ABOUT THE COVER

The figure on the cover represents a female mouse studying the impact of a western-type diet on her reproductive system. The western-type diet is characterized by food high in saturated fats and sugar, which is depicted here as pizza, hamburger, French fries, a hotdog and a soft drink. In this PhD thesis we investigated the effects of a western-type diet on the oviducts and ovaries of mice after different short and long-term periods of feeding.

This cover was made with the great help of one of my best friends, Kirsten Ryvers. All credits go to you for your artistic expertise in designing and creating a figure which clearly represents the focus of this PhD thesis. Thank you!

I would also like to thank Thomas Campaert for his help with the design of the cover and Silke Andries for brainstorming about the concept of the figure.

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Faculty of Pharmaceutical, Biomedical and Veterinary Sciences

Department of Veterinary Sciences

MATERNAL METABOLIC HEALTH AND FERTILITY:

ACUTE AND CHRONIC EFFECTS OF A WESTERN-TYPE DIET ON OVIDUCTAL AND OVARIAN CELLS
A multi-omic approach

Maternale metabole gezondheid en fertiliteit:

Acute en chronische effecten van een westers dieet op oviductale en ovariële cellen
Een multi-omic aanpak

Dissertation for the degree of Doctor in Veterinary Sciences (PhD) at the University of Antwerp to be defended by

KERLIJNE MOORKENS

Supervisors
Prof. dr. Jo Leroy
Prof. dr. Waleed Marei

Antwerp, 2024

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LIST OF ABBREVIATIONS

AO	Antioxidants
ART	Assisted reproductive technology
BMI	Body mass index
BP	Biological Process
B6	C57BL/6
CAT	Catalase
CC	Cumulus cell
COC	Cumulus oocyte complex
CRP	C-reactive protein
d	days
DIO	Diet-induced obesity
DM	Discriminative mass
ER	Endoplasmic reticulum
EV	Extracellular vesicle
FA	Fatty acid
FF	Follicular fluid
FFA	Free fatty acid
FSH	Follicle stimulating hormone
GC	Granulosa cell
GnRH	Gonadotropin releasing hormone
GO	Gene ontology
GPx	Glutathione peroxidase
GSEA	Gene Set Enrichment Analysis
GSH	Glutathione
hCG	Human chorionic gonadotropin
HDL	High-density lipoprotein
HF	High fat
HF/HS	High-fat high-sugar
HSL	Hormone sensitive lipase
IL	Interleukin
IL-6	Interleukin 6
IL-10	Interleukin 10
IL-1β	Interleukin 1β
IVF	In vitro fertilization
LDL	Low-density lipoprotein
LH	Luteinizing hormone

LPL	Lipoprotein lipase
MALDI	Matrix assisted laser desorption ionization
MF	Molecular Function
miRNA	microRNA
MMP	Mitochondrial membrane potential
MSI	Mass spectrometry imaging
mtDNA	Mitochondrial DNA
MUFA	Mono-unsaturated fatty acid
MW	Molecular weight
NADPH	Nicotinamide adenine dinucleotide phosphate
NEFA	Non-esterified fatty acid
OE	Oviductal epithelium
OEC	Oviductal epithelial cell
OF	Oviductal fluid
OS	Oxidative stress
PA	Palmitic acid
PCOS	Polycystic Ovarian Syndrome
PKA	Protein kinase A
PUFA	Poly-unsaturated fatty acid
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
SFA	Saturated fatty acid
SHBG	Sex hormone binding globulin
SOD	Sodium dismutase
TEM	Transmission electron microscopy
TG	Triglyceride
TNF-α	Tumour necrosis factor alpha
UPR	Unfolded protein response
VLDL	Very-low density lipoprotein
w	weeks
WHO	World Health Organization

CHAPTER 1: GENERAL INTRODUCTION

Content of General Introduction

1. Maternal metabolic health in relation to fertility

1.1 Western type diet, obesity and the impact on metabolic health and fertility

1.1.1 Obesity and the importance of subfertility: facts and figures

In the modern world, the overconsumption and large portion sizes of **obesogenic type diets** or western type diets**,** which are low in fruits and vegetables, high in long chain saturated fats and sodium and characterized by large portion sizes, a high amount of calories and excess sugar ((Haley et al., 2019b), in combination with a lack of physical activity, form the main cause of the increased **obesity** prevalence worldwide and is known to increase the risk of metabolic disorders, leading to metabolic changes, and eventually to the development of metabolic syndrome (Hu et al., 2003; London et al., 2017). The latter one, also called syndrome X or insulin resistance syndrome, is defined by the World Health Organization (WHO) as "a pathogenic condition characterized by abdominal obesity, insulin resistance, hypertension and hyperlipidaemia" (Saklayen, 2018). Metabolic syndrome is a condition that includes increased abdominal fat, hypertension, hyperglycemia, elevated triglyceride (TG) concentrations and low HDL cholesterol, and has found to be significantly impacting on female fertility (Hu, 2003). Metabolic syndrome has a global average prevalence of 31% and thus forms a major health hazard in today's world (Eckel et al., 2010; Engin, 2017a; Saklayen, 2018). Obesity is defined as an abnormal or excessive fat accumulation that impairs health, according to the WHO (Mahutte et al., 2018) and it is nowadays considered as the largest public health problem in industrialized and developing countries. As was recently stated by the WHO in their key facts on obesity and overweight, 1 in 8 people in the world were living with obesity in 2022 and worldwide adult obesity has more than doubled since 1990. Adolescent obesity has even quadrupled since 1990. The WHO stated that in 2022, 43% of adults aged 18 years and older were overweight and 16% were living with obesity (WHO, 2024). It was estimated that by 2030, 40% of the world population will be overweight, diagnosed at a BMI between 25.0kg/m^2 and

29.9kg/m^2, and 20% will be obese, diagnosed at a BMI that exceeds 30kg/m^2 (Bessesen, 2008; Fernandez-Sanchez et al., 2011; Oestreich and Moley, 2017; Liu et al., 2021). Next to the significant impact of obesity and overweight on metabolic health, reproductive health is also impaired in obese and overweight women.

The prevalence of **subfertility** and infertility among women has shown to progressively increase. As fertility is complex and depends on multiple factors, its impairment has been associated with complex interactions between nutritional, environmental and genetic factors. Subfertility is defined as any form or grade of reduced fertility in couples with prolonged time, being more than six cycles with unprotected intercourse, of unwanted non-conception (Gnoth et al., 2005), referring to the lacking ability of conception, maintaining a pregnancy or successfully producing healthy offspring. Infertility may be used synonymously with sterility and is clinically defined as one year or more of unwanted non-conception with regular unprotected intercourse in the fertile phase of the menstrual cycles (Gnoth et al., 2005; Zegers-Hochschild et al., 2009). Globally, an estimated one out of every six people are affected by the inability to have a child at some point in their life (WHO, 2021). In 2018, 50-80 million women worldwide encountered subfertility issues and in 20-30% of the cases, the cause was unknown. When discussing sub/infertility prevalence estimates it is however important to be aware of the variation in how researchers define and measure sub/infertility, which may result in imperfect estimates on subfertility prevalence (Cox et al., 2022). In Belgium, 10 to 15% of couples with difficulties to conceive at least once, seek reproductive assistance. According to the Belgian 2019 Assisted Reproductive Technology (ART) National Summary Report of the College of Physicians in Reproductive Medicine (published by the Belgian Register for Assisted Procreation (BELRAP)), 25% of the causes of infertility among ART patients are linked to pathologies in the female, 26% are male pathologies, 17% are due to a combined female and male pathology (17%), 24% remained unexplained with no clear cause of infertility found in

either partner and 8% is unknown. In 40% of the women, the pathologies relate to tubal defects, which may be, amongst others, a consequence of tubal infections with Chlamydia Trachomatis or Neisseria Gonorrhea (Silvestris et al., 2018). Other female pathologies were ovulatory dysfunction (in 40.88% of the women), endometriosis (35%), premature ovarian failure (8.5%), genetic anomaly (6.8%) and uterine factors (8.70%). Some patients had more than one cause identified per cycle (BELRAP, 2019).

Couples with subfertility may encounter feelings of reduced self-esteem, failure, anxiety and guilt, both in men and women, which may eventually even lead to grief and depression (Hasanpoor-Azghdy et al., 2014). Reproductively challenged couples also experience stigmatization, potential socio-isolation, exile and even being neglected by family and the community or being disinherited. In developing countries, the consequences of subfertility may form an added stress to couples as they directly rely on their children for future economic survival (Ombelet, 2014). New estimates by the WHO show some variation in sub/infertility prevalence across the WHO geographical regions, indicating lifetime prevalence of infertility is highest in the Western Pacific Region and African region and lowest in the Eastern Mediterranean Region. The available data was however based on a very limited number of studies, suggesting that the observed differences may not be substantial or conclusive (WHO, 2021).

Subfertility can be treated with ARTs, but, although the financial burden of ART treatment on society remains modest (Chambers et al., 2009; Chambers et al., 2013) as reproductive assistance only makes up less than 2% of the total health care costs in western countries (Katz et al., 2002), the economic impact for the patients remains significant (Chambers et al., 2009; Chambers et al., 2013). However, with the accessibility of treatment through social security systems and considering the increasing proportion of couples seeking reproductive assistance, fertility clinics providing ART are a thriving business (Chambers et al., 2009; Ombelet, 2014). In Belgium, 85% of the almost 41.000 ART cycles were covered by social security in 2021, as was reported by BELRAP. The total cost associated with an ART treatment, pregnancy

follow-up, delivery and child cost until the age of 2 years for singleton births was estimated to be 17.800 euros, of which the cost of the ART treatment is 2.417 euros (Peeraer et al., 2017).

Already 39% and 15% of the global female population is considered to be overweight or obese respectively, as was estimated by the WHO in 2016 (WHO, 2022)(Fig. 1). Various epidemiological studies have reported **impaired reproductive physiology in obese and overweight women** (Gesink Law et al., 2007; Wise et al., 2010; Loy et al., 2018). It is known that 60% of the women in the US with a wish to have children are overweight and that the reproductive outcomes of these women are lower compared to normal weight women (Luzzo et al., 2012). The risk of subfertility in obese women was even shown to be three times higher compared to non-obese women (Evers, 2002; Talmor and Dunphy, 2015). Furthermore, for every unit increase in BMI above 25kg/m2, chances for spontaneous conception decrease with 5% as was seen in a large cohort of subfertile couples across different ART centres. That same study reported a linear decline in the probability of spontaneous pregnancy above a BMI of 29 (van der Steeg et al., 2008). Generally, the prevalence of obesity is higher in adult women compared to men at all ages (Chooi et al., 2019) (Fig. 2) and obese women have shown to account for a larger percentage of seeking medical reproductive assistance compared to normal weight women (Vahratian and Smith, 2009). As the numbers of overweight and obese individuals continue to rise in western countries (WHO, 2017), female subfertility is expected to inevitably follow the same trend. Therefore, the economic, socio-cultural and psychological burden that female subfertility may have, is of growing significance.

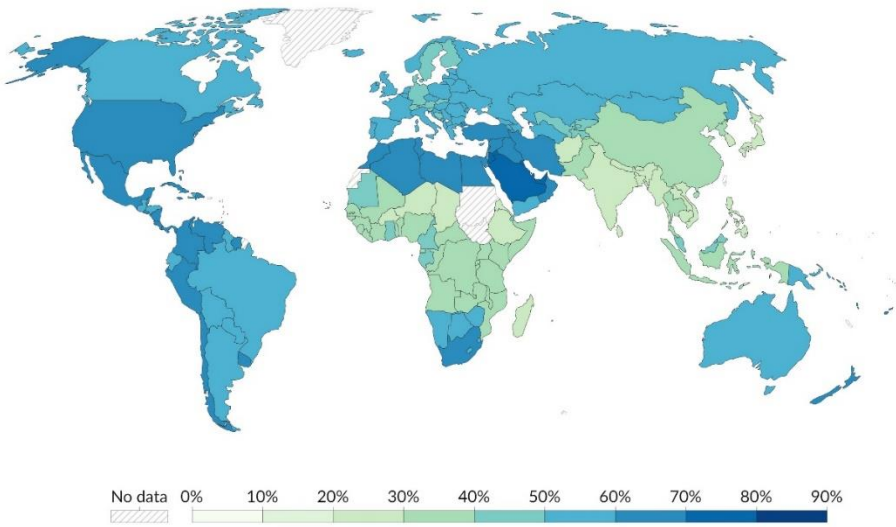

Figure 1. Share of women aged 18+ years who are overweight or obese (BMI>25), estimated by WHO in 2016 (WHO, 2022).

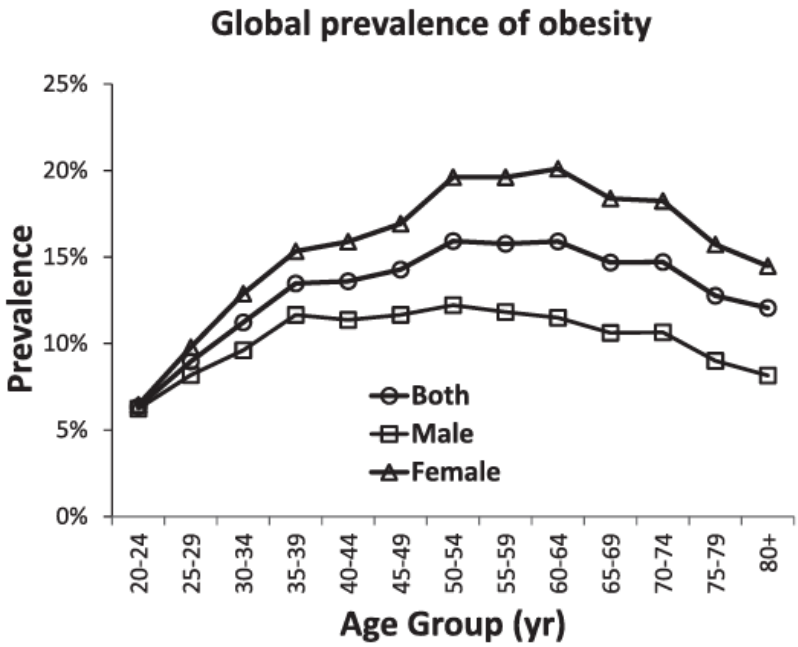

Figure 2. Global prevalence of obesity in adults > 20 years old by age group and sex (ca. 2015) (Chooi et al., 2019).

A disturbed metabolic state can result in many fertility problems such as menstrual irregularities, anovulation, longer times to conception, reduced conception rate, increased miscarriage rates, increased risk of pregnancy complications, dystocia and foetal abnormalities. Hampered reproduction in obese women can thus not be attributed to a single factor, but is a complex health issue evident at several levels (Robker, 2008; Zain and Norman, 2008). It has been reported that menstrual disturbances are fourfold more common in obese women (Seif et al., 2015). Anovulatory infertility has been associated with obesity in various human studies (Green et al., 1988; Jensen et al., 1999; Bolumar et al., 2000; Mola, 2009; Jungheim et al., 2012; Weiss and Clapauch, 2014; Giviziez et al., 2016). Women with an overweight of less than 20% of their body weight have 2.6% of anovulatory cycles, whereas a percentage of overweight exceeding 74% of their body weight, was linked with 8.4% anovulatory cycles. The risk of reproductive morbidity, including ovulatory disorders, is thus believed to increase when BMI is also increased (Jungheim et al., 2012; Giviziez et al., 2016). It has also been reported that greater degrees of obesity over a longer period of time is associated with worse clinical outcomes related to fertility, indicating that the effect of obesity is duration dependent (Best and Bhattacharya, 2015). Obese women, even when they have regular ovulation, have shown to have decreased rates of pregnancy and required a longer time to achieve spontaneous pregnancy (Gesink Law et al., 2007; Wise et al., 2010). Miscarriage rates have been reported to be increased as well in obese women (38.1%) compared to women with a normal BMI (13.3%) (Bellver et al., 2003; Lashen et al., 2004). Furthermore, obese women (25.3% IVF cycle cancellation rate) were found to have decreased success rates of ARTs compared to normal BMI women (10.9%) (Dokras et al., 2006; Luke et al., 2011). A 68% reduction has even been shown in chances of having a live birth following the first ART cycle for obese women compared to non-obese women (Sim et al., 2014). Besides higher chances for congenital anomalies, developmental delay and neurological deficits, children from obese mothers also have a higher chance to develop childhood obesity,

type II diabetes and cardiovascular diseases (Jungheim and Moley, 2010, Luzzo et al., 2012). In 2014, 41 million children under the age of 5 were overweight or obese. Researchers suggest that the global obesity pandemic is not only a result of the Western style diet and the sedentary lifestyle, but that the risk of obesity in the offspring is programmed before or during pregnancy (Oestreich and Moley, 2017). The 'First 1000 Days' campaign states that the nutritional status of both child and mother is most important in the fetal and neonatal period until 2 years after birth (total of 1000 days: 280 days before birth + 720 days after birth) (Suzuki, 2018). According to the Developmental Origins of Health and Diseases (DOHAD) hypothesis, adverse events at conception and during pregnancy, like maternal overnutrition, lead to an increased risk of illness later in life because of epigenetic consequences on the offspring (Oestreich and Moley, 2017). Epigenetic programming starts very early during development, particularly in the oviduct during pre-implantation embryo development. The mechanisms of epigenetic programming are not the focus of this work. However, it is important to understand that maternal metabolic stress and its impact on early embryo development does not only have an effect on fertility but can also lead to adverse effects in the offspring. **This highlights the importance of fundamental research to investigate the effects of metabolic stress on the reproductive system in order to further elucidate the underlying mechanisms of subfertility.**

1.1.2 Health and reproductive physiology: causative links

As subfertility comprises a complexity of events that can interfere with different aspects of reproductive physiology, it can be driven by many different pathways. Subfertility can, irrespective of species, be caused by immunological and infectious diseases, congenital abnormalities and extensive exposure to extreme environmental conditions such as endocrine disrupting chemicals, but also excessive caloric expenditure (for example by athletes) and chronic malnutrition (Evers, 2002). Aging is another risk factor and is known to reduce fertilization rates and increase foetal

chromosomal abnormalities (Kim et al., 2013). Furthermore, advanced maternal age is linked with significant morphological and functional alterations in the oocytes (for example atypical polar body morphology and intracytoplasmic anomalies such as vacuolization and intracellular lipid droplets) as well as with a reduced ovarian reserve and decreased oocyte/embryo competence. The overall success of female and mammalian reproduction is thus highly dependent upon maternal age (Cimadomo et al., 2018). Another key player contributing to the causes of infertility is **hormonal imbalance**. Disturbed hormone profiles, which are characterized by reduced oestradiol and progesterone levels, form the basis of fertility related disorders. Altered hormone balance can lead to ovulatory disorders, reduced oocyte and embryo quality, poor ART outcome, increased miscarriage rate and a range of gynaecological and obstetric comorbidities linked with obesity (Lash and Armstrong, 2009), which may even have lasting adverse effects on offspring (Jungheim et al., 2011a). Successful reproduction requires normal functionality of the hypothalamic-pituitary-gonadal axis, which is the 'command-centre' managing the mechanisms controlling reproductive function and metabolic state (Donato et al., 2011). The hypothalamus produces gonadotropin-releasing hormone (GnRH) which triggers the pituitary to produce gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), thereby regulating progesterone and estrogen production in the ovary (Schneider, 2004; Jain et al., 2007; van der Steeg et al., 2008). Progesteron and estrogen secreted by the ovaries can inhibit the hypothalamic synthesis of GnRH and gonadotropin synthesis by the pituitary gland via a feedback loop, hence playing a vital role in regulating reproductive function (Gupta P, 2021). Estrogen switches from being inhibitory (negative feedback) on GnRH secretion to stimulatory (positive feedback) on GnRH secretion upon ovulation (Kauffman, 2022). An overview of the hypothalamic-pituitary-ovarian (HPO) axis can be found in Figure 3. Disruptions at different levels of the HPO axis, as a consequence of a disturbed metabolic state, may have an impact on reproductive events such as follicular development and ovulation, eventually leading to decreased fertility. A number of

reproductive disorders, such as hypothalamic amenorrhea, hyperprolactinemia and PCOS in women, are associated with impaired GnRH pulsatility (Tsutsumi and Webster, 2009). Obese women showed to have lower GnRH, LH and FSH concentrations which may result in oligo- or anovulation, poor oocyte quality and aberrant follicle recruitment (Michalakis et al., 2013). In addition, sex steroid balance may also be disturbed by metabolic disorders. Insulin plays an important role in a multitude of ovarian functions such as steroidogenesis, regulating folliculogenesis, gene expression in the follicle, oocyte maturation etc. (Poretsky et al., 1999; Dupont and Scaramuzzi, 2016). The increased circulating insulin concentrations associated with obesity lead to the suppression of hepatic sex hormone-binding globulin (SHBG) synthesis, the carrier protein of androgens and estrogens in the blood (Hammond, 2011). A reduction in SHBG leads to elevated circulating free sex steroids, resulting in an increased metabolic clearance of these hormones. This is however compensated by increased androgen synthesis by the ovaries resulting in hyperandrogenism which has a pathophysiological effect on ovarian function and is also associated with menstrual disturbance and oligo-anovulation (Michalakis et al., 2013; Kazemi et al., 2020). Adipose tissue forms an important site for steroid hormone synthesis, metabolism and storage. Obese individuals have shown to have a greater steroid pool compared to normal weight individuals (Gambineri et al., 2002).

In addition, the impact of **lifestyle** on female fertility became a very important research focus the past years. More and more evidence shows that physiological stress, smoking, drugs, caffeine, environmental pollutants, metabolic stress and obesity adversely affect fertility. However, the underlying pathways and cellular mechanisms are yet often missing and not well understood (Silvestris et al., 2018). An in-depth knowledge of the involved pathways is crucial, though, in order to be able to propose advise on lifestyle, preconception care or therapeutic interventions.

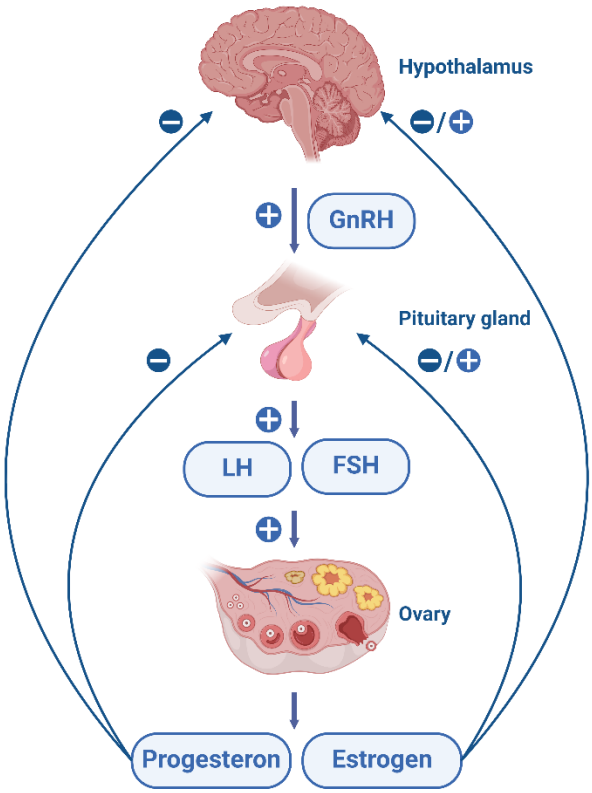

Figure 3. Overview of the hypothalamic-pituitary-ovarian axis. Estrogen switches from being inhibitory (negative feedback) on GnRH secretion to stimulatory (positive feedback) on GnRH secretion upon ovulation (Kauffman, 2022). GnRH = Gonadotropin Releasing Hormone; LH = Luteinizing Hormone; FSH = Follicle Stimulating Hormone (made with Biorender).

While the systemic metabolic disorders that are associated with obesity and obesogenic diets have their effects on overall body function, they specifically affect reproduction. **Metabolic stress** conditions are known to affect reproduction since diet (Leroy et al., 2014) and metabolic health (Robker et al., 2009b) were found to be reflected in the oocyte and embryo developmental environment, thus linking metabolic health and subfertility. Metabolic stress is defined as a physical imbalance resulting

from an overload or shortage in directly available energy sources (Sordillo and Raphael, 2013). The consequences of metabolic stress are evident and are not only focused on the direct impact on ovarian cyclicity (Leroy et al., 2011). Metabolic stress conditions affect fertility through interactions with reproductive processes at different levels of the female tract, thereby leading to reduced oocyte and embryo development, as was shown in *in vitro* bovine studies (Leroy et al., 2017). The impact may be mediated through systemic alterations or through direct detrimental effects during oocyte and embryo development and during pregnancy (Jungheim and Moley, 2010). Obese individuals show upregulated fat storage due to the excessive supply of TGs from high fat (HF) diets and simultaneous hyperglycaemic conditions (Morigny et al., 2016), of which the latter were reported to be directly toxic for *in vitro* embryo growth (Fraser et al., 2007).

Animal models are often used to thoroughly study the effects of obesity and metabolic changes on reproduction and specifically oocyte quality. Mice are commonly used as diet-induced obesity (DIO) models. Because of the absence of a specific marker and a consensus for mice that defines the presence or absence of obesity, some studies have established their own parameters such as the difference of 15% or 20 gram in body weight between test and control groups, adiposity index determination, creation of cutoff points, and calculation of body mass index. However, the majority of studies consider the differences in total body weight gain as the main parameter to assess the outcome of the development of obesity (reviewed by (Dias et al., 2021)).

The sequence of the pathophysiological changes, that occur at the cellular level in association with the development of a metabolic disorder such as obesity, varies from one tissue to another and is not clearly described. However, it is known that hyperlipidaemia, as a result of a high fat/high sugar (HF/HS) diet, leads to lipotoxicity, mitochondrial dysfunction and oxidative stress (OS) as well as to local inflammatory responses (Lin et al., 2005; Herdt, 2013; Engin, 2017b). These are the key mechanisms

by which peripheral tissues are affected during the development of metabolic disorders. These mechanisms may also apply on the temporal changes that are expected to occur in the reproductive tract (Herdt, 2013; Engin, 2017b).

In the following sections, the underlying mechanisms of the obesogenic diet-induced metabolic changes will be described in more detail (an overview can be found in Figure 5) as well as their impact on reproduction.

1.1.3 Diet-induced metabolic changes and dynamics that lead to systemic metabolic stress and metabolic disease

1.1.3.1 Hyperlipidaemia and lipotoxocity

In the intestinal lumen, TGs, from the diet, are digested to free fatty acids (FFAs) and taken up by the enterocytes. Cholesterol is taken up through a specific cholesterol transporter after which it is transformed in the enterocyte into cholesterol-esters. TGs are re-synthesized and packaged together with cholesterol-esters in chylomicrons (Hussain, 2014). When they are released in the blood stream, the chylomicrons interact with lipoprotein lipase (LPL), resulting in lipoprotein precursors which can be taken up by the liver. Lipoproteins of different sizes, very low density (VLDL), low density (LDL) and high density (HDL) lipoproteins, are released from the liver into the circulation (Goldstein and Brown, 2009; Klop et al., 2012; Lambert et al., 2012). The different lipoproteins contain TGs, cholesterol, phospholipids and proteins. In the bloodstream, chylomicrons and VLDL interact with LPL. LPL forms the primary enzyme for TG lipolysis in the circulation and is stimulated by insulin, which increases postprandially. It catalyzes the release of FFA which diffuse across the endothelial cells after which they can enter the adipose, muscle, mammary and other target cells (Karpe et al., 2011; McQuaid et al., 2011). The postprandial increase of insulin inhibits hormone sensitive lipase (HSL), a key enzyme for hydrolysis of FAs from TGs in adipose tissue, which results in glycerol and 3 free or non-esterified fatty acids (NEFAs) (Karpe et al., 2011) (for an

overview see Figure 4). When lipolysis occurs, chylomicrons and VLDL shrink and form respectively chylomicron remnants and dense LDL. Through the LDL receptor, LDL is taken up by the liver. Also the chylomicron remnants are taken up by the liver and they are broken down into FFA, cholesterol, glycerol and amino acids (Goldstein and Brown, 2009; Lambert et al., 2012). The liver also contributes to HDL particles synthesis. HDL stimulates the uptake of cholesterol from peripheral tissues and returns cholesterol to the liver.

Obesity is typically associated with dyslipidemia which consists of increased TGs and FFAs, decreased HDL cholesterol with HDL dysfunction and normal or slightly increased LDL cholesterol with increased small dense LDL. In addition, human obesity is commonly associated with increased FFA and cholesterol concentrations as well as hypertriglyceridemia in the serum (Klop et al., 2013). Hypertriglyceridemia is a form of hyperlipidaemia in which the blood TG levels are elevated (Karr, 2017). Lin et al. (2005) have demonstrated that 48 hours of feeding a HF diet, with high concentrations of saturated fats and trans fats, resulted in hypercholesterolemia and hypertriglyceridemia in C57BL/6 mice (Lin et al., 2005). Various other studies also reported higher levels of LDL cholesterol in the serum of HF diet fed mice compared to controls (Williams et al., 2014; Huang et al., 2019; Sadowska et al., 2019). Furthermore, mice fed a HF diet showed elevated glucose concentrations in their blood. The observed glucose intolerance was reported to develop in distinct phases. The first phase, after 3 days of feeding, resulted in elevated glucose concentrations, which improved after 1 week on the diet and remained stable until 12 weeks. Between 12 and 16 weeks on the HF diet the glucose concentration showed a second significant increase (Williams et al., 2014). Plasma NEFA concentrations are increased in obese women due to the upregulated basal lipolysis of the adipose tissue and the increased fat uptake from their diet (Alligier et al., 2013). The adipose tissue plays an important role in metabolic disorders. Under normal conditions, HSL moves from the cytosol to the surface of lipid droplets and interacts with perilipin-1 and neutral lipids. HSL then hydrolyses

triglycerides into glycerol and NEFAs. In the circulation, NEFAs are bound to albumin to facilitate their transport to different target cells (Bolsoni-Lopes and Alonso-Vale, 2015; Sun et al., 2019). Reduced circulating insulin or reduced insulin sensitivity, which is associated with metabolic disorders, results in the ineffective inhibition of HSL-mediated lipolysis in adipose tissue (Lan et al., 2019) (Fig. 4). Adipocytes undergoing uncontrolled lipolysis results in high levels of circulating NEFAs in obese individuals.

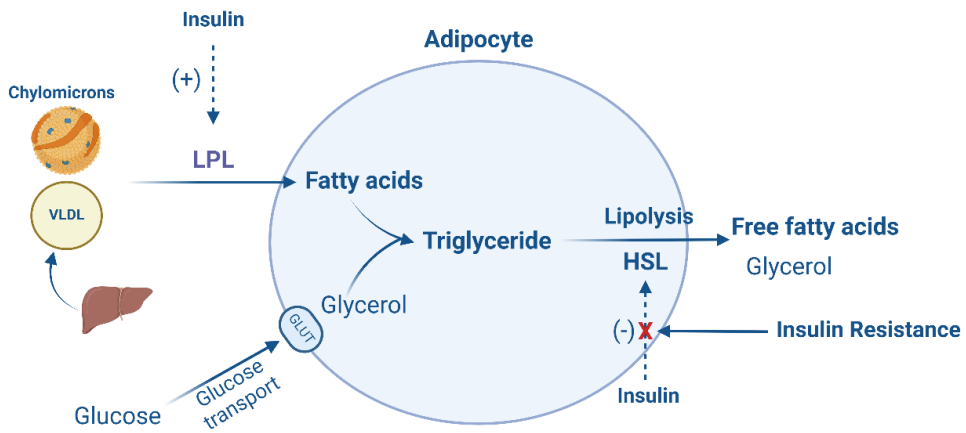

Figure 4. Summarized overview of the regulation of adipose storage and lipolysis by insulin. Insulin resistance, which is associated with metabolic disorders, results in the ineffective inhibition of HSL-mediated lipolysis in adipose tissue, causing adipocytes to undergo uncontrolled lipolysis, leading to high levels of circulating free fatty acids. LPL = Lipoprotein Lipase; VLDL = Very Low Density Lipoprotein; GLUT = Glucose Transporter; HSL = Hormone Sensitive Lipase (made with Biorender).

Additionally, increased caloric intake leads to increased storage of TGs in the adipocytes and as the adipocytes grow their capacity to limit lipolysis in response to insulin is impaired. With increasing lipolysis, the adipokine production by the adipose tissue increases as well (Arner, 2005). The metabolism of different tissues and organs, especially muscle, liver and pancreas, is influenced by adipokines. Adipocyte hypertrophy leads to infiltration of the adipose tissue with macrophages and T helper cells. The macrophages secrete pro-inflammatory cytokines and interleukins, such as

TNF-α and IL-6. Upon secretion by the adipose tissue, adipokines promote additional release of TNF-α and IL-6 (Snider and Wood, 2019). TNF-α has a big impact as it alters the normal insulin signalling pathway, stimulates lipid breakdown by altering the action of perilipin, decreases the insulin receptor substrate-1 activity and its substrate phosphorylation and decreases the glucose transporter GLUT4 synthesis and membrane translocation in the adipose tissue. Thereby, the plasma NEFA concentrations continue to increase which leads to increased lipid deposition in the liver, skeletal muscle, pancreas and heart (Bergman and Ader, 2000; Wajchenberg, 2000; Tamura and Shimomura, 2005; Karpe et al., 2011). Chronic elevation of circulating NEFAs can reach toxic levels of TG accumulation within non-adipose tissues. The deleterious effects of the intracellular lipid accumulation in non-adipose tissues are known as lipotoxicity, which promotes insulin resistance (Engin, 2017c). Lipotoxicity is typically associated with decreased cell function by affecting energy uptake and processing pathways, inducing oxidative stress and mitochondrial dysfunction.

Elevated serum NEFA concentrations are reflected in the FF, as well as oviductal fluid, as was shown in humans and cows (Leroy et al., 2005; Valckx et al., 2014a; Jordaens et al., 2017a) and can thus have a detrimental impact on oocyte and embryo quality (Jungheim et al., 2011a). NEFAs can reach the oocyte via the FF and can be taken up by the follicular cells and/or oocytes. In the oocyte, fatty acids have an important role in maturation, fertilization and preimplantation development as well as serving as an important source for energy production (Dunning et al., 2010). To protect itself from lipotoxicity the oocyte efficiently stores FAs intracellularly within lipid droplets (Warzych et al., 2017). Oocytes from follicular fluid rich in FAs, may thus contain a higher number of lipid droplets (Ferguson and Leese, 2006; Sturmey et al., 2009; Warzych et al., 2017). Oocytes were shown to actively take up and metabolize NEFAs out of their environment by fatty acid β-oxidation (Aardema et al., 2011). An excessive amount of lipid droplets can however have a large impact on oocyte and embryo quality. Intracellular lipids were shown to be dramatically increased in both oocytes

and cumulus cells of DIO mice (Wu et al., 2010). The increase in intracellular lipids in the oocyte may be caused by an upregulated active uptake of lipids from the FF or by the different nutrient availability which leads to a shift in energy pathways in the oocyte, however this is not clear yet. The accumulation of lipids in such cells other than adipocytes and the incapability of packaging the FAs into lipid droplets may however be considered as the initiation of lipotoxicity (Szendroedi and Roden, 2009; Hauck and Bernlohr, 2016; Engin, 2017c).

Next to the increased accumulation of neutral lipids in somatic cells, an obesogenic diet may also change the composition of lipids which are essential for the structure and functions of the plasma membrane and membranous organelles and play an important role in cell signalling and cell-to-cell interaction (Cockcroft, 2021). Cell membrane phospholipid composition is regulated by the FA composition of dietary fat, being especially sensitive to n-6 and n-3 polyunsaturated fatty acids (PUFAs), with a preference for the latter (Haag and Dippenaar, 2005; Perona et al., 2007). Membrane saturated fatty acid (SFA) and monounsaturated fatty acid content is not as dependent on the dietary FA profile, as these FAs can be synthesized endogenously. The type and amount of dietary lipids have shown to influence the lipid composition of cell membranes and impact interactions with proteins involved in different processes associated with the metabolic syndrome, such as dyslipidaemia, regulation of insulin sensitivity and hypertension. The effects were suggested to be partially mediated by the modification of the composition and structural properties of plasma membranes (Barcelo et al., 2009). The modification of plasma membrane lipid composition can change membrane biophysical properties and can thus influence protein-lipid interactions, enzymatic activity and regulation of surface receptors (Yang et al., 2011; Maulucci et al., 2016; Romero et al., 2019). Obesity has been linked to altered membrane lipid composition and fluidity (reviewed by (Ahyayauch, 2023)). Moreover, lipid bilayer stress can modulate the function of membrane proteins involved in the

pathogenesis of obesity-linked inflammatory and metabolic disorders (reviewed by (Gianfrancesco et al., 2018)). Many studies have reported a strong relationship between dietary lipids, membrane lipid profiles and insulin resistance, with SFAs rich diets leading to insulin resistance (Haag and Dippenaar, 2005; Chabowski et al., 2013). Different studies have demonstrated that alterations in the fat content of the diet and in the ratio between saturated and unsaturated FAs influence the lipid profile of reproductive cells and tissues. As such, n-3 polyunsaturated fatty acid (PUFA) supplementation of the diet increased n-3 PUFA content of endometrial epithelial cells of cattle (Childs et al., 2008). Additionally, Zachut et al (Zachut et al., 2011) reported increased concentration of n3-FA in plasma, FF and granulosa cells (GCs) of cows fed a diet supplemented with n-3 FAs. A similar study in sheep demonstrated an altered FA composition of both GCs and oocytes as well as an impact on ovarian steroidogenesis (Wonnacott et al., 2010).

1.1.3.2 Cellular stress and the role of mitochondria

In the mitochondria ATP is generated through oxidative phosphorylation in the mitochondrial matrix. By-products of this process are reactive oxygen species (ROS) (Menezo et al., 2016), which play a key role as signaling molecules. ROS are generated as some electrons in the mitochondrial transport chain 'leak' to oxygen thereby forming superoxide anion ($O_2^{\bullet-}$) (Kovacic et al., 2005). Superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) are considered the main ROS species (Engin, 2017c; Snider and Wood, 2019).

In the lipotoxic state, the metabolization of FFAs increases the rate of oxidative phosphorylation which leads to excessive production of ROS. The resulting high concentrations of ROS are harmful to different cellular structures and functions (Gehrmann et al., 2010). For example in the membrane of the cell or mitochondria, ROS can induce the oxidation of lipids, resulting in the formation of highly reactive lipid peroxides, which can then cause further disruption of several cellular processes (Su et al., 2019). Cells contain a large number of antioxidants to prevent or repair the damage

caused by ROS. The most important enzymes in the cellular antioxidant (AO) defense mechanisms are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and peroxiredoxins (like PRDX6). SOD converts $O_2^{\bullet-}$ in the mitochondria into H_2O_2 and molecular oxygen. Catalase and peroxidases convert H_2O_2 in the cytoplasm into water. Peroxiredoxins are able to reduce H_2O_2 and lipid peroxides (Weydert and Cullen, 2010; Rhee et al., 2012). When these important antioxidants no longer have the capacity to process excessive amounts of ROS and other radical species, **oxidative stress** (OS) occurs (Maier and Chan, 2002). Therefore, OS is defined as "an imbalance between the generation of free radicals and the AO capacity of the organism"(Ballesteros-Guzman et al., 2019). Ros can also damage the mitochondria, even though mitochondria possess several adaptive mechanisms to cope with oxidative stress (Marei and Leroy, 2022). When ROS levels exceed a certain threshold, uncoupling proteins (UCPs) in the inner mitochondrial membrane are activated and partially expel the proton gradient in the mitochondria. As this generates a bypass of electron transport from complex V (or ATP synthase) from the electron transport chain, it reduces the driving force of mitochondrial ROS production (Hass and Barnstable, 2021). This reduction in the mitochondrial membrane potential (MMP) also elicits a mitochondrial unfolded protein response (UPR^{MT}) in which the stress-activated transcription factor ATFS-1 is translocated to the nucleus, where it stimulates gene expression of mitochondrial chaperones. In addition, the UPR^{MT} initiates a global reduction in protein synthesis, inducing protein degradation of endoplasmic reticulum (ER)-associated proteins (Rutkowski and Kaufman, 2004). At low levels of stress, cellular homeostasis can be reestablished by chaperones, mtHsp70, Hsp60 and Hsp10, as they alleviate stress-induced damage and prevent the induction of apoptosis (Nargund et al., 2012; Runkel et al., 2014). When exposure to high levels of ROS persists, the translocation of misfolded proteins to the mitochondria increases, which may cause ultrastructural damage. Together with mitochondrial uncoupling, this can impact cellular mitochondrial bioenergetic capacity and metabolic functions (Marei and

Leroy, 2022). Higher stress levels may activate mitophagy, a process by which damaged mitochondria can be eliminated and replaced (Lemasters, 2014).

The endoplasmic reticulum (ER) is a cellular organelle important for regulating calcium homeostasis, lipid metabolism, protein synthesis, and posttranslational modification and trafficking (Han and Kaufman, 2016). The ER functions and protein folding processes are also sensitive to lipotoxicity and OS (Adachi et al., 2009; Pagliassotti et al., 2016). An increased stress level results in failure of protein folding or protein misfolding, which are then retained in the ER to be either repaired or degraded by the ER-associated degradation (ERAD) machinery (Nakatsukasa et al., 2008). High levels of intracellular lipids and high ROS concentrations can generate structural alterations of the ER, compromising ER functions such as lipid processing, Ca^{2+} homeostasis and protein folding, which leads to the accumulation of misfolded proteins and elicits specific ER unfolded protein responses (UPR^{ER}) (Borradaile et al., 2006). Lipotoxicity is known to increase membrane fluidity of the ER (Celik et al., 2023). ROS oxidizes nascent proteins and increases misfolded and unfolded proteins in the ER. Further, ROS acts on calcium channels in the ER membrane, followed by stimulation of calcium release from the ER. Decreased concentration of the total calcium in the ER lumen ultimately impairs protein folding of nascent proteins (Kawasaki et al., 2012). The resulting **ER stress** is defined as “an imbalance between the load of unfolded proteins that enter the ER and the capacity of the cellular machinery to handle this load”. UPR^{ER}, activated by ER stress, is associated with increased expression of *ATF4* and *ATF6* (Ron and Walter, 2007; Snider and Wood, 2019). The UPR^{ER} is characterized by three main actions, namely a reduction in the protein load that enters the ER, an increase in the capacity of the ER to handle unfolded proteins by activation of UPR target genes and cell death (Ron and Walter, 2007). Chronic ER stress can however lead to apoptosis (Tabas and Ron, 2011). ER stress mediates lipotoxicity in several peripheral organs through ER stress-induced apoptosis or modulation of membrane composition of phospholipids (Han and Kaufman, 2016).

A certain level of ROS may be needed for successful conception (Wiener-Megnazi et al., 2004) and is involved in different physiological processes related to oocyte and embryo development (Cardoso et al., 2019). Low levels of follicular ROS have been proposed as a candidate marker for predicting a successful pregnancy outcome in IVF patients (Attaran et al., 2000). The state of upregulated lipolysis in obese women can however lead to upregulated uptake of NEFAs during oocyte maturation, which disrupts oocyte metabolism and leads to increased mitochondrial activity (Gu et al., 2015). This results in excessive ROS generation and eventually leads to OS. ROS can damage mitochondria leading to mitochondrial dysfunction, further aggravating the OS (Agarwal et al., 2008; Jungheim et al., 2010; Wu et al., 2010). **Mitochondria** are the most prominent organelles within the oocyte and early embryo and are essential to ensure oocyte developmental competence as next to energy production they carry out various other functions within the oocyte, such as spindle formation, promoting cell survival, calcium signalling, regulating apoptosis and mitochondrial recovery through the UPR^{MT} (Van Blerkom, 2004; Richani et al., 2021). Maintaining the balance between ROS production and detoxification is thus crucial for proper embryo development (Halliwell and Aruoma, 1991; Kehrer and Lund, 1994; Cardoso et al., 2019). As it is generally believed that sperm mitochondrial proteins are degraded when entering the oocyte, mitochondria are exclusively maternally inherited (Song et al., 2014). The preimplantation embryo can only depend on the oocyte's mitochondria as mitochondrial replication does not start until the blastocyst stage. Therefore, mitochondrial dysfunction may have a severe negative impact on the quality of gametes, oocyte maturation and fertilization (Agarwal et al., 2012; Da Broi and Navarro, 2016; Budani et al., 2017; Abbasihormozi et al., 2019) and result in a detrimental impact on early embryo development (Richani et al., 2021). In contrast to somatic cells, mitochondrial dysfunction in oocytes does not lead to increased mitophagy (Boudoures et al., 2017). As a consequence, damaged mitochondria accumulate in the oocyte and

mitochondrial dysfunction may persist during early embryo development (Marei et al., 2019). Further exposure to lipotoxicity due to high concentrations of NEFAs in the oviductal fluid can aggravate this even more. The absence of mitophagy in the setting of a high-fat/high-sugar (HF/HS) exposure contributes to the oocyte-to-blastocyst transmission of dysfunctional mitochondria (Boudoures et al., 2017). The limited success rates in obese women going to IVF clinics were mainly attributed to poor mitochondrial quality in the oocytes (Shamsi et al., 2013). Bovine oocytes that were matured under lipotoxic conditions *in vitro* showed increased ROS levels in the oocytes and consequently a lower developmental competence compared to the controls (Marei et al., 2017). The resulting blastocysts were triggered by OS regulating mechanisms and showed signs of mitochondrial dysfunction. Diet-induced obesity in mice showed oocytes and zygotes with altered mitochondrial function and increased ROS, resulting in embryos with reduced developmental capacity (Igosheva et al., 2010). Furthermore, pre-implantation embryos resulting from oocyte maturation under lipotoxic conditions showed altered mitochondrial functions and DNA methylation (Desmet et al., 2016), as well as significant epigenetic modifications in developmentally important genes (Duranthon et al., 2008; Arnaud, 2010). A recent study from our laboratory demonstrated that zygotes derived from metabolically compromised bovine oocytes, by PA-exposure during final oocyte maturation, already exhibit altered histone modifications. During *in vitro* culture, a concentration of PA was used that resembles the serum and oviductal fluid pathophysiological concentrations (Jordaens et al., 2017a). With this continued exposure to a lipotoxic microenvironment during early embryo development, DNA and histone methylation marks were found to be increased in morulae (Meulders et al., 2023). Moreover, Meulders et al. (2024) reported that oocyte and early embryo epigenetic programming are dependent on mitochondrial ATP production during maturation, using a bovine *in vitro* experiment. The significant epigenetic modifications can distort implantation, placentation and foetal growth rate

and compromise postnatal health (Young et al., 1998; Godfrey et al., 2007; Rivera and Ross, 2013; Pisarska et al., 2019).

Abnormalities in mitochondrial function are evident at different levels. Mitochondrial morphology is one of the factors contributing to their functioning. Normal mitochondria in the oocyte have a smooth inner membrane, truncated cristae and a round shape (Fabozzi et al., 2021). The oocytes of obese mice exhibit mitochondria with a higher number of vacuoles, fewer cristae, increased cristae disarray and decreased electron density of the mitochondrial matrix compared to oocyte mitochondria from control mice (Luzzo et al., 2012). An unbalanced dietary intake also resulted in oocytes with dumbbell shaped mitochondria. This was linked to alterations of their dynamics, which may suggest the occurrence of fission and fusion (Boudoures et al., 2016). Swiss mice that were fed a HF diet for 13 weeks showed oocytes with significantly increased mitochondrial ultrastructural abnormalities. The abnormal mitochondria were vacuolated with loose membranous structures, elongated, dumbbell and rose petal in shape and contained highly electron dense foci or they were degenerated (Marei et al., 2020). To guarantee proper oocyte maturation, fertilization and embryo development, the number of mitochondria and their activity have to be regulated as well (Reynier et al., 2001; Cummins, 2002; Kirillova et al., 2021). In that perspective, mtDNA copy numbers have shown to be crucial to ensure acceptable fertilization rates. The analysis of mtDNA content in fertilized and non-fertilized oocytes has shown that the non-fertilized oocyte has a significant reduction of 35% in mtDNA copy numbers (Santos et al., 2006). Also in mice it was shown that low mtDNA copy number oocytes were more likely to be negatively affected than high mtDNA copy number oocytes (Ge et al., 2012). Mitochondrial DNA copy numbers are significantly higher in obese mice fed a HF diet compared to control, lean mice (Igosheva et al., 2010; Luzzo et al., 2012) The magnitude of polarity across the inner mitochondrial membrane is also involved in the mitochondrial activity. High NEFA exposure during bovine *in vitro* maturation resulted

in oocytes and cumulus cells with reduced inner MMP (Marei et al., 2017). Igosheva et al. 2010 have observed oocytes with an increased MMP in obese mice (Igosheva et al., 2010), whereas feeding a HF diet to mice resulted in oocytes with a significantly reduced MMP in the study of Wu et al. (2010). Reduced oocyte developmental competence has been associated with both decreased and increased MMP (Wilding et al., 2001; Acton et al., 2004). This could be explained by the process of mitochondrial uncoupling which in somatic cells is an actively regulated process in response to oxidative stress to reduce further ROS and it constitutes the loss of inner MMP as an attempt for cell survival (Brand, 2000). This may result in a heterogeneous population of hyperactive and inactive mitochondria within the same oocyte. Another important potential marker of oocyte developmental competence is the distribution pattern of mitochondria throughout the oocyte. It was shown that, after maturation, oocytes with larger mitochondrial clusters at the periphery were linked with higher developmental competence compared whereas oocytes linked with reduced developmental competence did not show this mitochondrial distribution pattern (Stojkovic et al., 2001). Mitochondria surrounding the spindle is another indicator demonstrating mitochondrial function in oocytes. During oocyte meiosis, mitochondria usually surround the spindle to meet the energy demand of spindle migration and chromosome segregation (Wang et al., 2020). Spindle defects and chromosome alignments are significantly higher in HF diet fed mice compared to control mice (Grindler and Moley, 2013). Studies in which mitochondrial function was inhibited demonstrated defects in meiotic and mitotic spindles and inhibited oocyte maturation and embryo development (Zhang et al., 2006; Dalton et al., 2014; Wu et al., 2015). An established measure of mitochondrial function is the oxygen consumption rate (OCR) of the oocytes. This can be measured using a Seahorse XF Bioanalyzer which detects extracellular changes in the cellular consumption of oxygen and the efflux of protons to measure rates of cellular mitochondrial respiration, glycolysis and ATP production. As the Seahorse is based on extracellular flux analysis, the technique is a non-invasive

technique and enables real-time determination of the impact of compounds on the OCR (Muller et al., 2019).

Feeding an obesogenic diet leads to abnormalities in mitochondrial morphology, mitochondrial distribution, metabolism and spindle formation in the oocyte. A direct result of the mitochondrial dysfunction is reduced oocyte quality which ultimately leads to subfertility.

In the reproductive tract, induction of stress responses have been documented in the oocytes and GCs collected from HF diet-induced obese mice. Markers of oxidative and cellular stress (e.g. ER stress markers: *ATF4* and *HSPA5* (also known as *GRP78* or *BiP*)) were found to be augmented compared to the controls (Wu et al., 2010). Expression and activity of antioxidant enzymes have also been shown to be altered as a result of obesity. Sadowska et al. (2019) for example showed an increase in *SOD* expression in the uterus of Wistar rats fed with a 16% sucrose diet for 8 weeks (Sadowska et al., 2019). In addition, Marei et al. (2020) reported an increase in *PRDX6* expression in the oocytes of Swiss mice fed with a HF diet for 13 weeks as well as a significant increase of *HSPE1*, related to mitochondrial UPRs, in both oocytes and CCs (Marei et al., 2020). Bovine oocytes that were matured under lipotoxic conditions showed higher ROS content and exhibited upregulation of reduction-oxidation-related genes (Van Hoeck et al., 2013). Moreover, Marei et al. (2019) reported that blastocysts produced from palmitic acid (PA)-exposed oocytes had a higher mRNA expression of *CAT, SOD, BiP* and *ATF6* (Marei et al., 2019). It can thus be concluded that the impact of lipotoxicity, caused by a HF diet, on oocyte quality is indeed mediated by OS, ER stress and mitochondrial dysfunction. **However, whereas the direct impact of obesity and obesogenic diets on the ovarian FF microenvironment and oocyte quality is relatively more documented, the effect on the microenvironment of other reproductive organs, particularly the oviduct, remains poorly understood. The oviduct, however, provides the earliest developmental environment for the embryo. Such unfavorable**

conditions associated with obesity and obesogenic diets in the oviductal tract may thus directly lead to a disturbed reproductive outcome.

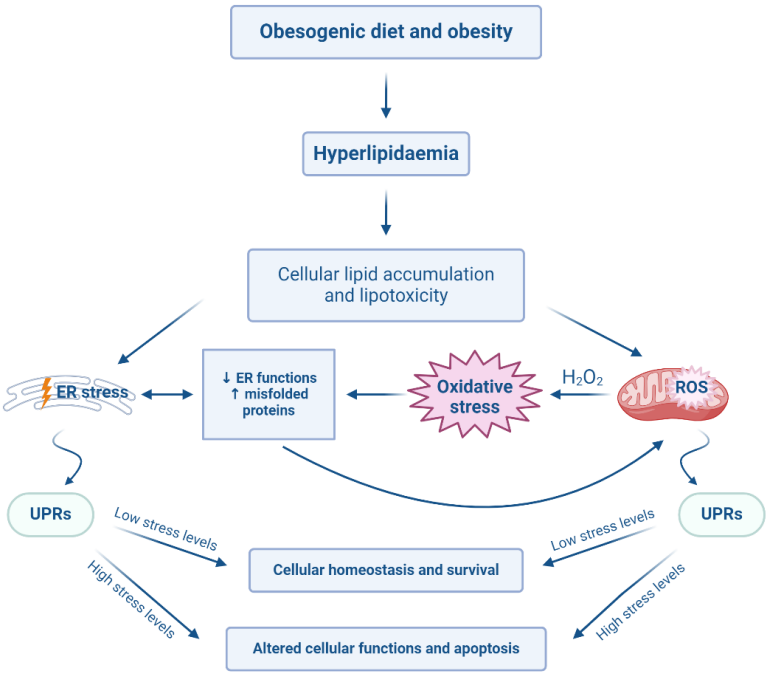

Figure 5: Link between lipotoxicity, oxidative stress, endoplasmic reticulum stress and UPRs. An overview of the sequence of the pathophysiological changes, that may occur at the cellular level of peripheral tissues in association with the development of a metabolic disorder such as obesity. Hyperlipidaemia due to an obesogenic diet results in lipotoxicity, which affects the function and structure of mitochondria, leading to incomplete beta-oxidation of free fatty acids (FFAs). This results in increased reactive oxygen species (ROS) (H_2O_2) levels and a disturbance of the redox balance (oxidative stress). Lipotoxicity and oxidative stress can disrupt endoplasmic reticulum (ER) functions leading to accumulation of mis-folded proteins, causing ER stress, more ROS production, and the activation of unfolded protein responses (UPRs). Under high levels of cellular stress this all leads to altered cell functions and apoptosis (made with Biorender).

1.1.3.3 Inflammation

Inflammation is defined as dysregulated and persistent chemokine and cytokine synthesis and secretion and has found to be linked to lipotoxicity pathways. As

discussed before, increased circulating TGs cause adipocyte hypertrophy which results in infiltration of adipose tissue with macrophages and T helper cells. These macrophages secrete TNF-α and other pro-inflammatory interleukins, like interleukin 6 (IL-6) (Snider and Wood, 2019). IL-6 and tumor necrosis factor alpha (TNF-α) are cytokines with an inflammatory function. For example, TNF-α activates the NF-κB signal transduction pathway resulting in the production of more cytokines and chemokines (IL-1β, IL-6, IL-12, IL-23, MCP-1, etc.) and an inflammatory state in the adipose tissue (Fernandez-Sanchez et al., 2011; Liu et al., 2017; Snider and Wood, 2019). In addition, it has been shown that activation of NF-κB may increase pro-oxidant genes such as NADPH oxidase in the adipose tissue, indicating the link between inflammation and the generation of ROS (Fernandez-Sanchez et al., 2011; Lingappan, 2018). Similar responses also occur in non-adipose tissues. The peri-ovarian adipose tissue of mice fed a HF diet for 7 months showed increased immune cell infiltration and an upregulation of pro-inflammatory cytokine/chemokine gene expression (Nteeba et al., 2013). In addition, excessive FA release can also influence insulin sensitivity by modulating adipocyte secretion of adipokines and lipokines and FAs promote cytokine production in macrophages causing adipose tissue inflammation, a known parameter that also contributes to insulin resistance. (Morigny et al., 2016). Adipokines are commonly involved in metabolic regulation and inflammatory processes and include adipose-specific cytokines, secreted by adipocytes, such as leptin, adiponectin, visfatin and resistin, and the non-adipose specific cytokines such as IL-6, interleukin 1β (IL-1β), and TNF-α (Silvestris et al., 2018). They are also known to maintain the integrity of the hypothalamic-pituitary axis and regulate ovulatory processes and successful embryo implantation (Tersigni et al., 2011; Silvestris et al., 2018). Alterations in adipokine signalling can affect female fertility (Mitchell et al., 2005) by impacting the hypothalamic-pituitary axis as well as ovarian follicles and early embryos in women (Michalakis et al., 2013). For example, the progressive expansion of adipose tissue in obese mothers culminates with hyperleptinemia and the establishment of leptin

resistance which result in major consequences in follicle and oocyte growth and maturation. The altered leptin signalling affects preovulatory follicle formation by altering steroidogenesis and the communication between the granulosa cells (GCs) and the oocyte, thereby hampering antrum formation and oocyte growth. Furthermore, changed leptin signalling hinders meiosis resumption and cytoplasmic maturation, thus drastically affecting oocyte maturation (Wolodko et al., 2021).

Lipotoxicity can lead to systemic inflammation already as early as 3 days after the start of the HF diet, illustrated by significant higher plasma levels of IL-6 in C57BL/6 mice. This may result in local inflammatory responses at the cellular level after long term HF diet feeding. After 12 weeks and 16 weeks on the HF diet, IL-1β and IL-6 expression in the muscles of the mice were increased as well as TNF-α in muscles and white adipose tissue (WAT) was increased after 16 weeks of HF diet feeding. The inflammatory effects appeared to be multiphasic, since no markers of inflammation were measurable between 1 week and 12 weeks after the start of the HF diet. Therefore, Williams et al. (2014) suggested that the inflammatory response develops in distinct phases (Williams et al., 2014). This is in contrast with the result of a similar study where researchers observed a gradual increase in inflammation in WAT after the start of a HF diet in mice (Kleemann et al., 2010). The fact that both studies were executed on different mouse strains, C57BL/6J mice (Williams et al., 2014) and E3L mice (Kleemann et al., 2010), might explain the differences in their results. Haley et al. (2019) also described contradicting results since their study showed no effects in the levels of IL-6 and TNF-α in liver and adipose tissue after short term HF diet feeding of mice (Haley et al., 2019a). However, are the cellular inflammatory changes in response to an obesogenic diet observed in the peripheral tissues also elicited at the level of the reproductive tract? Bovine endometrial cells that were treated *in vitro* with lipotoxic concentrations of FFAs showed increased levels of both IL-6 and IL-8 24 hours after treatment, in a dose-dependent manner (Chankeaw et al., 2018). In addition, increased expression of

IL-6, IL-8 and TNF-α was observed in ovaries from obese women and mice (Nteeba et al., 2013; Ruebel et al., 2016; Xie et al., 2016). Higher BMI appeared to be linked with elevated CRP concentrations in serum and FF, which is also characteristic for increased inflammation and involved in OS (Das, 2001; Agarwal et al., 2008; Combelles et al., 2009). Valckx et al observed that increased CRP in serum was reflected in the follicular micro-environment of the maturing oocyte in women undergoing ART (Valckx et al., 2012). In addition, intra-follicular lipid concentrations in overweight and obese patients were reported to be associated with both pro-inflammatory (CRP, sICAM-1, IL-6, TNF-α) and anti-inflammatory (adiponectin, IL-10) markers (Gonzalez et al., 2018). These results confirm that lipotoxicity can induce a pro-inflammatory response in the reproductive tissue which can have negative effects on the reproductive functions and embryo development (Chankeaw et al., 2018). However, the multiphasic dynamics of effects in response to an obesogenic diet as were described in the study of Williams et al. (2014), have not been described in reproductive tissues.

Taken together, the different studies demonstrate that feeding a HF diet may induce systemic or local inflammation. **However, the dynamics and progression of the effects in the reproductive tissue remains to be studied. In addition, whether feeding an obesogenic diet elicits such inflammatory responses at the level of the oviduct, thereby impacting the earliest environment for the growing embryo, is not known yet.**

1.2 Metabolic changes and their influence on oocyte quality

1.2.1 The follicular microenvironment

The micro-environment in which the oocyte grows and matures and the early embryo develops is of high importance to ensure optimal oocyte developmental capacity and subsequent embryo development and quality. Next to the uterine fluid, which consist of glandular secretions, the micro-milieu comprises the follicular and oviductal fluid (Rodgers and Irving-Rodgers, 2010; Bhusane et al., 2016; Saint-Dizier et al., 2019).

Appropriate oocyte and embryo development requires several well-orchestrated processes involved in oogenesis, folliculogenesis, fertilization and early embryogenesis. Thorough understanding of these processes is thus of key importance to acquire more insights in the impact of maternal metabolic disorders on oocyte quality and early embryo development, and when these alterations may develop over time, since the growing follicle and oocyte are very sensitive to changes in circulating metabolites in the follicular fluid during folliculo- and oogenesis (Best and Bhattacharya, 2015; Valckx et al., 2014b).

1.2.2 Importance of the follicular microenvironment in fertility

The follicle forms the functional unit in which the oocyte grows and matures until ovulation. The follicular fluid (FF) accumulates in the antrum of the follicle during follicular development and forms a specialized micro-environment that is responsible for supporting the oocyte until its release into the oviduct (Hennet and Combelles, 2012). The FF is an ultrafiltrate derived from two sources, namely the bloodstream that is connected via thecal capillaries in the cortical region of the ovary, and components that are secreted by cell layers within the follicle, particularly the GCs. The fluid from the bloodstream is filtrated through the blood-follicle barrier. The capillary network surrounding the follicle expands as the follicle increases in size (Rodgers and Irving-Rodgers, 2010; Hennet and Combelles, 2012). FF contains many components, such as steroid hormones, FSH, LH, proteins, enzymes, cytokines, anticoagulants, ROS, electrolytes, growth factors, enzymatic and non-enzymatic antioxidants, metabolites, extracellular vesicles (EVs) and RNAs. A major determining factor for the developmental competence of the oocyte is the nature of the follicular environment from which it originates. Various components of the FF have an influence on the growth and quality of the oocyte (Revelli et al., 2009; Hennet and Combelles, 2012; Basuino and Silveira, 2016). As such, intrafollicular hormones such as growth hormone, FSH, LH, progesterone and estretol, a natural estrogen (E4), are vital for folliculogenesis and oogenesis (Scaramuzzi et al., 2011). The FF of the pre-ovulatory follicle contains a group

of sterols, namely meiosis-activating sterols, that are involved in meiotic resumption (Sun et al., 2009). Also FF metabolites such as amino acids, fatty acids, nucleotides and other small molecules are essential for oocyte growth. Amino acids play an important role in cellular functions such as energy production and protein synthesis and are transported to the oocyte via the cumulus cells (CCs) (Van Winkle, 2001; Sutton et al., 2003). Fatty acids serve as a cellular energy source and have important biological functions in cell membrane biogenesis (Sturmey et al., 2009; Renaville et al., 2010) and signalling (McKeegan and Sturmey, 2012). The most abundant NEFAs in serum and FF are palmitic, stearic and oleic acid (Leroy et al., 2005; Valckx et al., 2014a). Other FF metabolic substrates, e.g. glucose and pyruvate, are used by oocytes during maturation (Rieger and Loskutoff, 1994). Taken together, this demonstrates the importance of the follicular microenvironment to support the needs of the oocyte and regulate the cross-talk between the oocyte and its surrounding cells. Changes in the composition of the FF can have a direct impact on oocyte maturation and development as well as on the metabolism of the oocyte's supporting cells, such as the CCs (Jungheim and Moley, 2010) and may influence early embryo development and subsequent pregnancy (Gode et al., 2011; Liu et al., 2012; Yang et al., 2015).

Increased inflammatory markers in FF may disrupt oocyte quality (Robker et al., 2011). For example higher TNF-α levels are associated with poor oocyte quality (Lee et al., 2000) and a lower number of fertilized oocytes (Gaafar et al., 2014). Higher levels of IL-6 were observed in the FF of older IVF patients compared to younger patients, which was related to a reduced chance of clinical pregnancy (Altun et al., 2011). Furthermore, oxidative stress in FF can also be associated to impaired oocyte quality (Chattopadhayay et al., 2010; Da Broi et al., 2016). Next to hormonal, paracrine and autocrine signalling pathways, the components of the FF can also be altered by systemic conditions (Chattopadhayay et al., 2010; Von Wald et al., 2010; Liu et al., 2012; Prieto et al., 2012; Moreno et al., 2015; Da Broi et al., 2018). Metabolic changes in the

blood were found to be reflected in the FF in both human and animal. Studying the correlation between serum and FF in cattle during the early postpartum period and in obese women indicated that metabolic changes in the serum, such as changes in the concentration of glucose, urea, β-hydroxybutyrate (BHB), TGs, total protein, NEFAs, total cholesterol as well as elevated insulin concentrations and increased C-reactive protein (CRP) were reflected in the follicular micro-environment of the maturing oocyte (Leroy et al., 2004; Valckx et al., 2012). Pantasri et al. (2015) reported that insulin, glucose and TG levels in both blood and FF positively correlated with increasing BMI and waist circumference. They also demonstrated that insulin increased in FF in association with metabolic syndrome (Pantasri et al., 2015). Additionally, female obesity was associated with FF alterations such as increased glucose and lactate, decreased human chorionic gonadotropin (hCG) concentrations and increased androgen activity, which may lead to poorer reproductive outcomes in these women (Carrell et al., 2001; Robker et al., 2009b). In the study of Ruebel et al, increased levels of FF leptin, CRP and TG levels were found compared to normal weight women (Ruebel et al., 2017). In the FF of obese women that were subjected to ovum pick-up, Yang et al. (2012) reported increased TG and NEFA concentrations which are negatively associated with human cumulus-oocyte-complex (COC) morphology (Jungheim et al., 2011b) and were reported to affect both murine oocyte maturation as well as embryo quality in cattle (Van Hoeck et al., 2011; Van Hoeck et al., 2013). Furthermore, total NEFA and TG concentrations in the FF were found to be affected by BMI (Valckx et al., 2014a). Moreover, murine oocytes that were matured in medium supplemented with TG/NEFA rich FF of obese women showed upregulation of genes related to ER stress and increased oocyte lipid content (Yang et al., 2012), further suggesting a deleterious effect of elevated TG and/or NEFA concentrations on oocyte quality. Addition of FF of obese women during *in vitro* maturation of bovine oocytes affected oocyte developmental competence and embryo quality (Valckx et al., 2015). The different

alterations observed in the FF of obese women undergoing ART treatment are shown in Table 1.

1.2.3 Cumulus-oocyte-complex interactions

The pre-ovulatory oocyte is known to be surrounded by multiple layers of specialized somatic cells, the cumulus cells (CCs), which are essential to sustain oocyte maturation and fertilization (Collado-Fernandez et al., 2012). Their cytoplasmic processes cross the zona pellucida and penetrate the oocyte membrane, thereby forming the cumulus-oocyte complex (COC). The CCs support the oocyte maturation and development by the transportation of small molecules through gap junctions at the ends of their processes. Amino acids, glucose metabolites, ions, GSH, nucleotides and RNA are transported to the oocyte (Eppig, 2001; Gilchrist et al., 2004; Macaulay et al., 2016). The oocyte on its turn regulates granulosa cell (GC) function by producing paracrine factors, defined as oocyte-secreted factors (Gilchrist et al., 2004). Crucial processes, such as cumulus expansion, have found to be disturbed or even prevented in the absence of an oocyte (Sugiura et al., 2007). In the absence of CCs or when gap junctions were inhibited, the *in vitro* maturation of bovine oocytes showed to result in oocytes with an aberrant metabolism and a hampered capacity to support fertilization (Geshi et al., 2000; Fatehi et al., 2002). Taken together, this shows that proper communication between the oocyte and its surrounding CCs is highly important to acquire developmental competence of the oocyte (Krisher, 2004).

Table 1. Overview of the alterations observed in follicular fluid collected upon ovum pick-up in obese women undergoing assisted reproductive treatment

Alterations	Reference	Patients info
↑ Triglyceride	(Robker et al., 2009b)	Averaged age: 35.2 ± 0.97 years Average BMI: ≥ 30 kg/m^2
	(Valckx et al., 2012)	Average age: 35 years (stated as 'representative cohort of all women of reproductive age') BMI: ≥ 30 kg/m^2
	(Yang et al., 2012)	Age : not indicated BMI: ≥ 30 kg/m^2
	(Valckx et al., 2014a)	Average age: 35 years old BMI: ≥ 30 kg/m^2
	(Pantasri et al., 2015)	Average age: 34.2 ± 4.6 years BMI: ≥ 30 kg/m^2
	(Ruebel et al., 2017)	Average age : 32.1 ± 1.5 years BMI: ≥ 25 kg/m^2 (overweight)
↑ NEFA	(Yang et al., 2012; Valckx et al., 2014a)	
↑ Insulin	(Robker et al., 2009a; Valckx et al., 2012; Pantasri et al., 2015)	
↑ Glucose	(Pantasri et al., 2015)	
↑ Lactate	(Robker et al., 2009a)	
↑ CRP	(Robker et al., 2009a) (Valckx et al., 2012; Ruebel et al., 2017)	
↑ Androgen activity	(Robker et al., 2009a)	
↓ hCG	(Carrell et al., 2001)	Average age: not indicated, stated 'no significant differences in age of patients' BMI: ≥ 30 kg/m^2
↑ Leptin	(Ruebel et al., 2017)	
↓ HDL-cholesterol	(Valckx et al., 2012)	

BMI = Body Mass Index; NEFA = Non-Esterified Fatty Acid; CRP = C-Reactive Protein; hCG = human chorionic gonadotropin; HDL-cholesterol = High Density Lipoprotein Cholesterol

1.2.4 Effects of metabolic stress on oocyte quality

Metabolic stress is demonstrated to be reflected in FF, thus surrounding the oocyte during maturation. As such, it was demonstrated that murine folliculogenesis was directly affected by metabolic stress. This was shown by reduced follicle diameter and antrum formation after exposure to high NEFA concentrations during *in vitro* development of follicles from B6CBAF1 mice (Valckx et al., 2014b). To obtain proper embryo development, the oocyte needs to be of high quality. Poor quality oocytes may be the cause of female subfertility and may hinder successful IVF. Various studies in obese women have attributed early pregnancy loss and increased rates of miscarriage to a reduced oocyte quality in these women (Fedorcsak et al., 2004; Lashen et al., 2004; Robker, 2008). For instance, oocytes from obese women showed a higher incidence of reduced quality with increased percentages of fractured zona pellucida or germinal vesicle stage and reduced nuclear maturity. These oocytes can also have impaired *in vitro* fertilization rates which frequently leads to inferior embryos. Disturbed environmental conditions during oocyte maturation may lead to epigenetic modifications to DNA and histone proteins, and compromised postnatal health (Robker, 2008). Severe obesity was associated with a greater prevalence of spindle anomalies and chromosome misalignment in failed fertilized oocytes, compared to oocytes from women with a normal BMI (Machtinger et al., 2012). Different studies have shown a reduced oocyte quality, size or maturity with elevated BMI in humans (Carrell et al., 2001; Dokras et al., 2006). Women with a BMI exceeding 25 kg/m^2 exhibited more germinal stage oocytes, postmature oocytes and oocytes with a damaged zona pellucida at ovulation, which are all considered bad quality oocytes, compared to women with a BMI between 20 and 25 kg/m^2 (Robker, 2008; Purcell and Moley, 2011). Overweight or obese women undergoing ART showed to have oocytes with a significantly smaller diameter (Marquard et al., 2011), which were less likely to reach blastocyst stage, than oocytes from normal BMI women (Leary et al., 2015).

Another study in obese women undergoing IVF has shown that elevated FFA levels in their FF were associated with abnormal COC morphology (Jungheim et al., 2011b). Female obese C57BL/6 mice that were fed a HF diet for 16 weeks demonstrated that they were less likely to ovulate compared to the lean, control mice. The HF diet fed mice showed a deterioration in oocyte quality which was manifested in slower developmental competence during *in vitro* fertilization and culturing of the oocytes that did ovulate (Minge et al., 2008). In addition, it has been shown that oocytes collected from HF-diet induced obese mice exhibit altered mitochondrial functions and OS, leading to alterations during subsequent embryo development. This was reported in the study of Marei et al. (2020) where 13 weeks of feeding a HF diet increased the MMP, ROS concentrations and mitochondrial ultrastructural abnormalities in the oocytes of both outbred Swiss and inbred C57BL/6 mice compared to their controls (Marei et al., 2020). Similar findings were observed by Igosheva et al. (2010) in C57BL/6 mice that were fed an obesogenic diet for 6 weeks. Furthermore, C57BL/6 mice fed a HF diet for 16 weeks had ovaries containing significantly more apoptotic ovarian follicles and fewer and smaller mature oocytes in comparison to the controls (Jungheim et al., 2010). These studies show the impact of the obese state on oocyte quality after long term periods of feeding an obesogenic diet and metabolic stress. **However, the dynamics of the effects on oocyte quality and the reproductive tissue and how quick these changes are detectable and progress over time have not been elucidated.**

The potential effects of diet and the different diet compositions on oocyte quality are important to be investigated as well (Ashworth et al., 2009) as maternal dietary intake has shown to be the main source of nourishment for the oocyte and early embryo (Leese, 2012). Studies investigating the effects of obesity and metabolic stress on oocyte quality often use diet-induced obese mouse models which are fed with different diets and different diet compositions, such as high fat only, high fat/ high sugar, high sugar only, the Western cafeteria diet and many more (Panchal and Brown, 2011). All

different diets and their composition may have a differential impact on oocyte quality, and ultimately on fertility. It has been reported that adding 6% fat to the diet causes alteration of the FA profile of both serum and FF in cows and this altered FF FA composition was reflected in the COC's FA content and profile (Adamiak et al., 2005). In human, reduced quality and developmental morpho-kinetics of preimplantation embryos have been associated with insufficient maternal vegetable and fruit intake during the periconception period (Hoek et al., 2020). On the other hand, a 'high fish, olive oil and low meat' maternal dietary pattern during the early first trimester, was linked with accelerated embryonic development in human (Parisi et al., 2018). Consumption of a low-dose of sucrose for 6 months by healthy monkeys has shown to inhibit oocyte maturation and early embryo gene expression (Chaffin et al., 2014). **Very little is known however about the importance of the duration of feeding and the impact of duration on the development of effects. The consumption of an obesogenic diet may already directly impact reproductive physiology pathways without immediately inducing an obese metabolic state. Therefore, thorough investigation of the impact of duration of feeding is needed, as oocyte quality and embryo development may already be impacted directly after an obesogenic meal, long before the development of the obese metabolic state.**

1.2.4.1 Importance of used mouse model and strain in studying effects of metabolic stress on oocyte quality

In the different studies investigating the effects of metabolic changes on oocyte quality that were described above, different mouse strains were used as the model. Effects on fertility were however reported to be strain dependent. Therefore, strain differences should be considered when interpreting reproductive experiments using mouse models (Kaleta, 1977; Ibanez et al., 2005). In addition, Marei et al. (2020) have recently shown that the sensitivity to HF diet-induced obesity and its influence on the metabolic profile and oocyte quality was dependent on the mitochondrial and/or genetic

background of the mouse model. This was demonstrated by comparing the outbred Swiss mice with the inbred C57BL/6N mice (Marei et al., 2020). The C57BL/6 strain is the most commonly used polygenic strain in studies investigating the impact of HF/HS diet exposure and obesity on metabolic health and fertility. The genetic homogeneity in inbred strains minimizes variability in experimental settings (Nicholson et al., 2010). However, the use of an inbred strain limits the extrapolation of acquired data and conclusions to the human (outbred) physiology. In contrast, outbred strains show more variation in responses to changes in environmental conditions due to the wide genetic variability. An outbred strain, such as the Swiss strain, mimics however the human genetic diversity and may therefore be more relevant when investigating the impact of obesogenic diets, obesity and metabolic health on fertility.

1.3 What about the oviductal microenvironment?

In the previous sections we learned that the consumption of an obesogenic diet and metabolic disorders such as obesity are associated with lipotoxicity which results in oxidative stress, leading to mitochondrial dysfunction as well as ER stress. These distinct effects are evident at the level of the oocyte, negatively impacting its development and quality, thereby linking maternal metabolic disorders with subfertility. In contrast to the effects on oocytes and the follicular microenvironment, the effect of maternal metabolic stress on the oviduct is much less characterized. Whether the introduction of an obesogenic diet or metabolic stress causes similar responses that are locally initiated in the oviductal cells, potentially affecting pre-implantation embryo development, has not been previously investigated. However, several crucial reproductive events take place in the oviduct, such as fertilization, and early embryo development (Besenfelder et al., 2012; Maillo et al., 2016a). During the short period within the oviduct, the embryo will undergo embryo genome activation and DNA methylation, thus comprising a sensitive and important period that can determine subsequent stages of its development (Latham and Schultz, 2001; Inbar-

Feigenberg et al., 2013). Improper establishment or erasure of epigenetic marks may lead to permanently altered gene expression, abnormal development and early onset of disease (Godfrey et al., 2007; Rivera and Ross, 2013). Such important processes make the early developmental stages of the embryo most susceptible for environmental changes. The oviduct can provide a favorable and dynamic microenvironment for ideal functioning and development of these processes. The adverse reproductive outcome linked with metabolic disorders may therefore be directly related to the conditions within the oviductal tract. For that reason, this will be the focus in the next few sections.

1.3.1 The oviductal microenvironment

The oviductal micro-environment is composed of the oviductal epithelial cells (OECs) and the oviductal fluid (OF). It provides nutrients, growth factors and antioxidants to support key reproductive processes such as early embryo cleavage and survival and can therefore have a significant impact on the overall reproductive outcome. Exposing *in vitro* matured porcine oocytes to bovine OF has shown to improve porcine blastocyst rates and quality (Lloyd et al., 2009). In addition, coincubation of matured oocytes with OF resulted in altered gene expression through upregulation of antioxidative mechanisms (Cebrian-Serrano et al., 2013), as well as improving bovine blastocyst quality through increased cryotolerance and altered gene expression (Lopera-Vasquez et al., 2017). Being the first interface between the female genital tract and the conceptus, the oviduct mediates an early embryo-maternal dialog through cell contacts, secreted molecules and extracellular vesicles (EVs) (Hunter, 2012a; Alminana et al., 2017; Banliat et al., 2022). Exposure of bovine embryos during *in vitro* culture to EVs derived from OF resulted in improved blastocyst quality (Alminana et al., 2017; Lopera-Vasquez et al., 2017; Leal et al., 2022).

1.3.2 Anatomy and histology of the oviduct

The mammalian oviduct is composed of four segments: the infundibulum, responsible for collecting ovulated oocytes; the ampulla, where fertilization occurs; the isthmus for

preimplantation embryo development and the uterotubal junction which transits embryos to the uterus (Li and Winuthayanon, 2017). The oviductal epithelium consists of ciliary and secretory cells (Leese, 1988; Aviles et al., 2010; Maillo et al., 2016b; Li and Winuthayanon, 2017). The distal (infundibulum and ampulla) and proximal (isthmus and uterotubal junction) regions of the oviduct have shown functional (Ardon et al., 2016) and transcriptomic differences (Bauersachs et al., 2003).

1.3.3 The role of the oviduct in fertility

For many years, researchers tended to underestimate the physiological importance of the oviduct as the arrival of in vitro fertilization techniques in the 1970s allowed procreation even when completely bypassing the oviduct. However, since then different studies have reported that the morphology, gene expression and lipid and protein composition of *in vitro* produced embryos were markedly different from their *in vivo* counterparts. These differences were associated with lower pregnancy outcomes and more health issues after birth (Goncalves et al., 2009; Ghersevich et al., 2015; Lamy et al., 2018; Ferraz et al., 2019). This may highlight the vital role of the oviduct, also called fallopian tube, in fertility. Researchers nowadays consider the oviduct as the most suitable microenvironment for early embryo development (Saint-Dizier et al., 2019) and therefore its dynamic functions have been studied substantially the past years.

Due to its characteristic structure, the oviduct can be of crucial importance for fertilization by acting as a sperm reservoir, serving the environment for sperm capacitation and directing the spermatozoa and oocyte to each other (Alberts B, 2008). Furthermore, the oviduct forms the environment for the earliest, though essential, steps in embryo development. The human embryo spends approximately 3.5 days and the murine embryo approximately 3 days in the oviduct (Croxatto, 2002) during which events such as embryonic genome activation (which is crucial for further cell differentiation, embryo implantation and fetal development (Latham and Schultz,

2001)) and embryo epigenetic programming (including methylation and acetylation) take place (Aviles et al., 2010; Besenfelder et al., 2012; Maillo et al., 2016a). Thus, the oviduct does not serve merely as a tube to guide female and male gametes and to lead the pre-implantation embryo in the direction of the uterus. Hampered environmental conditions in the oviduct may interact with various crucial reproductive processes and may lead to early termination of the pregnancy. However, information on this assumption is very scarce and certainly, more research is needed.

When the oocyte surrounded by cumulus cells gets released in the oviduct, it needs sufficient time to prepare for fertilization. To ensure this, the oocyte will be firmly attached to the ciliated epithelium of the ampulla. The adhesion will also prevent discarding viable oocytes by the oviductal flow, due to forceful ciliary beating (Kolle et al., 2009). Oocyte growth is arrested at the metaphase stage of meiosis II until fertilization (Sadler, 2009). For successful fertilization a mature and healthy oocyte is essential. Therefore, the oocyte has to progress to metaphase II as the final step in oocyte maturation to become developmentally competent. After ovulation and with the extrusion of the second polar body at fertilization, the oocyte completes final maturation (Coticchio et al., 2015). Oviductal hyaluronidase production causes the oocyte to get released from the oviduct and to progress it's journey towards the uterus (Kolle et al., 2009).

Many thousands, even millions, of sperm cells are inseminated by the male and various regulatory processes control their passage into and through the oviduct. After insemination, the spermatozoa's motility, as well as the muscular activity of the female reproductive tract and chemotaxis, cause the spermatozoa to progress towards the oocyte. This is in contrast with the accidental connection of sperm with the oocyte during *in vitro* fertilization and thus indicates that the oviduct accommodates a signalling cascade efficiently bringing the male and female gametes together (Kolle et al., 2009).

Only the fittest spermatozoa are permitted to enter the oviduct. The oviductal fluid does not contain polymorph nuclear cells, unlike the uterine environment, and is therefore considered a protective and safe environment for the privileged sperm that reaches it (Holt and Fazeli, 2016a; Holt and Fazeli, 2016b). Most of the lumen of the oviduct is occupied by extensive mucosal folding which can also occlude the lumen by secretion of OF and/or contraction of smooth muscle cells. Thus, the luminal capacity of the oviduct is limited (Suarez, 2008). The luminal fluid is known to be rich in mucinous secretions and proteins. Up until after ovulation, sperm-oviduct interaction can be strengthened by a plug, formed by oviductal mucus and forcing the spermatozoa in the crypts of the oviduct (Palermo et al., 2014). The passage of spermatozoa is made even more difficult by the luminal fluid's effects on beat frequency and flagellar movement of the sperm cells as well as the fluid's high viscosity (Kirkman-Brown and Smith, 2011). The latter can also prevent the passage of DNA-damaged spermatozoa through the oviduct, which is needed as DNA damage is often linked to decreased motility in humans. OECs appear to be able to identify altered sperm DNA through differences in electronegative charge at the sperm membrane. Therefore, the oviduct is capable of discriminating between mature and immature spermatozoa (Palermo et al., 2014). Thus, the oviduct subjects the spermatozoa to various sperm selection processes to either inhibit or facilitate their advancement towards the oocyte (Holt and Fazeli, 2016a).

When the spermatozoa pass the uterotubal junction and enter the isthmus of the oviduct, they become trapped and held in a reservoir, by binding of the sperm to the epithelial surface of the isthmus. This sperm reservoir function of the oviduct allows an extension of sperm fertility from ejaculation or insemination until ovulation (Kolle et al., 2009) as well as a gradual release during the periovulatory period, thereby reducing the incidence of polyspermia, and to acquire the ability to fertilize the oocyte (Suarez, 2008). Only uncapacitated sperm can be bound to the ciliary epithelial cells. In addition, sperm cell capacitation is inhibited as long as the sperm is bound to the oviduct

(Rodriguez-Martinez, 2007). In human, spermatozoa can be stored for 5 days (Birkhead and Moller, 1993; Kolle, 2022). Around ovulation, part of the stored spermatozoa are gradually directed towards the ampullary-isthmic junction and sperm capacitation takes place (Rodriguez-Martinez, 2007). Capacitation encompasses the series of biochemical and physiological modifications that the sperm must undergo before the acrosome reaction. Fast capacitation events take place when the sperm is released from the epididymis and the flagellum is activated. Slow capacitation events occur in the oviduct. For the slow events the presence of oviductal bicarbonate, calcium and serum albumin are essential. The first stages of capacitation comprise membrane destabilising changes and are primarily mediated by bicarbonate (Rodriguez-Martinez, 2007). By redistributing cholesterol in the cranial part of the sperm membrane, bicarbonate achieves a cholesterol efflux due to the increased receptivity for serum albumins. Furthermore, there will be an increased permeability for bicarbonate and calcium ions as the sperm membrane fluidity is changed. Production of cyclic adenosine monophosphate activates protein kinase A (PKA), which leads to the hyperactivated sperm motility, which is vital for successful fertilization. Eventually, the sperm acrosome reaction is needed to effectuate the fusion of the sperm and the oocyte membrane during fertilization. This is carried out by the release of acrosomal enzymes (Ickowicz et al., 2012). The OECs secrete extracellular vesicles which enhance fertilization, protect against polyspermy and sperm entry and aid in embryonic development (Alcantara-Neto et al., 2020; Harris et al., 2020). Taken together, the OECs are triggered by the presence of spermatozoa to initiate various reactions influencing sperm storage, survival, motility and capacitation (Holt and Fazeli, 2016a; Holt and Fazeli, 2016b). In a relatively short period of time many steps, each being able to modify the early embryonic microenvironment, take place before embryo development in the oviduct (an overview is shown in Figure 7). After successful fertilization, the oviduct supports the early embryo by providing it with an optimal environment as described later.

Thus, primarily the oviduct comprises the microenvironment in which the first very critical stages in the development of the pre-implantation embryo take place. Therefore, suboptimal growth conditions in the oviduct can have a severe impact on overall reproductive outcome.

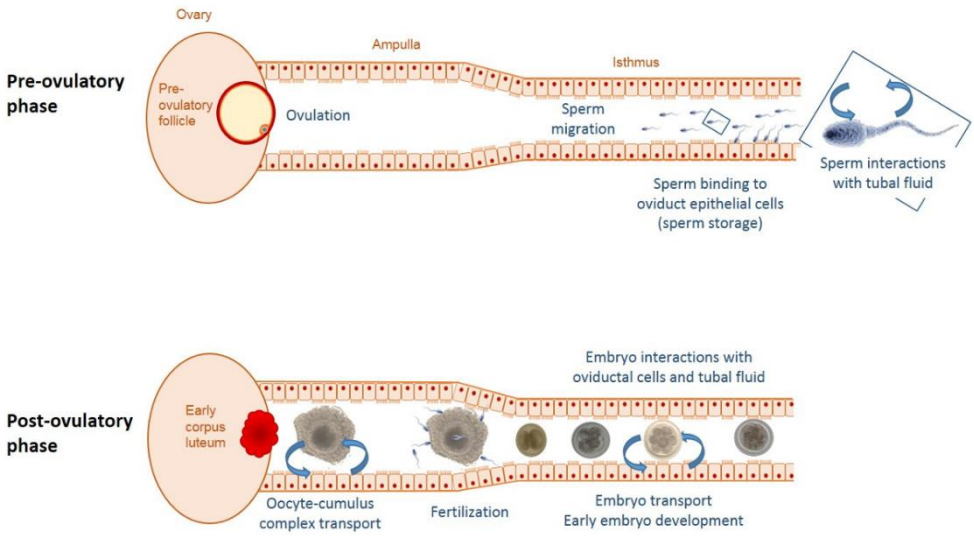

Figure 7. Overview of the different processes taking place in the oviductal micro-environment before and during embryonic development (Image from (Saint-Dizier et al., 2019))

1.3.4 Physiological characteristics of the oviductal micro-environment

Many components of the oviductal micro-environment influence or may contribute to the optimal development of the early embryo such as growth factors, ions, vitamins and energy substrates (Aviles et al., 2010; Hu and Yu, 2017), but also the specific physiological characteristics of the oviductal micro-environment, such as temperature, pH, oxygen pressure and redox potential. These physiological parameters show dynamic changes linked with the anatomical location and time, thereby matching the needs of the embryo in each specific developmental stage (Gardner, 1998; Hugentobler et al., 2008; Ghersevich et al., 2015). During the different phases of the menstrual or

oestrus cycle the temperature in the mammalian oviduct was found to vary. These temperature gradients are thought to be important for oocyte and sperm maturation and may thus play a vital role in events that occur shortly before and after fertilization (Hunter, 2012b). In contrast to temperature, pH does not appear to be changing according to the estrous cycle stage. The mammalian oviductal fluid has a pH of around 7.6 to 7.9, which is typically higher than uterine and venous pH. High levels of carbonic anhydrase in the oviductal epithelium lead to the production of bicarbonate which regulates the pH of the oviductal fluid. (Hugentobler et al., 2004). In different species the oxygen tension of the oviduct was found to be much less than half the atmospheric 0_2, ranging from 60 mm HG to as low as 11 mm Hg. Such low oxygen tension in the oviductal micro-environment was shown to improve embryo development as it reduces the amount of reactive oxygen species (Bermejo-Alvarez et al., 2010).

1.3.5 The oviductal fluid: formation and composition

The OF is essential to support all reproductive events that occur in the oviduct and its components contribute to optimal early embryo development. It is predominantly composed by secretions of the oviductal epithelium, complemented by a limited amount of FF and CCs at ovulation (Hansen et al., 1991). It consists of components that are either de novo secreted by the OECs or passively and actively transported over the epithelial barrier from the circulating blood or the interstitial tissue (Leese, 1988; Leese et al., 2001; Aviles et al., 2010). Oviductal secretions are regulated by a range of factors and processes, such as ovulation, insemination, the presence of gametes, the embryo, sex steroid hormones, metabolism and energy balance (reviewed by (Saint-Dizier et al., 2019)). OF production is driven by catecholamines, ATP and acetylcholamine and appears to be increased during estrous (Gott et al., 1988; Leese et al., 2001). Recent years, the wider use of mass spectrometry techniques has increased the understanding of the complex composition of the OF (for reviews see (Aviles et al., 2010; Saint-Dizier et al., 2019; Bastos et al., 2022)).

Energy substrates in the OF, such as glucose, lactate, pyruvate, support both female and male survival as well as early embryo development. The early embryo mostly uses oxidative phosphorylation (mainly from pyruvate and lactate) (Thompson et al., 2000). Lipids are also highly abundant in the OF and can significantly influence the oviductal and embryonic cell signalling and molecular functions (Saint-Dizier et al., 2019). They provide a potential source of energy and contribute to cell proliferation and differentiation. A variety of lipids have been identified in the OF, including TGs, cholesterol and FAs (Rizos et al., 2010b), as well as HDLs and LDLs (Gad et al., 2012; Marey et al., 2016), glycerophospholipids and sphingolipids (Enright et al., 2000; Gad et al., 2012). Amino acids in the OF support protein synthesis and supply nitrogen needed for normal cell function and proliferation (Tay et al., 1997; Hugentobler et al., 2007). They also serve as an energy source, signalling molecules and osmolytes (Gardner et al., 1989; Leese et al., 2008; Tartia et al., 2009). Steroid hormones can be introduced in the OF via the peritoneal fluid, blood, seminal plasma after ejaculation and also GCs after ovulation of a cumulus-oocyte complex (Hunter, 2012a), and may participate in the optimal transport of gametes and embryo (Bahat and Eisenbach, 2006). Studying the proteome of the OF has shown that the most abundant proteins in oviductal secretions include serum albumin, oviductin (OVGP1), heat shock proteins (HSPs), annexins, tubulin subunits, complement C3 and myosins (Wijayagunawardane et al., 1998; Smits et al., 2016; Rodriguez-Alonso et al., 2019). A recent study identified 56 proteins that were involved in embryo-maternal interactions in the bovine oviduct (Banliat et al., 2020). A majority of proteins in the OF were also found in extracellular vesicle (EV) fractions isolated from bovine OF (Salzano et al., 2018), which may suggest that a large part of the OF proteins are actively released from the OECs as EV cargos. Furthermore, a bovine study demonstrated, using mass spectrometry, that more than 50 OF proteins interacted with the embryo (Fontes et al., 2014) and the majority of these embryo-interacting proteins were also identified in bovine and feline oviductal

exosomes (Ulbrich et al., 2003; Salzano et al., 2018), indicating the role of EVs in the embryo-maternal dialogue in the oviduct.

EVs originating from the oviduct epithelium have recently been identified as major components of the OF (Alminana and Bauersachs, 2019). They are also present in a wide variety of other biofluids such as the uterine, seminal and follicular fluid. EVs are nano-sized membranous vesicles and by exocytosis they transfer signal and regulatory molecules. Their molecular cargo includes micro RNAs (miRNAs), mRNAs, proteins, lipids, and metabolites and can be easily delivered and fused with cell plasma membranes (Kim et al., 2017; Jeppesen et al., 2019). The EVs serve a crucial role in transmitting information and organelles between living cells (Mathieu et al., 2019; D'Souza et al., 2021) and can modify the epigenetic signature by transferring small molecule RNAs between donor cells (Mannavola et al., 2019; Willis et al., 2021). Through their contents, EVs can ensure proper cell communication between the mother and the embryo. The molecular cargo of bovine oviductal EVs was reported to dynamically change across the estrous cycle (Alminana et al., 2017; Alminana et al., 2018). EVs present in the female genital tract fluid are crucial for gamete fertilization and early preimplantation embryonic development (Saadeldin et al., 2015; Alminana et al., 2017; Alminana and Bauersachs, 2019; Alminana and Bauersachs, 2020; Bridi et al., 2020). They have shown to positively impact bovine embryo development and quality during *in vitro* embryo culture (Lopera-Vasquez et al., 2016). EVs from the preovulatory follicle may also enter the oviduct at ovulation. In cattle, EVs from FF were reported to be internalized by developing embryos *in vitro* and to modulate metabolic and developmental-related genes as well as miRNA and global DNA methylation (Saint-Dizier et al., 2019). Furthermore, oviductal epithelial cell extracellular vesicles (OEC-EVs) reduced apoptosis and improved porcine embryo quality by reducing ROS (Fang et al., 2022). A recent study demonstrated that the molecular cargo, specifically upregulated miR-378-3p, of EVs secreted by blastocysts plays a crucial role in promoting blastocyst hatching, which is essential for embryo implantation (Pavani et

al., 2022). In addition, porcine OEC-EV treatment during porcine IVF was reported to significantly improve concentration of cortical granules in the oocytes and increase oocyte mitochondrial activity, reduce polyspermy and increase the IVF success rate (Fang et al., 2023). Improved blastocyst quality was reported when bovine embryos were exposed to EVs derived from oviductal fluid, as well as uterine fluid, in a sequential *in vitro* culture system that mimics the anatomical developmental sequence which would happen during early development in vivo (Leal et al., 2022). In addition, co-culture of murine embryos with human oviductal EVs resulted in increased murine embryo viability *in vitro* (Li et al., 2023).

Overall, the components of the oviductal fluid are well characterized and highlight the vital role and importance of the strongly regulated oviductal micro-environment for embryo development.

1.3.6 Impact of gametes and embryos on the oviduct micro-environment and vice versa

Ovulation and/or insemination are followed by a dialogue between the oviduct and the gametes and embryo. Different studies have shown that oviduct-gamete communication is an intricate dialogue leading to successful fertilization (Liao et al., 2011; Maillo et al., 2016a; Yousef et al., 2016). The introduction of sperm or oocytes elicits different responses of the oviduct, for example alterations in the oviductal proteome (Georgiou et al., 2005) and oviductal ER signalling pathways (Orihuela et al., 2009). The crosstalk between oocyte, sperm and oviduct may be a way to guarantee the connection of oocyte and sperm at an appropriate time (Fernandez-Fuertes et al., 2018). Survival of spermatozoa within the oviduct is supported by the oviductal cells and their secretory products as they contribute to the regulation of sperm capacitation, motility and acrosome reaction (Bergqvist and Rodriguez-Martinez, 2006; Leese et al., 2008). The impact of oviductal secretions on gamete functionality and the interaction between gametes has been reviewed by Ghersevich et al. (2015).

The oviduct-embryo crosstalk is a two-way process in which signals can be sent and received from both the oviduct and the embryo (Fernandez-Fuertes et al., 2018). Studies in different species have shown changes in the oviductal vascularization, number of secretory cells (Kolle et al., 2009), immune functions (Alminana et al., 2012; Maillo et al., 2015) and OECs gene expression (Schmaltz-Panneau et al., 2014) in response to the presence of an embryo. In addition, a recent study on bovine embryos highlighted the great contribution of the maternal environment to the embryo proteome as it led to higher degradation of mitochondrial proteins at early developmental stages, lower abundance of proteins involved in protein synthesis at the time of embryonic genome activation and a global upregulation of carbohydrate and small molecule metabolic pathways compared to *in vitro* production. They also demonstrated that embryos developed *in vivo* uptake large amounts of OVGP1 and probably other oviduct fluid-derived proteins as soon as the 4-6 cell stage (Banliat et al., 2022). Furthermore, as described before, an important component of the OF are EVs. They also contribute to the embryo-oviduct communication and have been the focus of many studies (reviewed by (Bastos et al., 2022)). Already in the first moments of embryonic-maternal communication in the oviduct, both maternal and embryonic EVs are exchanged to prepare for the maternal recognition of pregnancy (Mazzarella et al., 2021).

It is evident from the previous sections that the oviduct serves as a dynamic environment in which optimal conditions are provided for various reproductive processes, including early embryo development (Aviles et al., 2010). The oviduct itself also has to adjust its micro-environment to the specific needs of each of these processes. The early embryonic environment has been shown to have a substantial influence on the developing organism, despite the short period of this developmental stage. Studies on both human and animal embryos discovered that *in vitro* fertilized embryos, bypassing the gamete/embryo-oviductal communications, show genomic

imprinting alterations and poorer developmental potential (Rizos et al., 2002; Rizos et al., 2008; Kleijkers et al., 2014; Lazaraviciute et al., 2014; Duranthon and Chavatte-Palmer, 2018). These studies indicate the pivotal role of oviduct-embryo interactions on early embryo development. The cells of the zygote and cleavage-stage embryo hold the potential to form all cell lineages of the embryonic and extra-embryonic tissues. As such, these cells have the potential to influence the phenotype of all successive cell types as the organism grows, differentiates and ages. The implication is, therefore, that adverse conditions which alter the developmental trajectory of these cells may have long-term implications for the health and development of the resulting offspring. The translation of environmental cues to phenotypic outcome can be due by epigenetic modification of the genome to modulate chromatin packaging and gene expression in a cell- and lineage-specific manner. Different studies in both human and animal have suggested epigenetic changes in embryonic and extra-embryonic tissues to contribute to adverse outcomes of ARTs (Niemitz and Feinberg, 2004; Katari et al., 2009; El Hajj and Haaf, 2013; Lucas, 2013; de Waal et al., 2014; de Waal et al., 2015) and reviewed by (Mani et al., 2020).

1.3.7 Effects of metabolic stress on the oviductal microenvironment

In most mammalian species, the embryo spends the first 3-4 days of life in the oviduct. During the early developmental stages the embryo is specifically sensitive to changes in its growth environment (Maillo et al., 2016a; Li and Winuthayanon, 2017). Changes in the biochemical composition of the oviductal microenvironment such as nutrient availability and OS, were found to induce molecular and metabolic dysfunction in mammalian preimplantation embryos which was demonstrated by impaired mitochondrial function, high lipid content, smaller size and altered DNA methylation in obese rodent models or metabolically stressed cows (reviewed by (Velazquez, 2015)). Such alterations may lead to embryonic death or long- term defects that puts the subsequent development at risk (Robertson et al., 2018). As the earliest developmental

environment for the embryo is provided by the oviduct, the conditions within the oviductal tract may directly contribute to an adverse reproductive outcome during metabolic disorders. Information regarding the impact of metabolic stress on the oviductal microenvironment is however limited. According to the "Quiet Embryo" Hypothesis , the viability of preimplantation embryos requires a metabolism that is 'quiet' rather than 'active' (Baumann et al., 2007). An 'active' metabolism during early development, due to e.g. nutritional overload, is associated with poorer outcomes in later life. In line with this hypothesis, nutritional overload in the oviduct may alter the embryo metabolic phenotype which can have negative effects on further development (Leese et al., 2007).

Jordaens et al. (2017) showed that plasma concentrations of NEFAs are reflected in the OF (Jordaens et al., 2017a). Thus, next to modulating blood and follicular metabolites, the maternal metabolic and nutritional state may also influence OF composition. This further highlights it is crucial to consider oviductal conditions as the OF forms the earliest embryonic environment. Although the oviductal epithelium functions as a gatekeeper selectively controlling molecular transfer from the blood to the oviduct to control the environment of the early embryo, NEFAs were shown to be either selectively transferred across the *in vitro* bovine oviductal epithelium or intracellularly metabolized, thereby impairing the barrier function of the BOECs (Jordaens et al., 2017b). Additionally, *in vitro* exposure of BOECs to elevated NEFA concentrations resulted in direct embryo toxicity and a reduced ability of the oviduct to support and protect early embryo development (Jordaens et al., 2020).

Only a few studies have demonstrated a possible direct impact of maternal metabolic disorders on the oviductal microenvironment. Researchers investigated the effect of nutrition and metabolism on the early embryonic microenvironment through embryo transfer in the oviducts of metabolically compromised cattle. These studies demonstrated that the oviductal environment in metabolically stressed lactating and intensively fed dairy cattle was less supportive for embryo development and survival

compared to heifers (Rizos et al., 2010a) and to non-lactating (metabolically healthy) cows fed a maintenance diet (Maillo et al., 2012). This indicates that the conditions in the reproductive tract define its ability to sustain early embryo development (Rizos et al., 2010a; Maillo et al., 2012; Matoba et al., 2012). *In vitro* research has shown that NEFA exposure during bovine embryo culture hampered embryo quality through reduced blastocyst formation and cell number, accompanied by an increase in apoptosis (Van Hoeck et al., 2013) which may potentially be caused by internalization of FAs (Listenberger et al., 2003; Leroy et al., 2010). Similar observations were made in murine embryos as exposure to pathological NEFA-concentrations during *in vitro* culture showed to induce effects on embryo growth and metabolism (Jungheim et al., 2011a). In addition, elevated NEFA concentrations during the *in vitro* culture period directly impacted early bovine embryo development, as they affected embryo DNA methylation and transcript patterns (Desmet et al., 2016). Moreover, recently it has been reported that zygotes from metabolically compromised (PA-exposed) oocytes had increased histone acetylation and methylation levels and that continued exposure to the lipotoxic environment during early embryo development, resulted in increased DNA and histone methylation marks in morulae. These effects were associated with embryo ATP production and mitochondrial dysfunction (Meulders et al., 2023). Furthermore, Yousif et al (Yousif et al., 2020) aimed to gain more insights in how a high-fat environment in obese patients affects preimplantation development by studying the direct effects of FFA exposure on preimplantation embryos. For this, the researchers treated 2-cell mouse embryos *in vitro* with increasing concentrations of palmitic acid (PA), the most abundant NEFA in circulation (Abdelmagid et al., 2015) and the Western diet (Ricchi et al., 2009), and oleic acid (OA), the most abundant monounsaturated NEFA in human serum (Abdelmagid et al., 2015), previously shown to counteract the detrimental effects of saturated FAs (Aardema et al., 2011). Their results showed that PA treatment reduced blastocyst development and altered ER stress pathway transcript levels. OA co-treatment with PA reversed the PA effects on

blastocyst development and ER stress transcript levels but also increased lipid droplet formation and reduced mitochondrial stress. Yousif et al (Yousif et al., 2020) concluded that the preimplantation embryo employs stress mechanisms to avoid deleterious effects of PA exposure, but that their protective ability may be overwhelmed by exposure to increasing concentrations of PA. However, more time course experiments are needed to fully understand such stage-specific effects.

Various studies investigating the follicular microenvironment have shown that other maternal metabolic stress factors such as circulating inflammatory cytokines and ROS are also reflected in the ovarian FF (Nteeba et al., 2013; Ruebel et al., 2016; Xie et al., 2016; Gonzalez et al., 2018). Whether maternal metabolic stress can be associated with oxidative stress or inflammatory responses in the oviduct and the dynamics and progression of these responses after the start of an obesogenic diet have not been previously investigated in animal models or in humans. One study has shown that exposure of BOECs to PA *in vitro* resulted in an increase in IL-8 concentrations already after 24 hours (Ohtsu et al., 2017).

While differences in the response to an obesogenic diet and obesity and the impact on oocyte quality between outbred en inbred mouse strains have been described (Marei et al., 2020), this has not been studied at the oviductal level and will therefore be considered in the current PhD thesis.

Overall, the embryonic growth conditions in the oviduct can thus be a key factor contributing to the reduced embryo quality that is associated with the maternal metabolic state. Suboptimal growth conditions significantly contribute to early embryonic loss and may alter the metabolism of the reduced number of surviving blastocysts, even potentially leading to lasting effects for the offspring (Jungheim et al., 2011a). Although the oviductal epithelium is well characterized in recent studies, the mechanisms by which consumption of an obesogenic diet and/or the development of obesity *in vivo* may affect the function of the oviductal cells have not

been elucidated. This may however provide new insights in whether the impact of metabolic stress on fertility is only mediated through reduced oocyte quality at the ovarian level or also by a direct impact on early embryo development in the oviduct.

1.4 The development of effects of metabolic stress on reproductive functions over time

1.4.1 When does an obesogenic diet start to affect the oocyte and/or oviduct?

The many adverse effects of metabolic stress and/or obesity on reproductive health have been previously discussed in detail. Various studies using animal models have elucidated some of the underlying mechanisms that result in reduced oocyte quality (Jungheim et al., 2010; Wu et al., 2011; Jungheim et al., 2012; Valckx et al., 2014b; Marei et al., 2017). Studies investigating the impact of metabolic stress on the oviductal microenvironment are scarce (Rizos et al., 2010a; Jordaens et al., 2020). As discussed, HF diets are commonly used to induce obesity in mouse models and have shown to lead to hyperlipidaemia, systemic lipotoxicity and oxidative stress, which is reflected in the ovarian follicular microenvironment (Reynolds et al., 2015). The effects of the HF diet and obesity on cellular functions depend on the time period of exposure to the dietary components. The timeframe, however, during which these adverse effects may develop in the reproductive tract has not been elucidated. This could however provide insights in the potential distinct acute effects elicited after short-term exposure to an obesogenic diet or chronic effects detected after long-term feeding of an obesogenic.

Mice that were fed a HF diet for 16 weeks showed induced mtDNA damage in their liver and skeletal muscle, which is linked to mitochondrial dysfunction and increased OS (Yuzefovych et al., 2013). Feeding mice a HF diet for a short period of only 3 days, thus in the absence of obesity, has shown to impair glucose tolerance and increase plasma glucose and insulin. Compared to long-term feeding of 7.5 months (30 weeks), a short-term feeding period did not impact body weight, adipose tissue, lipid profile or inflammatory markers (Haley et al., 2019a). Already 1 hour after a high-fat meal,

circulating endotoxin levels, irrespective of metabolic state, were shown to be increased in human (Harte et al., 2012). London et al (2017) investigated the different effects of short or long-term exposure to a HF diet on PKA regulation in mice. HF diet-feeding for 4 days already resulted in a suppression of hepatic PKA as a protective peripheral effect against obesity, whereas long-term HF diet feeding of 14 weeks resulted in dysregulated hepatic and hypothalamic PKA signalling as a signature of obesity (London et al., 2017). As previously described, Williams et al. (2014) suggested that the inflammatory effects due to feeding a HF diet in mice developed in distinct phases. Although the phases or the timeframe during which a HF diet may induce inflammatory changes is inconsistent among different mouse studies (Kleemann et al., 2010; Haley et al., 2019a), it shows that different periods of exposure to an obesogenic diet elicits different responses in the peripheral tissues over time. Few studies investigated the effects after different periods of exposure to a HF diet on the reproductive tract. Long-term HF diet feeding of 7 months in mice elevated expression of inflammatory-mediator genes in both the ovary and surrounding adipose depot, potentially negatively affecting ovarian function (Nteeba et al., 2013). Feeding of a HF diet for 16 weeks resulted in female obese mice that were less likely to ovulate compared to lean, control animals and *in vitro* fertilization and culture of the oocytes that did ovulate showed slower developmental competence (Minge et al., 2008). In addition, ovaries that contained significantly more apoptotic ovarian follicles and fewer, smaller mature oocytes were also observed in obese mice after 16 weeks of HF diet feeding compared to control mice (Jungheim et al., 2010). Feeding mice a HF diet for 13 weeks resulted in increased oocyte lipid droplet accumulation (Marei et al., 2020), a lower average number of oocytes collected from the oviduct after hormonal stimulation and a significantly increased proportion of oocyte mitochondrial structural abnormalities (Smits et al., 2021). Furthermore, oocytes collected from mice fed a HF diet for 4 weeks displayed higher rates of meiotic aneuploidy with fragmented disorganized meiotic spindles and chromosomes that were not properly aligned on the

metaphase plate (Luzzo et al., 2012). Four and 6 weeks of HF diet feeding also resulted in increased mitochondrial ultrastructural abnormalities in oocytes, altered mitochondrial inner membrane potential and ATP production, altered mitochondrial biogenesis and mtDNA copy numbers, compared to control mice (Igosheva et al., 2010; Wu et al., 2010). An overview of all these reported changes after different feeding periods of an obesogenic diet is presented in Figure 8.

However, unlike the impact of feeding a HF diet on the ovarian microenvironment and oocyte quality, the effects of obesogenic diets and obesity on the oviduct are much less characterized. In addition, the development of effects on the ovarian follicular and oviductal microenvironment after different periods of consuming an obesogenic diet remains to be investigated. The consumption of a Western type diet over the course of several weeks may lead to an exaggerated impact on metabolism, inflammation and many more health effects (reviewed by Clemente-Suarez et al. (2023)). However, it has been reported that even a single HF meal can already have significant health effects. As such, a single oral dose of saturated fat in healthy individuals increased hepatic TG accumulation, insulin resistance, gluconeogenesis and ATP concentrations in the human liver and induced peripheral insulin resistance in skeletal muscle and adipose tissue. Furthermore, a single saturated fat load in mice induced hepatic insulin resistance and affected hepatic gene expression (Hernandez et al., 2017). Thus, regardless of an individual being obese or metabolically stressed, they may already be affected after the consumption of a single obesogenic meal. Whether such an acute impact is evident at the reproductive level is not known yet. **Therefore, studying the pathophysiological changes in the reproductive tract that may develop after different durations of feeding an obesogenic diet, may provide fundamental insights in the potential impact on embryo development and the timeframe at which the early embryo might be at risk. Even short-term consumption of an obesogenic diet, may already influence oocyte quality and early embryo development, ultimately leading**

to reduced fertility. However, this is only an assumption and thorough investigation is needed.

1.4.2 What are the dynamics and progressive nature of the effects that may be detected in the oocyte and oviduct?

While just one week of feeding a HF diet induces glucose intolerance and hepatic insulin resistance in mice (Burchfield et al., 2018; Small et al., 2018), other features of the metabolic phenotype have shown to become apparent after more than 4 weeks of feeding (Buettner et al., 2007; Reuter, 2007). As mentioned before, mice consuming a HF diet for only 3 days showed systemic inflammation and reduced glucose intolerance. The development of these responses to the obesogenic diet showed to be multiphasic and limited to distinct phases of short-term and long-term HF diet feeding. In between these phases an adaptation period was observed in which glucose tolerance was stable and no markers of inflammation were detected (Williams et al., 2014). These findings indicates that HF diet exposure can lead to temporal changes over time which may be mediated by short-term feeding and progress over time with prolonged obesogenic feeding and the development of the obese phenotype. It is important to mention that in contrast to human studies in which patients are categorised as obese based on their BMI (≥ 30 kg/m^2) (Purnell, 2000), there is no specific marker or consensus to define obesity in mice (as was previously discussed and reviewed by Dias et al. (2021)). Researchers define the state of obesity based on their own parameters and assumptions. Since defining a specific timepoint for the onset of obesity after the start of an obesogenic diet is not straight forward, the responses to an obesogenic diet may also be described dependent on the duration of exposure, namely as acute and chronic effects that occur after short and long-term feeding respectively. While a lot of research has been conducted to investigate the direct impact of obesity and obesogenic diets on the ovarian FF microenvironment and oocyte quality, only few studies aimed to investigate the distinct impact of obesity after long-term feeding and the responses

elicited after short-term HF diet exposure in the reproductive tract. As such, a study using a diet-induced obese mouse model demonstrated the time-dependent development of gene expression changes due to feeding a HF diet. After 4 weeks on the diet, during early obesity, as was stated by the researchers, the transcriptome of CCs was affected by increased leptin signalling and mainly alterations in genes involved in membrane trafficking, cytoskeleton organisation and glucose metabolism were detected. On the other hand, during late obesity, 16 weeks of DIO, leptin resistance was established and transcriptomic analysis of the CCs indicated the activation of the inflammatory response and cellular anatomical morphogenesis, with inhibition of metabolism and transport. As a consequence, it was suggested that obesity conditions are able to contribute to considerable time-dependent changes in gene expression of CCs, which in the early stages of obesity may be causally related to increased leptin signalling in the ovary whereas in the late stages they are possibly results of the metabolic changes taking place in the obese mothers (Wolodko et al., 2020). Another study demonstrated that the consumption of the HF diet resulted in hampered fertility, higher proinflammatory cytokine levels and increased ovarian macrophage infiltration, independent of obesity (Skaznik-Wikiel et al., 2016).

Although these studies show distinct effects after short and long-term exposure to a HF diet or in the absence of obesity, the pattern of the development of effects over time remains unclear. It is, for example, not known whether the locally initiated changes in the oviduct in response to obesogenic diet exposure for different durations of feeding, would show similar dynamics and progression over time as was observed by Williams et al. (2014) in the reduced glucose tolerance and the systemic inflammatory response after consuming an obesogenic diet over time.

Studying the timeframe of the adverse effects in the follicular and oviductal micro-environment due to the introduction of an obesogenic diet and obesity might provide us with a better insight in whether these effects are acute, after short-term exposure,

or chronic, after long-term exposure and the development of obesity and its associated altered metabolic state. This may aid in understanding the mechanisms by which these pathophysiological changes develop and the timeframe at which oocyte and embryo development might be affected. Such insights are important for increasing awareness and implementing preventative measures during the periconceptional period to enhance fertility.

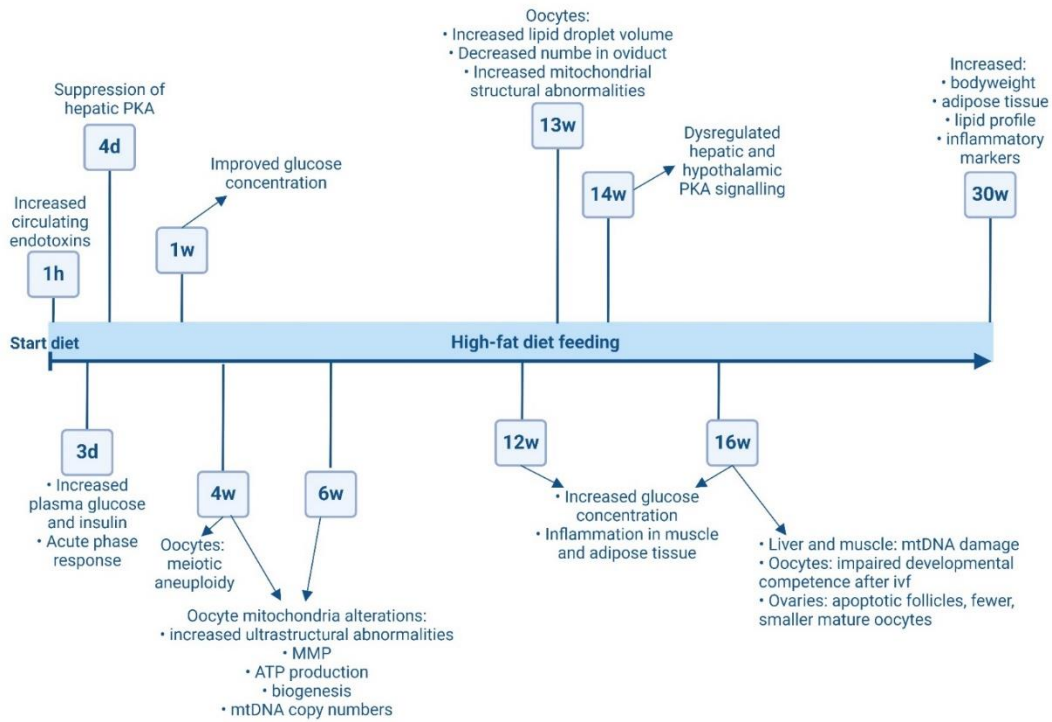

Figure 8: Presentation of the different observed effects after different feeding periods of an obesogenic diet in mice (h = hour; d = days; w = weeks; PKA = Protein Kinase A; MMP = mitochondrial membrane potential; ATP = Adenosine TriPhosphate; mtDNA = mitochondrial DNA; ivf = in vitro fertilisation) (made with Biorender). (Igosheva et al., 2010; Jungheim et al., 2010; Kleemann et al., 2010; Wu et al., 2010; Harte et al., 2012; Luzzo et al., 2012; Nteeba et al., 2013; Yuzefovych et al., 2013; Williams et al., 2014; London et al., 2017; Haley et al., 2019b; Marei et al., 2020; Smits et al., 2021)

2. References

AARDEMA, H., VOS, P. L., LOLICATO, F., ROELEN, B. A., KNIJN, H. M., VAANDRAGER, A. B., HELMS, J. B. & GADELLA, B. M. 2011. Oleic acid prevents detrimental effects of saturated fatty acids on bovine oocyte developmental competence. *Biol Reprod,* 85**,** 62-9.

ABBASIHORMOZI, S. H., BABAPOUR, V., KOUHKAN, A., NIASARI NASLJI, A., AFRAZ, K., ZOLFAGHARY, Z. & SHAHVERDI, A. H. 2019. Stress Hormone and Oxidative Stress Biomarkers Link Obesity and Diabetes with Reduced Fertility Potential. *Cell J,* 21**,** 307-313.

ABDELMAGID, S. A., CLARKE, S. E., NIELSEN, D. E., BADAWI, A., EL-SOHEMY, A., MUTCH, D. M. & MA, D. W. 2015. Comprehensive profiling of plasma fatty acid concentrations in young healthy Canadian adults. *PLoS One,* 10**,** e0116195.

ACTON, B. M., JURISICOVA, A., JURISICA, I. & CASPER, R. F. 2004. Alterations in mitochondrial membrane potential during preimplantation stages of mouse and human embryo development. *Mol Hum Reprod,* 10**,** 23-32.

ADACHI, M., LIU, Y., FUJII, K., CALDERWOOD, S. K., NAKAI, A., IMAI, K. & SHINOMURA, Y. 2009. Oxidative stress impairs the heat stress response and delays unfolded protein recovery. *PLoS One,* 4**,** e7719.

ADAMIAK, S. J., MACKIE, K., WATT, R. G., WEBB, R. & SINCLAIR, K. D. 2005. Impact of nutrition on oocyte quality: cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. *Biol Reprod,* 73**,** 918-26.

AGARWAL, A., APONTE-MELLADO, A., PREMKUMAR, B. J., SHAMAN, A. & GUPTA, S. 2012. The effects of oxidative stress on female reproduction: a review. *Reprod Biol Endocrinol,* 10**,** 49.

AGARWAL, A., GUPTA, S., SEKHON, L. & SHAH, R. 2008. Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications. *Antioxid Redox Signal,* 10**,** 1375-403.

AHYAYAUCH, H. 2023. Relationship between obesity, insulin resistance and cell membrane properties. *Eur J Clin Exp Med,* 2023**,** 357-364.

ALBERTS B, J. A., LEWIS J, RAFF M, ROBERTS K AND WALTER P 2008. Sexual Reproduction: meiosis, germ cells and fertilization. *Molecular Biology of the Cell,* Fifth edition.

ALCANTARA-NETO, A. S., FERNANDEZ-RUFETE, M., CORBIN, E., TSIKIS, G., UZBEKOV, R., GARANINA, A. S., COY, P., ALMINANA, C. & MERMILLOD, P. 2020. Oviduct fluid extracellular vesicles regulate polyspermy during porcine in vitro fertilisation. *Reprod Fertil Dev,* 32**,** 409-418.

ALLIGIER, M., GABERT, L., MEUGNIER, E., LAMBERT-PORCHERON, S., CHANSEAUME, E., PILLEUL, F., DEBARD, C., SAUVINET, V., MORIO, B., VIDAL-PUIG, A., VIDAL, H. &

LAVILLE, M. 2013. Visceral fat accumulation during lipid overfeeding is related to subcutaneous adipose tissue characteristics in healthy men. *J Clin Endocrinol Metab,* 98**,** 802-10.

ALMINANA, C. & BAUERSACHS, S. 2019. Extracellular Vesicles in the Oviduct: Progress, Challenges and Implications for the Reproductive Success. *Bioengineering (Basel),* 6.

ALMINANA, C. & BAUERSACHS, S. 2020. Extracellular vesicles: Multi-signal messengers in the gametes/embryo-oviduct cross-talk. *Theriogenology,* 150**,** 59-69.

ALMINANA, C., CORBIN, E., TSIKIS, G., ALCANTARA-NETO, A. S., LABAS, V., REYNAUD, K., GALIO, L., UZBEKOV, R., GARANINA, A. S., DRUART, X. & MERMILLOD, P. 2017. Oviduct extracellular vesicles protein content and their role during oviduct-embryo cross-talk. *Reproduction,* 154**,** 153-168.

ALMINANA, C., HEATH, P. R., WILKINSON, S., SANCHEZ-OSORIO, J., CUELLO, C., PARRILLA, I., GIL, M. A., VAZQUEZ, J. L., VAZQUEZ, J. M., ROCA, J., MARTINEZ, E. A. & FAZELI, A. 2012. Early developing pig embryos mediate their own environment in the maternal tract. *PLoS One,* 7**,** e33625.

ALMINANA, C., TSIKIS, G., LABAS, V., UZBEKOV, R., DA SILVEIRA, J. C., BAUERSACHS, S. & MERMILLOD, P. 2018. Deciphering the oviductal extracellular vesicles content across the estrous cycle: implications for the gametes-oviduct interactions and the environment of the potential embryo. *BMC Genomics,* 19**,** 622.

ALTUN, T., JINDAL, S., GREENSEID, K., SHU, J. & PAL, L. 2011. Low follicular fluid IL-6 levels in IVF patients are associated with increased likelihood of clinical pregnancy. *J Assist Reprod Genet,* 28**,** 245-51.

ARDON, F., MARKELLO, R. D., HU, L., DEUTSCH, Z. I., TUNG, C. K., WU, M. M. & SUAREZ, S. S. 2016. Dynamics of Bovine Sperm Interaction with Epithelium Differ Between Oviductal Isthmus and Ampulla. *Biology of Reproduction,* 95.

ARNAUD, P. 2010. Genomic imprinting in germ cells: imprints are under control. *Reproduction,* 140**,** 411-23.

ARNER, P. 2005. Human fat cell lipolysis: biochemistry, regulation and clinical role. *Best Pract Res Clin Endocrinol Metab,* 19**,** 471-82.

ASHWORTH, C. J., TOMA, L. M. & HUNTER, M. G. 2009. Nutritional effects on oocyte and embryo development in mammals: implications for reproductive efficiency and environmental sustainability. *Philos Trans R Soc Lond B Biol Sci,* 364**,** 3351-61.

ATTARAN, M., PASQUALOTTO, E., FALCONE, T., GOLDBERG, J. M., MILLER, K. F., AGARWAL, A. & SHARMA, R. K. 2000. The effect of follicular fluid reactive oxygen species on the outcome of in vitro fertilization. *Int J Fertil Womens Med,* 45**,** 314-20.

AVILES, M., GUTIERREZ-ADAN, A. & COY, P. 2010. Oviductal secretions: will they be key factors for the future ARTs? *Mol Hum Reprod,* 16**,** 896-906.

BAHAT, A. & EISENBACH, M. 2006. Sperm thermotaxis. *Mol Cell Endocrinol,* 252**,** 115-9.

BALLESTEROS-GUZMAN, A. K., CARRASCO-LEGLEU, C. E., LEVARIO-CARRILLO, M., CHAVEZ-CORRAL, D. V., SANCHEZ-RAMIREZ, B., MARINELARENA-CARRILLO, E. O., GUERRERO-SALGADO, F. & REZA-LOPEZ, S. A. 2019. Prepregnancy Obesity, Maternal Dietary Intake, and Oxidative Stress Biomarkers in the Fetomaternal Unit. *Biomed Res Int,* 2019**,** 5070453.

BANLIAT, C., MAHE, C., LAVIGNE, R., COM, E., PINEAU, C., LABAS, V., GUYONNET, B., MERMILLOD, P. & SAINT-DIZIER, M. 2022. The proteomic analysis of bovine embryos developed in vivo or in vitro reveals the contribution of the maternal environment to early embryo. *BMC Genomics,* 23**,** 839.

BANLIAT, C., TSIKIS, G., LABAS, V., TEIXEIRA-GOMES, A. P., COM, E., LAVIGNE, R., PINEAU, C., GUYONNET, B., MERMILLOD, P. & SAINT-DIZIER, M. 2020. Identification of 56 Proteins Involved in Embryo-Maternal Interactions in the Bovine Oviduct. *Int J Mol Sci,* 21.

BARCELO, F., PERONA, J. S., PRADES, J., FUNARI, S. S., GOMEZ-GRACIA, E., CONDE, M., ESTRUCH, R. & RUIZ-GUTIERREZ, V. 2009. Mediterranean-style diet effect on the structural properties of the erythrocyte cell membrane of hypertensive patients: the Prevencion con Dieta Mediterranea Study. *Hypertension,* 54**,** 1143-50.

BASTOS, N. M., FERST, J. G., GOULART, R. S. & COELHO DA SILVEIRA, J. 2022. The role of the oviduct and extracellular vesicles during early embryo development in bovine. *Anim Reprod,* 19**,** e20220015.

BASUINO, L. & SILVEIRA, C. F., JR. 2016. Human follicular fluid and effects on reproduction. *JBRA Assist Reprod,* 20**,** 38-40.

BAUERSACHS, S., BLUM, H., MALLOK, S., WENIGERKIND, H., RIEF, S., PRELLE, K. & WOLF, E. 2003. Regulation of ipsilateral and contralateral bovine oviduct epithelial cell function in the postovulation period: a transcriptomics approach. *Biol Reprod,* 68**,** 1170-7.

BAUMANN, C. G., MORRIS, D. G., SREENAN, J. M. & LEESE, H. J. 2007. The quiet embryo hypothesis: molecular characteristics favoring viability. *Mol Reprod Dev,* 74**,** 1345-53.

BELLVER, J., ROSSAL, L. P., BOSCH, E., ZUNIGA, A., CORONA, J. T., MELENDEZ, F., GOMEZ, E., SIMON, C., REMOHI, J. & PELLICER, A. 2003. Obesity and the risk of spontaneous abortion after oocyte donation. *Fertil Steril,* 79**,** 1136-40.

BELRAP, B. R. F. A. P. 2019. Assisted Reproductive Technology National Summary Report.

BERGMAN, R. N. & ADER, M. 2000. Free fatty acids and pathogenesis of type 2 diabetes mellitus. *Trends Endocrinol Metab,* 11**,** 351-6.

BERGQVIST, A. S. & RODRIGUEZ-MARTINEZ, H. 2006. Sulphated glycosaminoglycans (S-GAGs) and syndecans in the bovine oviduct. *Anim Reprod Sci,* 93**,** 46-60.

BERMEJO-ALVAREZ, P., LONERGAN, P., RIZOS, D. & GUTIERREZ-ADAN, A. 2010. Low oxygen tension during IVM improves bovine oocyte competence and enhances anaerobic glycolysis. *Reprod Biomed Online,* 20**,** 341-9.

BESENFELDER, U., HAVLICEK, V. & BREM, G. 2012. Role of the oviduct in early embryo development. *Reprod Domest Anim,* 47 Suppl 4**,** 156-63.

BESSESEN, D. H. 2008. Update on obesity. *J Clin Endocrinol Metab,* 93**,** 2027-34.

BEST, D. & BHATTACHARYA, S. 2015. Obesity and fertility. *Horm Mol Biol Clin Investig,* 24**,** 5-10.

BHUSANE, K., BHUTADA, S., CHAUDHARI, U., SAVARDEKAR, L., KATKAM, R. & SACHDEVA, G. 2016. Secrets of Endometrial Receptivity: Some Are Hidden in Uterine Secretome. *Am J Reprod Immunol,* 75**,** 226-36.

BIRKHEAD, T. R. & MOLLER, A. P. 1993. Sexual Selection and the Temporal Separation of Reproductive Events - Sperm Storage Data from Reptiles, Birds and Mammals. *Biological Journal of the Linnean Society,* 50**,** 295-311.

BOLSONI-LOPES, A. & ALONSO-VALE, M. I. 2015. Lipolysis and lipases in white adipose tissue - An update. *Arch Endocrinol Metab,* 59**,** 335-42.

BOLUMAR, F., OLSEN, J., REBAGLIATO, M., SAEZ-LLORET, I. & BISANTI, L. 2000. Body mass index and delayed conception: a European Multicenter Study on Infertility and Subfecundity. *Am J Epidemiol,* 151**,** 1072-9.

BORRADAILE, N. M., HAN, X., HARP, J. D., GALE, S. E., ORY, D. S. & SCHAFFER, J. E. 2006. Disruption of endoplasmic reticulum structure and integrity in lipotoxic cell death. *J Lipid Res,* 47**,** 2726-37.

BOUDOURES, A. L., CHI, M., THOMPSON, A., ZHANG, W. & MOLEY, K. H. 2016. The effects of voluntary exercise on oocyte quality in a diet-induced obese murine model. *Reproduction,* 151**,** 261-70.

BOUDOURES, A. L., SABEN, J., DRURY, A., SCHEAFFER, S., MODI, Z., ZHANG, W. & MOLEY, K. H. 2017. Obesity-exposed oocytes accumulate and transmit damaged mitochondria due to an inability to activate mitophagy. *Dev Biol,* 426**,** 126-138.

BRIDI, A., PERECIN, F. & SILVEIRA, J. C. D. 2020. Extracellular Vesicles Mediated Early Embryo-Maternal Interactions. *Int J Mol Sci,* 21.

BUDANI, M. C., CARLETTI, E. & TIBONI, G. M. 2017. Cigarette smoke is associated with altered expression of antioxidant enzymes in granulosa cells from women undergoing in vitro fertilization. *Zygote,* 25**,** 296-303.

BUETTNER, R., SCHOLMERICH, J. & BOLLHEIMER, L. C. 2007. High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring),* 15**,** 798-808.

BURCHFIELD, J. G., KEBEDE, M. A., MEOLI, C. C., STOCKLI, J., WHITWORTH, P. T., WRIGHT, A. L., HOFFMAN, N. J., MINARD, A. Y., MA, X., KRYCER, J. R., NELSON, M. E., TAN, S. X., YAU, B., THOMAS, K. C., WEE, N. K. Y., KHOR, E. C., ENRIQUEZ, R. F., VISSEL, B., BIDEN, T. J., BALDOCK, P. A., HOEHN, K. L., CANTLEY, J.,

COONEY, G. J., JAMES, D. E. & FAZAKERLEY, D. J. 2018. High dietary fat and sucrose results in an extensive and time-dependent deterioration in health of multiple physiological systems in mice. *J Biol Chem,* 293**,** 5731-5745.

CARDOSO, J. P., COCUZZA, M. & ELTERMAN, D. 2019. Optimizing male fertility: oxidative stress and the use of antioxidants. *World J Urol,* 37**,** 1029-1034.

CARRELL, D. T., JONES, K. P., PETERSON, C. M., AOKI, V., EMERY, B. R. & CAMPBELL, B. R. 2001. Body mass index is inversely related to intrafollicular HCG concentrations, embryo quality and IVF outcome. *Reprod Biomed Online,* 3**,** 109-111.

CEBRIAN-SERRANO, A., SALVADOR, I., GARCIA-ROSELLO, E., PERICUESTA, E., PEREZ-CEREZALES, S., GUTIERREZ-ADAN, A., COY, P. & SILVESTRE, M. A. 2013. Effect of the bovine oviductal fluid on in vitro fertilization, development and gene expression of in vitro-produced bovine blastocysts. *Reprod Domest Anim,* 48**,** 331-8.

CELIK, C., LEE, S. Y. T., YAP, W. S. & THIBAULT, G. 2023. Endoplasmic reticulum stress and lipids in health and diseases. *Prog Lipid Res,* 89**,** 101198.

CHABOWSKI, A., ZENDZIAN-PIOTROWSKA, M., KONSTANTYNOWICZ, K., PANKIEWICZ, W., MIKLOSZ, A., LUKASZUK, B. & GORSKI, J. 2013. Fatty acid transporters involved in the palmitate and oleate induced insulin resistance in primary rat hepatocytes. *Acta Physiol (Oxf),* 207**,** 346-57.

CHAFFIN, C. L., LATHAM, K. E., MTANGO, N. R., MIDIC, U. & VANDEVOORT, C. A. 2014. Dietary sugar in healthy female primates perturbs oocyte maturation and in vitro preimplantation embryo development. *Endocrinology,* 155**,** 2688-95.

CHAMBERS, G. M., ADAMSON, G. D. & EIJKEMANS, M. J. 2013. Acceptable cost for the patient and society. *Fertil Steril,* 100**,** 319-27.

CHAMBERS, G. M., SULLIVAN, E. A., ISHIHARA, O., CHAPMAN, M. G. & ADAMSON, G. D. 2009. The economic impact of assisted reproductive technology: a review of selected developed countries. *Fertil Steril,* 91**,** 2281-94.

CHANKEAW, W., GUO, Y. Z., BAGE, R., SVENSSON, A., ANDERSSON, G. & HUMBLOT, P. 2018. Elevated non-esterified fatty acids impair survival and promote lipid accumulation and pro-inflammatory cytokine production in bovine endometrial epithelial cells. *Reprod Fertil Dev,* 30**,** 1770-1784.

CHATTOPADHAYAY, R., GANESH, A., SAMANTA, J., JANA, S. K., CHAKRAVARTY, B. N. & CHAUDHURY, K. 2010. Effect of follicular fluid oxidative stress on meiotic spindle formation in infertile women with polycystic ovarian syndrome. *Gynecol Obstet Invest,* 69**,** 197-202.

CHILDS, S., HENNESSY, A. A., SREENAN, J. M., WATHES, D. C., CHENG, Z., STANTON, C., DISKIN, M. G. & KENNY, D. A. 2008. Effect of level of dietary n-3 polyunsaturated fatty acid supplementation on systemic and tissue fatty acid concentrations and on selected reproductive variables in cattle. *Theriogenology,* 70**,** 595-611.

CHOOI, Y. C., DING, C. & MAGKOS, F. 2019. The epidemiology of obesity. *Metabolism,* 92**,** 6-10.

CIMADOMO, D., FABOZZI, G., VAIARELLI, A., UBALDI, N., UBALDI, F. M. & RIENZI, L. 2018. Impact of Maternal Age on Oocyte and Embryo Competence. *Front Endocrinol (Lausanne),* 9**,** 327.

CLEMENTE-SUAREZ, V. J., BELTRAN-VELASCO, A. I., REDONDO-FLOREZ, L., MARTIN-RODRIGUEZ, A. & TORNERO-AGUILERA, J. F. 2023. Global Impacts of Western Diet and Its Effects on Metabolism and Health: A Narrative Review. *Nutrients,* 15.

COCKCROFT, S. 2021. Mammalian lipids: structure, synthesis and function. *Essays Biochem,* 65**,** 813-845.

COLLADO-FERNANDEZ, E., PICTON, H. M. & DUMOLLARD, R. 2012. Metabolism throughout follicle and oocyte development in mammals. *International Journal of Developmental Biology,* 56**,** 799-808.

COMBELLES, C. M., GUPTA, S. & AGARWAL, A. 2009. Could oxidative stress influence the in-vitro maturation of oocytes? *Reprod Biomed Online,* 18**,** 864-80.

COTICCHIO, G., DAL CANTO, M., MIGNINI RENZINI, M., GUGLIELMO, M. C., BRAMBILLASCA, F., TURCHI, D., NOVARA, P. V. & FADINI, R. 2015. Oocyte maturation: gamete-somatic cells interactions, meiotic resumption, cytoskeletal dynamics and cytoplasmic reorganization. *Hum Reprod Update,* 21**,** 427-54.

COX, C. M., THOMA, M. E., TCHANGALOVA, N., MBURU, G., BORNSTEIN, M. J., JOHNSON, C. L. & KIARIE, J. 2022. Infertility prevalence and the methods of estimation from 1990 to 2021: a systematic review and meta-analysis. *Hum Reprod Open,* 2022**,** hoac051.

CROXATTO, H. B. 2002. Physiology of gamete and embryo transport through the fallopian tube. *Reprod Biomed Online,* 4**,** 160-9.

CUMMINS, J. M. 2002. The role of maternal mitochondria during oogenesis, fertilization and embryogenesis. *Reprod Biomed Online,* 4**,** 176-82.

D'SOUZA, A., BURCH, A., DAVE, K. M., SREERAM, A., REYNOLDS, M. J., DOBBINS, D. X., KAMTE, Y. S., ZHAO, W., SABATELLE, C., JOY, G. M., SOMAN, V., CHANDRAN, U. R., SHIVA, S. S., QUILLINAN, N., HERSON, P. S. & MANICKAM, D. S. 2021. Microvesicles transfer mitochondria and increase mitochondrial function in brain endothelial cells. *J Control Release,* 338**,** 505-526.

DA BROI, M. G., DE ALBUQUERQUE, F. O., DE ANDRADE, A. Z., CARDOSO, R. L., JORDAO JUNIOR, A. A. & NAVARRO, P. A. 2016. Increased concentration of 8-hydroxy-2'-deoxyguanosine in follicular fluid of infertile women with endometriosis. *Cell Tissue Res,* 366**,** 231-42.

DA BROI, M. G., GIORGI, V. S. I., WANG, F., KEEFE, D. L., ALBERTINI, D. & NAVARRO, P. A. 2018. Influence of follicular fluid and cumulus cells on oocyte quality: clinical implications. *J Assist Reprod Genet,* 35**,** 735-751.

DA BROI, M. G. & NAVARRO, P. A. 2016. Oxidative stress and oocyte quality: ethiopathogenic mechanisms of minimal/mild endometriosis-related infertility. *Cell Tissue Res,* 364**,** 1-7.

DALTON, C. M., SZABADKAI, G. & CARROLL, J. 2014. Measurement of ATP in single oocytes: impact of maturation and cumulus cells on levels and consumption. *J Cell Physiol,* 229**,** 353-61.

DAS, U. N. 2001. Is obesity an inflammatory condition? *Nutrition,* 17**,** 953-66.

DE WAAL, E., MAK, W., CALHOUN, S., STEIN, P., ORD, T., KRAPP, C., COUTIFARIS, C., SCHULTZ, R. M. & BARTOLOMEI, M. S. 2014. In vitro culture increases the frequency of stochastic epigenetic errors at imprinted genes in placental tissues from mouse concepti produced through assisted reproductive technologies. *Biol Reprod,* 90**,** 22.

DE WAAL, E., VROOMAN, L. A., FISCHER, E., ORD, T., MAINIGI, M. A., COUTIFARIS, C., SCHULTZ, R. M. & BARTOLOMEI, M. S. 2015. The cumulative effect of assisted reproduction procedures on placental development and epigenetic perturbations in a mouse model. *Hum Mol Genet,* 24**,** 6975-85.

DESMET, K. L., VAN HOECK, V., GAGNE, D., FOURNIER, E., THAKUR, A., O'DOHERTY, A. M., WALSH, C. P., SIRARD, M. A., BOLS, P. E. & LEROY, J. L. 2016. Exposure of bovine oocytes and embryos to elevated non-esterified fatty acid concentrations: integration of epigenetic and transcriptomic signatures in resultant blastocysts. *BMC Genomics,* 17**,** 1004.

DIAS, M. D. E., DOS REIS, S. A., DA CONCEIÇAO, L. L., SEDIYAMA, C. M. N. D., PEREIRA, S. S., DE OLIVEIRA, L. L., PELUZIO, M. D. G., MARTINEZ, J. A. & MILAGRO, F. I. 2021. Diet-induced obesity in animal models: points to consider and influence on metabolic markers. *Diabetology & Metabolic Syndrome,* 13.

DOKRAS, A., BAREDZIAK, L., BLAINE, J., SYROP, C., VANVOORHIS, B. J. & SPARKS, A. 2006. Obstetric outcomes after in vitro fertilization in obese and morbidly obese women. *Obstet Gynecol,* 108**,** 61-9.

DONATO, J., JR., CRAVO, R. M., FRAZAO, R. & ELIAS, C. F. 2011. Hypothalamic sites of leptin action linking metabolism and reproduction. *Neuroendocrinology,* 93**,** 9-18.

DUNNING, K. R., CASHMAN, K., RUSSELL, D. L., THOMPSON, J. G., NORMAN, R. J. & ROBKER, R. L. 2010. Beta-oxidation is essential for mouse oocyte developmental competence and early embryo development. *Biol Reprod,* 83**,** 909-18.

DUPONT, J. & SCARAMUZZI, R. J. 2016. Insulin signalling and glucose transport in the ovary and ovarian function during the ovarian cycle. *Biochem J,* 473**,** 1483-501.

DURANTHON, V. & CHAVATTE-PALMER, P. 2018. Long term effects of ART: What do animals tell us? *Mol Reprod Dev,* 85**,** 348-368.

DURANTHON, V., WATSON, A. J. & LONERGAN, P. 2008. Preimplantation embryo programming: transcription, epigenetics, and culture environment. *Reproduction,* 135**,** 141-50.

ECKEL, R. H., ALBERTI, K. G., GRUNDY, S. M. & ZIMMET, P. Z. 2010. The metabolic syndrome. *Lancet,* 375**,** 181-3.

EL HAJJ, N. & HAAF, T. 2013. Epigenetic disturbances in in vitro cultured gametes and embryos: implications for human assisted reproduction. *Fertil Steril,* 99**,** 632-41.

ENGIN, A. 2017a. The Definition and Prevalence of Obesity and Metabolic Syndrome. *Adv Exp Med Biol,* 960**,** 1-17.

ENGIN, A. B. 2017b. Obesity and Lipotoxocity. *In:* ENGIN, A. (ed.). New York, NY, USA: Springer.

ENGIN, A. B. 2017c. What Is Lipotoxicity? *Adv Exp Med Biol,* 960**,** 197-220.

ENRIGHT, B. P., LONERGAN, P., DINNYES, A., FAIR, T., WARD, F. A., YANG, X. & BOLAND, M. P. 2000. Culture of in vitro produced bovine zygotes in vitro vs in vivo: implications for early embryo development and quality. *Theriogenology,* 54**,** 659-73.

EPPIG, J. J. 2001. Oocyte control of ovarian follicular development and function in mammals. *Reproduction,* 122**,** 829-38.

EVERS, J. L. 2002. Female subfertility. *Lancet,* 360**,** 151-9.

FABOZZI, G., IUSSIG, B., CIMADOMO, D., VAIARELLI, A., MAGGIULLI, R., UBALDI, N., UBALDI, F. M. & RIENZI, L. 2021. The Impact of Unbalanced Maternal Nutritional Intakes on Oocyte Mitochondrial Activity: Implications for Reproductive Function. *Antioxidants (Basel),* 10.

FANG, X., BANG, S., TANGA, B. M., SEO, C., ZHOU, D., SEONG, G., SAADELDIN, I. M., LEE, S., CUI, X. S. & CHO, J. 2023. Oviduct epithelial cell-derived extracellular vesicles promote the developmental competence of IVF porcine embryos. *Mol Med Rep,* 27.

FANG, X., TANGA, B. M., BANG, S., SEONG, G., SAADELDIN, I. M., LEE, S. & CHO, J. 2022. Oviduct epithelial cells-derived extracellular vesicles improve preimplantation developmental competence of in vitro produced porcine parthenogenetic and cloned embryos. *Mol Reprod Dev,* 89**,** 54-65.

FATEHI, A. N., ZEINSTRA, E. C., KOOIJ, R. V., COLENBRANDER, B. & BEVERS, M. M. 2002. Effect of cumulus cell removal of in vitro matured bovine oocytes prior to in vitro fertilization on subsequent cleavage rate. *Theriogenology,* 57**,** 1347-55.

FEDORCSAK, P., DALE, P. O., STORENG, R., ERTZEID, G., BJERCKE, S., OLDEREID, N., OMLAND, A. K., ABYHOLM, T. & TANBO, T. 2004. Impact of overweight and underweight on assisted reproduction treatment. *Hum Reprod,* 19**,** 2523-8.

FERGUSON, E. M. & LEESE, H. J. 2006. A potential role for triglyceride as an energy source during bovine oocyte maturation and early embryo development. *Mol Reprod Dev,* 73**,** 1195-201.

FERNANDEZ-FUERTES, B., RODRÍGUEZ-ALONSO, B., SÁNCHEZ, J. M., SIMINTIRAS, C. A., LONERGAN, P. & RIZOS, D. 2018. Looking at the big picture: understanding how the oviduct's dialogue with gametes and the embryo shapes reproductive success. *Animal Reproduction,* 15**,** 751-764.

FERNANDEZ-SANCHEZ, A., MADRIGAL-SANTILLAN, E., BAUTISTA, M., ESQUIVEL-SOTO, J., MORALES-GONZALEZ, A., ESQUIVEL-CHIRINO, C., DURANTE-MONTIEL, I., SANCHEZ-RIVERA, G., VALADEZ-VEGA, C. & MORALES-GONZALEZ, J. A. 2011. Inflammation, oxidative stress, and obesity. *Int J Mol Sci,* 12**,** 3117-32.

FERRAZ, M., CAROTHERS, A., DAHAL, R., NOONAN, M. J. & SONGSASEN, N. 2019. Oviductal extracellular vesicles interact with the spermatozoon's head and mid-piece and improves its motility and fertilizing ability in the domestic cat. *Sci Rep,* 9**,** 9484.

FONTES, P. K., CASTILHO, A. C., RAZZA, E. M., ERENO, R. L., SATRAPA, R. A. & BARROS, C. M. 2014. Prostaglandin receptors (EP2 and EP4) and angiotensin receptor (AGTR2) mRNA expression increases in the oviducts of Nelore cows submitted to ovarian superstimulation. *Anim Reprod Sci,* 151**,** 112-8.

FRASER, R. B., WAITE, S. L., WOOD, K. A. & MARTIN, K. L. 2007. Impact of hyperglycemia on early embryo development and embryopathy: in vitro experiments using a mouse model. *Hum Reprod,* 22**,** 3059-68.

GAAFAR, T. M., HANNA, M. O., HAMMADY, M. R., AMR, H. M., OSMAN, O. M., NASEF, A. & OSMAN, A. M. 2014. Evaluation of cytokines in follicular fluid and their effect on fertilization and pregnancy outcome. *Immunol Invest,* 43**,** 572-84.

GAD, A., HOELKER, M., BESENFELDER, U., HAVLICEK, V., CINAR, U., RINGS, F., HELD, E., DUFORT, I., SIRARD, M. A., SCHELLANDER, K. & TESFAYE, D. 2012. Molecular mechanisms and pathways involved in bovine embryonic genome activation and their regulation by alternative in vivo and in vitro culture conditions. *Biol Reprod,* 87**,** 100.

GAMBINERI, A., PELUSI, C., VICENNATI, V., PAGOTTO, U. & PASQUALI, R. 2002. Obesity and the polycystic ovary syndrome. *Int J Obes Relat Metab Disord,* 26**,** 883-96.

GARDNER, D. K. 1998. Changes in requirements and utilization of nutrients during mammalian preimplantation embryo development and their significance in embryo culture. *Theriogenology,* 49**,** 83-102.

GARDNER, D. K., CLARKE, R. N., LECHENE, C. P. & BIGGERS, J. D. 1989. Development of a Noninvasive Ultramicrofluorometric Method for Measuring Net Uptake of Glutamine by Single Preimplantation Mouse Embryos. *Gamete Research,* 24**,** 427-438.

GE, H., TOLLNER, T. L., HU, Z., DAI, M., LI, X., GUAN, H., SHAN, D., ZHANG, X., LV, J., HUANG, C. & DONG, Q. 2012. The importance of mitochondrial metabolic activity and mitochondrial DNA replication during oocyte maturation in vitro on oocyte quality and subsequent embryo developmental competence. *Mol Reprod Dev,* 79**,** 392-401.

GEHRMANN, W., ELSNER, M. & LENZEN, S. 2010. Role of metabolically generated reactive oxygen species for lipotoxicity in pancreatic beta-cells. *Diabetes Obes Metab,* 12 Suppl 2**,** 149-58.

GEORGIOU, A. S., SOSTARIC, E., WONG, C. H., SNIJDERS, A. P., WRIGHT, P. C., MOORE, H. D. & FAZELI, A. 2005. Gametes alter the oviductal secretory proteome. *Mol Cell Proteomics,* 4**,** 1785-96.

GESHI, M., TAKENOUCHI, N., YAMAUCHI, N. & NAGAI, T. 2000. Effects of sodium pyruvate in nonserum maturation medium on maturation, fertilization, and subsequent development of bovine oocytes with or without cumulus cells. *Biol Reprod,* 63**,** 1730-4.

GESINK LAW, D. C., MACLEHOSE, R. F. & LONGNECKER, M. P. 2007. Obesity and time to pregnancy. *Hum Reprod,* 22**,** 414-20.

GHERSEVICH, S., MASSA, E. & ZUMOFFEN, C. 2015. Oviductal secretion and gamete interaction. *Reproduction,* 149**,** R1-R14.

GIANFRANCESCO, M. A., PAQUOT, N., PIERRE, J. & LEGRAND-POELS, S. 2018. Lipid bilayer stress in obesity-linked inflammatory and metabolic disorders. *Biochemical Pharmacology,* 153**,** 168-183.

GILCHRIST, R. B., RITTER, L. J. & ARMSTRONG, D. T. 2004. Oocyte-somatic cell interactions during follicle development in mammals. *Anim Reprod Sci,* 82-83**,** 431-46.

GIVIZIEZ, C. R., SANCHEZ, E. G., APPROBATO, M. S., MAIA, M. C., FLEURY, E. A. & SASAKI, R. S. 2016. Obesity and anovulatory infertility: A review. *JBRA Assist Reprod,* 20**,** 240-245.

GNOTH, C., GODEHARDT, E., FRANK-HERRMANN, P., FRIOL, K., TIGGES, J. & FREUNDL, G. 2005. Definition and prevalence of subfertility and infertility. *Hum Reprod,* 20**,** 1144-7.

GODE, F., GULEKLI, B., DOGAN, E., KORHAN, P., DOGAN, S., BIGE, O., CIMRIN, D. & ATABEY, N. 2011. Influence of follicular fluid GDF9 and BMP15 on embryo quality. *Fertil Steril,* 95**,** 2274-8.

GODFREY, K. M., LILLYCROP, K. A., BURDGE, G. C., GLUCKMAN, P. D. & HANSON, M. A. 2007. Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. *Pediatr Res,* 61**,** 5R-10R.

GOLDSTEIN, J. L. & BROWN, M. S. 2009. The LDL receptor. *Arterioscler Thromb Vasc Biol,* 29**,** 431-8.

GONCALVES, J. S., VANNUCCHI, C. I., BRAGA, F. C., PAULA-LOPES, F. F., MILAZZOTTO, M. P., ASSUMPCAO, M. E. & VISINTIN, J. A. 2009. Oestrogen and progesterone receptor gene expression in canine oocytes and cumulus cells throughout the oestrous cycle. *Reprod Domest Anim,* 44 Suppl 2**,** 239-42.

GONZALEZ, M. B., LANE, M., KNIGHT, E. J. & ROBKER, R. L. 2018. Inflammatory markers in human follicular fluid correlate with lipid levels and Body Mass Index. *J Reprod Immunol,* 130**,** 25-29.

GOTT, A. L., GRAY, S. M., JAMES, A. F. & LEESE, H. J. 1988. The mechanism and control of rabbit oviduct fluid formation. *Biol Reprod,* 39**,** 758-63.

GREEN, B. B., WEISS, N. S. & DALING, J. R. 1988. Risk of ovulatory infertility in relation to body weight. *Fertil Steril,* 50**,** 721-6.

GRINDLER, N. M. & MOLEY, K. H. 2013. Maternal obesity, infertility and mitochondrial dysfunction: potential mechanisms emerging from mouse model systems. *Mol Hum Reprod,* 19**,** 486-94.

GU, L., LIU, H., GU, X., BOOTS, C., MOLEY, K. H. & WANG, Q. 2015. Metabolic control of oocyte development: linking maternal nutrition and reproductive outcomes. *Cell Mol Life Sci,* 72**,** 251-71.

GUPTA P, M. A., SUMAN A, SINGH R K 2021. Effect of Endocrine Disrupting Chemicals on HPG axis: A Reproductive Endocrine Homeostasis. *Hot Topics in Endocrionology and Metabolism.* 1 ed.: IntechOpen.

HAAG, M. & DIPPENAAR, N. G. 2005. Dietary fats, fatty acids and insulin resistance: short review of a multifaceted connection. *Med Sci Monit,* 11**,** RA359-67.

HALEY, M. J., KRISHNAN, S., BURROWS, D., DE HOOG, L., THAKRAR, J., SCHIESSL, I., ALLAN, S. M. & LAWRENCE, C. B. 2019a. Acute high-fat feeding leads to disruptions in glucose homeostasis and worsens stroke outcome. *J Cereb Blood Flow Metab,* 39**,** 1026-1037.

HALEY, M. J., KRISHNAN, S., BURROWS, D., DE HOOG, L., THAKRAR, J., SCHIESSL, I., ALLAN, S. M. & LAWRENCE, C. B. 2019b. Acute high-fat feeding leads to disruptions in glucose homeostasis and worsens stroke outcome. *Journal of Cerebral Blood Flow and Metabolism,* 39**,** 1026-1037.

HALLIWELL, B. & ARUOMA, O. I. 1991. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. *FEBS Lett,* 281**,** 9-19.

HAMMOND, G. L. 2011. Diverse roles for sex hormone-binding globulin in reproduction. *Biol Reprod,* 85**,** 431-41.

HAN, J. & KAUFMAN, R. J. 2016. The role of ER stress in lipid metabolism and lipotoxicity. *J Lipid Res,* 57**,** 1329-38.

HANSEN, C., SRIKANDAKUMAR, A. & DOWNEY, B. R. 1991. Presence of follicular fluid in the porcine oviduct and its contribution to the acrosome reaction. *Mol Reprod Dev,* 30**,** 148-53.

HARRIS, E. A., STEPHENS, K. K. & WINUTHAYANON, W. 2020. Extracellular Vesicles and the Oviduct Function. *Int J Mol Sci,* 21.

HARTE, A. L., VARMA, M. C., TRIPATHI, G., MCGEE, K. C., AL-DAGHRI, N. M., AL-ATTAS, O. S., SABICO, S., O'HARE, J. P., CERIELLO, A., SARAVANAN, P., KUMAR, S. & MCTERNAN, P. G. 2012. High fat intake leads to acute postprandial exposure to circulating endotoxin in type 2 diabetic subjects. *Diabetes Care,* 35**,** 375-82.

HASANPOOR-AZGHDY, S. B., SIMBAR, M. & VEDADHIR, A. 2014. The emotional-psychological consequences of infertility among infertile women seeking treatment: Results of a qualitative study. *Iran J Reprod Med,* 12**,** 131-8.

HASS, D. T. & BARNSTABLE, C. J. 2021. Uncoupling proteins in the mitochondrial defense against oxidative stress. *Prog Retin Eye Res,* 83**,** 100941.

HAUCK, A. K. & BERNLOHR, D. A. 2016. Oxidative stress and lipotoxicity. *J Lipid Res,* 57**,** 1976-1986.

HENNET, M. L. & COMBELLES, C. M. 2012. The antral follicle: a microenvironment for oocyte differentiation. *Int J Dev Biol,* 56**,** 819-31.

HERDT, T. H. 2013. Metabolic diseases of dairy cattle. *Vet Clin North Am Food Anim Pract,* 29**,** xi-xii.

HERNANDEZ, E. A., KAHL, S., SEELIG, A., BEGOVATZ, P., IRMLER, M., KUPRIYANOVA, Y., NOWOTNY, B., NOWOTNY, P., HERDER, C., BAROSA, C., CARVALHO, F., ROZMAN, J., NESCHEN, S., JONES, J. G., BECKERS, J., DE ANGELIS, M. H. & RODEN, M. 2017. Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. *J Clin Invest,* 127**,** 695-708.

HOEK, J., SCHOENMAKERS, S., BAART, E. B., KOSTER, M. P. H., WILLEMSEN, S. P., VAN MARION, E. S., STEEGERS, E. A. P., LAVEN, J. S. E. & STEEGERS-THEUNISSEN, R. P. M. 2020. Preconceptional Maternal Vegetable Intake and Paternal Smoking Are Associated with Pre-implantation Embryo Quality. *Reprod Sci,* 27**,** 2018-2028.

HOLT, W. V. & FAZELI, A. 2016a. Sperm selection in the female mammalian reproductive tract. Focus on the oviduct: Hypotheses, mechanisms, and new opportunities. *Theriogenology,* 85**,** 105-12.

HOLT, W. V. & FAZELI, A. 2016b. Sperm Storage in the Female Reproductive Tract. *Annu Rev Anim Biosci,* 4**,** 291-310.

HU, F. B. 2003. Overweight and obesity in women: Health risks and consequences. *Journal of Womens Health & Gender-Based Medicine,* 12**,** 163-172.

HU, F. B., LI, T. Y., COLDITZ, G. A., WILLETT, W. C. & MANSON, J. E. 2003. Television watching and other sedentary behaviors in relation to risk of obesity and type 2 diabetes mellitus in women. *JAMA,* 289**,** 1785-91.

HU, K. & YU, Y. 2017. Metabolite availability as a window to view the early embryo microenvironment in vivo. *Mol Reprod Dev,* 84**,** 1027-1038.

HUANG, B. B., LIU, X. C., QIN, X. Y., CHEN, J., REN, P. G., DENG, W. F. & ZHANG, J. 2019. Effect of High-Fat Diet on Immature Female Mice and Messenger and Noncoding RNA Expression Profiling in Ovary and White Adipose Tissue. *Reprod Sci,* 26**,** 1360-1372.

HUGENTOBLER, S., MORRIS, D. G., KANE, M. T. & SREENAN, J. M. 2004. In situ oviduct and uterine pH in cattle. *Theriogenology,* 61**,** 1419-27.

HUGENTOBLER, S. A., DISKIN, M. G., LEESE, H. J., HUMPHERSON, P. G., WATSON, T., SREENAN, J. M. & MORRIS, D. G. 2007. Amino acids in oviduct and uterine fluid and blood plasma during the estrous cycle in the bovine. *Mol Reprod Dev,* 74**,** 445-54.

HUGENTOBLER, S. A., HUMPHERSON, P. G., LEESE, H. J., SREENAN, J. M. & MORRIS, D. G. 2008. Energy substrates in bovine oviduct and uterine fluid and blood plasma during the oestrous cycle. *Mol Reprod Dev,* 75**,** 496-503.

HUNTER, R. H. 2012a. Components of oviduct physiology in eutherian mammals. *Biol Rev Camb Philos Soc,* 87**,** 244-55.

HUNTER, R. H. 2012b. Temperature gradients in female reproductive tissues. *Reprod Biomed Online,* 24**,** 377-80.

HUSSAIN, M. M. 2014. Intestinal lipid absorption and lipoprotein formation. *Curr Opin Lipidol,* 25**,** 200-6.

IBANEZ, E., ALBERTINI, D. F. & OVERSTROM, E. W. 2005. Effect of genetic background and activating stimulus on the timing of meiotic cell cycle progression in parthenogenetically activated mouse oocytes. *Reproduction,* 129**,** 27-38.

ICKOWICZ, D., FINKELSTEIN, M. & BREITBART, H. 2012. Mechanism of sperm capacitation and the acrosome reaction: role of protein kinases. *Asian J Androl,* 14**,** 816-21.

IGOSHEVA, N., ABRAMOV, A. Y., POSTON, L., ECKERT, J. J., FLEMING, T. P., DUCHEN, M. R. & MCCONNELL, J. 2010. Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. *PLoS One,* 5**,** e10074.

INBAR-FEIGENBERG, M., CHOUFANI, S., BUTCHER, D. T., ROIFMAN, M. & WEKSBERG, R. 2013. Basic concepts of epigenetics. *Fertil Steril,* 99**,** 607-15.

JAIN, A., POLOTSKY, A. J., ROCHESTER, D., BERGA, S. L., LOUCKS, T., ZEITLIAN, G., GIBBS, K., POLOTSKY, H. N., FENG, S., ISAAC, B. & SANTORO, N. 2007. Pulsatile luteinizing hormone amplitude and progesterone metabolite excretion are reduced in obese women. *J Clin Endocrinol Metab,* 92**,** 2468-73.

JENSEN, T. K., SCHEIKE, T., KEIDING, N., SCHAUMBURG, I. & GRANDJEAN, P. 1999. Fecundability in relation to body mass and menstrual cycle patterns. *Epidemiology,* 10**,** 422-8.

JEPPESEN, D. K., FENIX, A. M., FRANKLIN, J. L., HIGGINBOTHAM, J. N., ZHANG, Q., ZIMMERMAN, L. J., LIEBLER, D. C., PING, J., LIU, Q., EVANS, R., FISSELL, W. H., PATTON, J. G., ROME, L. H., BURNETTE, D. T. & COFFEY, R. J. 2019. Reassessment of Exosome Composition. *Cell,* 177**,** 428-445 e18.

JORDAENS, L., VAN HOECK, V., DE BIE, J., BERTH, M., MAREI, W. F. A., DESMET, K. L. J., BOLS, P. E. J. & LEROY, J. 2017a. Non-esterified fatty acids in early luteal bovine oviduct fluid mirror plasma concentrations: An ex vivo approach. *Reprod Biol,* 17**,** 281-284.

JORDAENS, L., VAN HOECK, V., MAILLO, V., GUTIERREZ-ADAN, A., MAREI, W. F. A., VLAEMINCK, B., THYS, S., STURMEY, R. G., BOLS, P. E. J. & LEROY, J. 2017b. Maternal metabolic stress may affect oviduct gatekeeper function. *Reproduction,* 153**,** 759-773.

JORDAENS, L., VAN HOECK, V., PINTELON, I., THYS, S., BOLS, P. E. J., MAREI, W. F. A. & LEROY, J. 2020. Altered embryotrophic capacities of the bovine oviduct under

elevated free fatty acid conditions: an in vitro embryo--oviduct co-culture model. *Reprod Fertil Dev,* 32**,** 553-563.

JUNGHEIM, E. S., LOUDEN, E. D., CHI, M. M. Y., FROLOVA, A. I., RILEY, J. K. & MOLEY, K. H. 2011a. Preimplantation Exposure of Mouse Embryos to Palmitic Acid Results in Fetal Growth Restriction Followed by Catch-Up Growth in the Offspring. *Biology of Reproduction,* 85**,** 678-683.

JUNGHEIM, E. S., MACONES, G. A., ODEM, R. R., PATTERSON, B. W., LANZENDORF, S. E., RATTS, V. S. & MOLEY, K. H. 2011b. Associations between free fatty acids, cumulus oocyte complex morphology and ovarian function during in vitro fertilization. *Fertil Steril,* 95**,** 1970-4.

JUNGHEIM, E. S. & MOLEY, K. H. 2010. Current knowledge of obesity's effects in the pre- and periconceptional periods and avenues for future research. *Am J Obstet Gynecol,* 203**,** 525-30.

JUNGHEIM, E. S., SCHOELLER, E. L., MARQUARD, K. L., LOUDEN, E. D., SCHAFFER, J. E. & MOLEY, K. H. 2010. Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. *Endocrinology,* 151**,** 4039-46.

JUNGHEIM, E. S., TRAVIESO, J. L., CARSON, K. R. & MOLEY, K. H. 2012. Obesity and reproductive function. *Obstet Gynecol Clin North Am,* 39**,** 479-93.

KALETA, E. 1977. Influence of genetic factors on the fertilization of mouse ova in vitro. *J Reprod Fertil,* 51**,** 375-81.

KARPE, F., DICKMANN, J. R. & FRAYN, K. N. 2011. Fatty acids, obesity, and insulin resistance: time for a reevaluation. *Diabetes,* 60**,** 2441-9.

KARR, S. 2017. Epidemiology and management of hyperlipidemia. *Am J Manag Care,* 23**,** S139-S148.

KATARI, S., TURAN, N., BIBIKOVA, M., ERINLE, O., CHALIAN, R., FOSTER, M., GAUGHAN, J. P., COUTIFARIS, C. & SAPIENZA, C. 2009. DNA methylation and gene expression differences in children conceived in vitro or in vivo. *Hum Mol Genet,* 18**,** 3769-78.

KATZ, P., NACHTIGALL, R. & SHOWSTACK, J. 2002. The economic impact of the assisted reproductive technologies. *Nat Cell Biol,* 4 Suppl**,** s29-32.

KAUFFMAN, A. S. 2022. Neuroendocrine mechanisms underlying estrogen positive feedback and the LH surge. *Front Neurosci,* 16**,** 953252.

KAWASAKI, N., ASADA, R., SAITO, A., KANEMOTO, S. & IMAIZUMI, K. 2012. Obesity-induced endoplasmic reticulum stress causes chronic inflammation in adipose tissue. *Sci Rep,* 2**,** 799.

KAZEMI, M., JARRETT, B. Y., VANDEN BRINK, H., LIN, A. W., HOEGER, K. M., SPANDORFER, S. D. & LUJAN, M. E. 2020. Obesity, Insulin Resistance, and Hyperandrogenism Mediate the Link between Poor Diet Quality and Ovarian Dysmorphology in Reproductive-Aged Women. *Nutrients,* 12.

KEHRER, J. P. & LUND, L. G. 1994. Cellular Reducing Equivalents and Oxidative Stress. *Free Radical Biology and Medicine,* 17**,** 65-75.

KIM, K. M., ABDELMOHSEN, K., MUSTAPIC, M., KAPOGIANNIS, D. & GOROSPE, M. 2017. RNA in extracellular vesicles. *Wiley Interdiscip Rev RNA,* 8.

KIM, Y. J., LEE, J. E., KIM, S. H., SHIM, S. S. & CHA, D. H. 2013. Maternal age-specific rates of fetal chromosomal abnormalities in Korean pregnant women of advanced maternal age. *Obstet Gynecol Sci,* 56**,** 160-6.

KIRILLOVA, A., SMITZ, J. E. J., SUKHIKH, G. T. & MAZUNIN, I. 2021. The Role of Mitochondria in Oocyte Maturation. *Cells,* 10.

KIRKMAN-BROWN, J. C. & SMITH, D. J. 2011. Sperm motility: is viscosity fundamental to progress? *Mol Hum Reprod,* 17**,** 539-44.

KLEEMANN, R., VAN ERK, M., VERSCHUREN, L., VAN DEN HOEK, A. M., KOEK, M., WIELINGA, P. Y., JIE, A., PELLIS, L., BOBELDIJK-PASTOROVA, I., KELDER, T., TOET, K., WOPEREIS, S., CNUBBEN, N., EVELO, C., VAN OMMEN, B. & KOOISTRA, T. 2010. Time-resolved and tissue-specific systems analysis of the pathogenesis of insulin resistance. *PLoS One,* 5**,** e8817.

KLEIJKERS, S. H., VAN MONTFOORT, A. P., SMITS, L. J., VIECHTBAUER, W., ROSEBOOM, T. J., NELISSEN, E. C., COONEN, E., DERHAAG, J. G., BASTINGS, L., SCHREURS, I. E., EVERS, J. L. & DUMOULIN, J. C. 2014. IVF culture medium affects post-natal weight in humans during the first 2 years of life. *Hum Reprod,* 29**,** 661-9.

KLOP, B., ELTE, J. W. & CABEZAS, M. C. 2013. Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients,* 5**,** 1218-40.

KLOP, B., JUKEMA, J. W., RABELINK, T. J. & CABEZAS, M. C. 2012. A physician's guide for the management of hypertriglyceridemia: the etiology of hypertriglyceridemia determines treatment strategy. *Panminerva Medica,* 54**,** 91-103.

KOLLE, S. 2022. Sperm-oviduct interactions: Key factors for sperm survival and maintenance of sperm fertilizing capacity. *Andrology,* 10**,** 837-843.

KOLLE, S., DUBIELZIG, S., REESE, S., WEHREND, A., KONIG, P. & KUMMER, W. 2009. Ciliary transport, gamete interaction, and effects of the early embryo in the oviduct: ex vivo analyses using a new digital videomicroscopic system in the cow. *Biol Reprod,* 81**,** 267-74.

KOVACIC, P., POZOS, R. S., SOMANATHAN, R., SHANGARI, N. & O'BRIEN, P. J. 2005. Mechanism of mitochondrial uncouplers, inhibitors, and toxins: focus on electron transfer, free radicals, and structure-activity relationships. *Curr Med Chem,* 12**,** 2601-23.

KRISHER, R. L. 2004. The effect of oocyte quality on development. *J Anim Sci,* 82 E-Suppl**,** E14-23.

LAMBERT, G., SJOUKE, B., CHOQUE, B., KASTELEIN, J. J. & HOVINGH, G. K. 2012. The PCSK9 decade. *J Lipid Res,* 53**,** 2515-24.

LAMY, J., NOGUES, P., COMBES-SOIA, L., TSIKIS, G., LABAS, V., MERMILLOD, P., DRUART, X. & SAINT-DIZIER, M. 2018. Identification by proteomics of oviductal sperm-interacting proteins. *Reproduction,* 155**,** 457-466.

LAN, Y. L., LOU, J. C., LYU, W. & ZHANG, B. 2019. Update on the synergistic effect of HSL and insulin in the treatment of metabolic disorders. *Ther Adv Endocrinol Metab,* 10**,** 2042018819877300.

LASH, M. M. & ARMSTRONG, A. 2009. Impact of obesity on women's health. *Fertil Steril,* 91**,** 1712-6.

LASHEN, H., FEAR, K. & STURDEE, D. W. 2004. Obesity is associated with increased risk of first trimester and recurrent miscarriage: matched case-control study. *Human Reproduction,* 19**,** 1644-1646.

LATHAM, K. E. & SCHULTZ, R. M. 2001. Embryonic genome activation. *Front Biosci,* 6**,** D748-59.

LAZARAVICIUTE, G., KAUSER, M., BHATTACHARYA, S., HAGGARTY, P. & BHATTACHARYA, S. 2014. A systematic review and meta-analysis of DNA methylation levels and imprinting disorders in children conceived by IVF/ICSI compared with children conceived spontaneously. *Hum Reprod Update,* 20**,** 840-52.

LEAL, C. L. V., CANON-BELTRAN, K., CAJAS, Y. N., HAMDI, M., YARYES, A., MILLAN DE LA BLANCA, M. G., BELTRAN-BRENA, P., MAZZARELLA, R., DA SILVEIRA, J. C., GUTIERREZ-ADAN, A., GONZALEZ, E. M. & RIZOS, D. 2022. Extracellular vesicles from oviductal and uterine fluids supplementation in sequential in vitro culture improves bovine embryo quality. *J Anim Sci Biotechnol,* 13**,** 116.

LEARY, C., LEESE, H. J. & STURMEY, R. G. 2015. Human embryos from overweight and obese women display phenotypic and metabolic abnormalities. *Hum Reprod,* 30**,** 122-32.

LEE, K. S., JOO, B. S., NA, Y. J., YOON, M. S., CHOI, O. H. & KIM, W. W. 2000. Relationships between concentrations of tumor necrosis factor-alpha and nitric oxide in follicular fluid and oocyte quality. *J Assist Reprod Genet,* 17**,** 222-8.

LEESE, H. J. 1988. The formation and function of oviduct fluid. *J Reprod Fertil,* 82**,** 843-56.

LEESE, H. J. 2012. Metabolism of the preimplantation embryo: 40 years on. *Reproduction,* 143**,** 417-27.

LEESE, H. J., HUGENTOBLER, S. A., GRAY, S. M., MORRIS, D. G., STURMEY, R. G., WHITEAR, S. L. & SREENAN, J. M. 2008. Female reproductive tract fluids: composition, mechanism of formation and potential role in the developmental origins of health and disease. *Reprod Fertil Dev,* 20**,** 1-8.

LEESE, H. J., STURMEY, R. G., BAUMANN, C. G. & MCEVOY, T. G. 2007. Embryo viability and metabolism: obeying the quiet rules. *Hum Reprod,* 22**,** 3047-50.

LEESE, H. J., TAY, J. I., REISCHL, J. & DOWNING, S. J. 2001. Formation of Fallopian tubal fluid: role of a neglected epithelium. *Reproduction,* 121**,** 339-46.

LEMASTERS, J. J. 2014. Variants of mitochondrial autophagy: Types 1 and 2 mitophagy and micromitophagy (Type 3). *Redox Biol,* 2**,** 749-54.

LEROY, J. L., RIZOS, D., STURMEY, R., BOSSAERT, P., GUTIERREZ-ADAN, A., VAN HOECK, V., VALCKX, S. & BOLS, P. E. 2011. Intrafollicular conditions as a major link between maternal metabolism and oocyte quality: a focus on dairy cow fertility. *Reprod Fertil Dev,* 24**,** 1-12.

LEROY, J. L., STURMEY, R. G., VAN HOECK, V., DE BIE, J., MCKEEGAN, P. J. & BOLS, P. E. 2014. Dietary fat supplementation and the consequences for oocyte and embryo quality: hype or significant benefit for dairy cow reproduction? *Reprod Domest Anim,* 49**,** 353-61.

LEROY, J. L., VANHOLDER, T., MATEUSEN, B., CHRISTOPHE, A., OPSOMER, G., DE KRUIF, A., GENICOT, G. & VAN SOOM, A. 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. *Reproduction,* 130**,** 485-95.

LEROY, J. L. M. R., DE BIE, J., JORDAENS, L., DESMET, K., SMITS, A., MAREI, W. F. A., BOLS, P. E. J. & VAN HOECK, V. 2017. Negative energy balance and metabolic stress in relation to oocyte and embryo quality: an update on possible pathways reducing fertility in dairy cows. *Animal Reproduction,* 14**,** 497-506.

LEROY, J. L. M. R., VAN HOECK, V., CLEMENTE, M., RIZOS, D., GUTIERREZ-ADAN, A., VAN SOOM, A., UYTTERHOEVEN, M. & BOLS, P. E. J. 2010. The effect of nutritionally induced hyperlipidaemia on

bovine embryo quality. *Human Reproduction,* 25**,** 768-778.

LEROY, J. L. M. R., VANHOLDER, T., DELANGHE, J. R., OPSOMER, G., VAN SOOM, A., BOLS, P. E. J., DEWULF, J. & DE KRUIF, A. 2004. Metabolic changes in follicular fluid of the dominant follicle in high-yielding dairy cows early post partum. *Theriogenology,* 62**,** 1131-1143.

LI, S. & WINUTHAYANON, W. 2017. Oviduct: roles in fertilization and early embryo development. *J Endocrinol,* 232**,** R1-R26.

LI, Y., LIU, C., GUO, N., CAI, L., WANG, M., ZHU, L., LI, F., JIN, L. & SUI, C. 2023. Extracellular vesicles from human Fallopian tubal fluid benefit embryo development in vitro. *Hum Reprod Open,* 2023**,** hoad006.

LIAO, S. B., HO, J. C., TANG, F. & O, W. S. 2011. Adrenomedullin increases ciliary beat frequency and decreases muscular contraction in the rat oviduct. *Reproduction,* 141**,** 367-72.

LIN, J., YANG, R., TARR, P. T., WU, P. H., HANDSCHIN, C., LI, S., YANG, W., PEI, L., ULDRY, M., TONTONOZ, P., NEWGARD, C. B. & SPIEGELMAN, B. M. 2005. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. *Cell,* 120**,** 261-73.

LINGAPPAN, K. 2018. NF-kappaB in Oxidative Stress. *Curr Opin Toxicol,* 7**,** 81-86.

LISTENBERGER, L. L., HAN, X., LEWIS, S. E., CASES, S., FARESE, R. V., JR., ORY, D. S. & SCHAFFER, J. E. 2003. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci U S A,* 100**,** 3077-82.

LIU, C., SHAO, M., LU, L., ZHAO, C., QIU, L. & LIU, Z. 2021. Obesity, insulin resistance and their interaction on liver enzymes. *PLoS One,* 16**,** e0249299.

LIU, N., MA, Y., LI, R., JIN, H., LI, M., HUANG, X., FENG, H. L. & QIAO, J. 2012. Comparison of follicular fluid amphiregulin and EGF concentrations in patients undergoing IVF with different stimulation protocols. *Endocrine,* 42**,** 708-16.

LIU, T., ZHANG, L., JOO, D. & SUN, S. C. 2017. NF-kappaB signaling in inflammation. *Signal Transduct Target Ther,* 2**,** 17023-.

LLOYD, R. E., ROMAR, R., MATAS, C., GUTIERREZ-ADAN, A., HOLT, W. V. & COY, P. 2009. Effects of oviductal fluid on the development, quality, and gene expression of porcine blastocysts produced in vitro. *Reproduction,* 137**,** 679-87.

LONDON, E., NESTEROVA, M. & STRATAKIS, C. A. 2017. Acute vs chronic exposure to high fat diet leads to distinct regulation of PKA. *J Mol Endocrinol,* 59**,** 1-12.

LOPERA-VASQUEZ, R., HAMDI, M., FERNANDEZ-FUERTES, B., MAILLO, V., BELTRAN-BRENA, P., CALLE, A., REDRUELLO, A., LOPEZ-MARTIN, S., GUTIERREZ-ADAN, A., YANEZ-MO, M., RAMIREZ, M. A. & RIZOS, D. 2016. Extracellular Vesicles from BOEC in In Vitro Embryo Development and Quality. *PLoS One,* 11**,** e0148083.

LOPERA-VASQUEZ, R., HAMDI, M., MAILLO, V., GUTIERREZ-ADAN, A., BERMEJO-ALVAREZ, P., RAMIREZ, M. A., YANEZ-MO, M. & RIZOS, D. 2017. Effect of bovine oviductal extracellular vesicles on embryo development and quality in vitro. *Reproduction,* 153**,** 461-470.

LOY, S. L., CHEUNG, Y. B., SOH, S. E., NG, S., TINT, M. T., ARIS, I. M., BERNARD, J. Y., CHONG, Y. S., GODFREY, K. M., SHEK, L. P., TAN, K. H., LEE, Y. S., TAN, H. H., CHERN, B. S. M., LEK, N., YAP, F., CHAN, S. Y., CHI, C. & CHAN, J. K. Y. 2018. Female adiposity and time-to-pregnancy: a multiethnic prospective cohort. *Hum Reprod,* 33**,** 2141-2149.

LUCAS, E. 2013. Epigenetic effects on the embryo as a result of periconceptional environment and assisted reproduction technology. *Reprod Biomed Online,* 27**,** 477-85.

LUKE, B., BROWN, M. B., STERN, J. E., MISSMER, S. A., FUJIMOTO, V. Y., LEACH, R. & GROUP, S. W. 2011. Female obesity adversely affects assisted reproductive technology (ART) pregnancy and live birth rates. *Hum Reprod,* 26**,** 245-52.

LUZZO, K. M., WANG, Q., PURCELL, S. H., CHI, M., JIMENEZ, P. T., GRINDLER, N., SCHEDL, T. & MOLEY, K. H. 2012. High fat diet induced developmental defects in the mouse: oocyte meiotic aneuploidy and fetal growth retardation/brain defects. *PLoS One,* 7**,** e49217.

MACAULAY, A. D., GILBERT, I., SCANTLAND, S., FOURNIER, E., ASHKAR, F., BASTIEN, A., SAADI, H. A., GAGNE, D., SIRARD, M. A., KHANDJIAN, E. W., RICHARD, F. J., HYTTEL, P. & ROBERT, C. 2016. Cumulus Cell Transcripts Transit to the Bovine Oocyte in Preparation for Maturation. *Biol Reprod,* 94**,** 16.

MACHTINGER, R., COMBELLES, C. M., MISSMER, S. A., CORREIA, K. F., FOX, J. H. & RACOWSKY, C. 2012. The association between severe obesity and characteristics of failed fertilized oocytes. *Hum Reprod,* 27**,** 3198-207.

MAHUTTE, N., KAMGA-NGANDE, C., SHARMA, A. & SYLVESTRE, C. 2018. Obesity and Reproduction. *J Obstet Gynaecol Can,* 40**,** 950-966.

MAIER, C. M. & CHAN, P. H. 2002. Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. *Neuroscientist,* 8**,** 323-34.

MAILLO, V., GAORA, P. O., FORDE, N., BESENFELDER, U., HAVLICEK, V., BURNS, G. W., SPENCER, T. E., GUTIERREZ-ADAN, A., LONERGAN, P. & RIZOS, D. 2015. Oviduct-Embryo Interactions in Cattle: Two-Way Traffic or a One-Way Street? *Biol Reprod,* 92**,** 144.

MAILLO, V., RIZOS, D., BESENFELDER, U., HAVLICEK, V., KELLY, A. K., GARRETT, M. & LONERGAN, P. 2012. Influence of lactation on metabolic characteristics and embryo development in postpartum Holstein dairy cows. *J Dairy Sci,* 95**,** 3865-76.

MAILLO, V., SANCHEZ-CALABUIG, M. J., LOPERA-VASQUEZ, R., HAMDI, M., GUTIERREZ-ADAN, A., LONERGAN, P. & RIZOS, D. 2016a. Oviductal response to gametes and early embryos in mammals. *Reproduction,* 152**,** R127-41.

MAILLO, V., SÁNCHEZ-CALABUIG, M. J., LOPERA-VASQUEZ, R., HAMDI, M., GUTIERREZ-ADAN, A., LONERGAN, P. & RIZOS, D. 2016b. Oviductal response to gametes and early embryos in mammals. *Reproduction,* 152**,** R127-R141.

MANI, S., GHOSH, J., COUTIFARIS, C., SAPIENZA, C. & MAINIGI, M. 2020. Epigenetic changes and assisted reproductive technologies. *Epigenetics,* 15**,** 12-25.

MANNAVOLA, F., D'ORONZO, S., CIVES, M., STUCCI, L. S., RANIERI, G., SILVESTRIS, F. & TUCCI, M. 2019. Extracellular Vesicles and Epigenetic Modifications Are Hallmarks of Melanoma Progression. *Int J Mol Sci,* 21.

MAREI, W. F. A., ALVAREZ, M. A., VAN HOECK, V., GUTIERREZ-ADAN, A., BOLS, P. E. J. & LEROY, J. 2017. Effect of nutritionally induced hyperlipidaemia on in vitro bovine embryo quality depends on the type of major fatty acid in the diet. *Reprod Fertil Dev,* 29**,** 1856-1867.

MAREI, W. F. A. & LEROY, J. 2022. Cellular Stress Responses in Oocytes: Molecular Changes and Clinical Implications. *Adv Exp Med Biol,* 1387**,** 171-189.

MAREI, W. F. A., SMITS, A., MOHEY-ELSAEED, O., PINTELON, I., GINNEBERGE, D., BOLS, P. E. J., MOERLOOSE, K. & LEROY, J. 2020. Differential effects of high fat diet-induced obesity on oocyte mitochondrial functions in inbred and outbred mice. *Sci Rep,* 10**,** 9806.

MAREI, W. F. A., VAN DEN BOSCH, L., PINTELON, I., MOHEY-ELSAEED, O., BOLS, P. E. J. & LEROY, J. 2019. Mitochondria-targeted therapy rescues development and quality of embryos derived from oocytes matured under oxidative stress conditions: a bovine in vitro model. *Hum Reprod,* 34**,** 1984-1998.

MAREY, M. A., YOUSEF, M. S., KOWSAR, R., HAMBRUCH, N., SHIMIZU, T., PFARRER, C. & MIYAMOTO, A. 2016. Local immune system in oviduct physiology and pathophysiology: attack or tolerance? *Domest Anim Endocrinol,* 56 Suppl**,** S204-11.

MARQUARD, K. L., STEPHENS, S. M., JUNGHEIM, E. S., RATTS, V. S., ODEM, R. R., LANZENDORF, S. & MOLEY, K. H. 2011. Polycystic ovary syndrome and maternal obesity affect oocyte size in in vitro fertilization/intracytoplasmic sperm injection cycles. *Fertil Steril,* 95**,** 2146-9, 2149 e1.

MATHIEU, M., MARTIN-JAULAR, L., LAVIEU, G. & THERY, C. 2019. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat Cell Biol,* 21**,** 9-17.

MATOBA, S., O'HARA, L., CARTER, F., KELLY, A. K., FAIR, T., RIZOS, D. & LONERGAN, P. 2012. The association between metabolic parameters and oocyte quality early and late postpartum in Holstein dairy cows. *J Dairy Sci,* 95**,** 1257-66.

MAULUCCI, G., COHEN, O., DANIEL, B., SANSONE, A., PETROPOULOU, P. I., FILOU, S., SPYRIDONIDIS, A., PANI, G., DE SPIRITO, M., CHATGILIALOGLU, C., FERRERI, C., KYPREOS, K. E. & SASSON, S. 2016. Fatty acid-related modulations of membrane fluidity in cells: detection and implications. *Free Radic Res,* 50**,** S40-S50.

MAZZARELLA, R., BASTOS, N. M., BRIDI, A., DEL COLLADO, M., ANDRADE, G. M., PINZON, J., PRADO, C. M., SILVA, L. A., MEIRELLES, F. V., PUGLIESI, G., PERECIN, F. & DA SILVEIRA, J. C. 2021. Changes in Oviductal Cells and Small Extracellular Vesicles miRNAs in Pregnant Cows. *Front Vet Sci,* 8**,** 639752.

MCKEEGAN, P. J. & STURMEY, R. G. 2012. The role of fatty acids in oocyte and early embryo development. *Reproduction Fertility and Development,* 24**,** 59-67.

MCQUAID, S. E., HODSON, L., NEVILLE, M. J., DENNIS, A. L., CHEESEMAN, J., HUMPHREYS, S. M., RUGE, T., GILBERT, M., FIELDING, B. A., FRAYN, K. N. & KARPE, F. 2011. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition? *Diabetes,* 60**,** 47-55.

MENEZO, Y. J., SILVESTRIS, E., DALE, B. & ELDER, K. 2016. Oxidative stress and alterations in DNA methylation: two sides of the same coin in reproduction. *Reprod Biomed Online,* 33**,** 668-683.

MEULDERS, B., LEROY, J., XHONNEUX, I., BOLS, P. E. J. & MAREI, W. F. A. 2024. In vitro reduction of bovine oocyte ATP production with oligomycin affects embryo epigenome. *Reproduction,* 167.

MEULDERS, B., MAREI, W. F. A., XHONNEUX, I., BOLS, P. E. J. & LEROY, J. 2023. Effect of lipotoxicity on mitochondrial function and epigenetic programming during bovine in vitro embryo production. *Sci Rep,* 13**,** 21664.

MICHALAKIS, K., MINTZIORI, G., KAPRARA, A., TARLATZIS, B. C. & GOULIS, D. G. 2013. The complex interaction between obesity, metabolic syndrome and reproductive axis: a narrative review. *Metabolism,* 62**,** 457-78.

MINGE, C. E., BENNETT, B. D., NORMAN, R. J. & ROBKER, R. L. 2008. Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reverses the adverse effects of diet-induced obesity on oocyte quality. *Endocrinology,* 149**,** 2646-56.

MITCHELL, M., ARMSTRONG, D. T., ROBKER, R. L. & NORMAN, R. J. 2005. Adipokines: implications for female fertility and obesity. *Reproduction,* 130**,** 583-97.

MOLA, J. R. L. D. 2009. Obesity and its relationship to infertility in men and women. *Obstet Gynecol Clin North Am,* 36**,** 333-46.

MORENO, J. M., NUNEZ, M. J., QUINONERO, A., MARTINEZ, S., DE LA ORDEN, M., SIMON, C., PELLICER, A., DIAZ-GARCIA, C. & DOMINGUEZ, F. 2015. Follicular fluid and mural granulosa cells microRNA profiles vary in in vitro fertilization patients depending on their age and oocyte maturation stage. *Fertil Steril,* 104**,** 1037-1046 e1.

MORIGNY, P., HOUSSIER, M., MOUISEL, E. & LANGIN, D. 2016. Adipocyte lipolysis and insulin resistance. *Biochimie,* 125**,** 259-66.

MULLER, B., LEWIS, N., ADENIYI, T., LEESE, H. J., BRISON, D. R. & STURMEY, R. G. 2019. Application of extracellular flux analysis for determining mitochondrial function in mammalian oocytes and early embryos. *Sci Rep,* 9**,** 16778.

NAKATSUKASA, K., HUYER, G., MICHAELIS, S. & BRODSKY, J. L. 2008. Dissecting the ER-associated degradation of a misfolded polytopic membrane protein. *Cell,* 132**,** 101-12.

NARGUND, A. M., PELLEGRINO, M. W., FIORESE, C. J., BAKER, B. M. & HAYNES, C. M. 2012. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. *Science,* 337**,** 587-90.

NICHOLSON, A., REIFSNYDER, P. C., MALCOLM, R. D., LUCAS, C. A., MACGREGOR, G. R., ZHANG, W. & LEITER, E. H. 2010. Diet-induced obesity in two C57BL/6 substrains with intact or mutant nicotinamide nucleotide transhydrogenase (Nnt) gene. *Obesity (Silver Spring),* 18**,** 1902-5.

NIEMITZ, E. L. & FEINBERG, A. P. 2004. Epigenetics and assisted reproductive technology: a call for investigation. *Am J Hum Genet,* 74**,** 599-609.

NTEEBA, J., ORTINAU, L. C., PERFIELD, J. W., 2ND & KEATING, A. F. 2013. Diet-induced obesity alters immune cell infiltration and expression of inflammatory cytokine genes in mouse ovarian and peri-ovarian adipose depot tissues. *Mol Reprod Dev,* 80**,** 948-58.

OESTREICH, A. K. & MOLEY, K. H. 2017. Developmental and Transmittable Origins of Obesity-Associated Health Disorders. *Trends Genet,* 33**,** 399-407.

OHTSU, A., TANAKA, H., SENO, K., IWATA, H., KUWAYAMA, T. & SHIRASUNA, K. 2017. Palmitic acid stimulates interleukin-8 via the TLR4/NF-kappaB/ROS pathway and induces mitochondrial dysfunction in bovine oviduct epithelial cells. *Am J Reprod Immunol,* 77.

OMBELET, W. 2014. Is global access to infertility care realistic? The Walking Egg Project. *Reprod Biomed Online,* 28**,** 267-72.

ORIHUELA, P. A., ZUNIGA, L. M., RIOS, M., PARADA-BUSTAMANTE, A., SIERRALTA, W. D., VELASQUEZ, L. A. & CROXATTO, H. B. 2009. Mating changes the subcellular distribution and the functionality of estrogen receptors in the rat oviduct. *Reprod Biol Endocrinol,* 7**,** 139.

PAGLIASSOTTI, M. J., KIM, P. Y., ESTRADA, A. L., STEWART, C. M. & GENTILE, C. L. 2016. Endoplasmic reticulum stress in obesity and obesity-related disorders: An expanded view. *Metabolism,* 65**,** 1238-46.

PALERMO, G. D., NERI, Q. V., COZZUBBO, T. & ROSENWAKS, Z. 2014. Perspectives on the assessment of human sperm chromatin integrity. *Fertil Steril,* 102**,** 1508-17.

PANCHAL, S. K. & BROWN, L. 2011. Rodent models for metabolic syndrome research. *J Biomed Biotechnol,* 2011**,** 351982.

PANTASRI, T., WU, L. L., HULL, M. L., SULLIVAN, T. R., BARRY, M., NORMAN, R. J. & ROBKER, R. L. 2015. Distinct localisation of lipids in the ovarian follicular environment. *Reprod Fertil Dev,* 27**,** 593-601.

PARISI, F., ROUSIAN, M., STEEGERS-THEUNISSEN, R. P. M., KONING, A. H. J., WILLEMSEN, S. P., DE VRIES, J. H. M., CETIN, I. & STEEGERS, E. A. P. 2018. Early first trimester maternal 'high fish and olive oil and low meat' dietary pattern is associated with accelerated human embryonic development. *Eur J Clin Nutr,* 72**,** 1655-1662.

PAVANI, K. C., MEESE, T., PASCOTTINI, O. B., GUAN, X., LIN, X., PEELMAN, L., HAMACHER, J., VAN NIEUWERBURGH, F., DEFORCE, D., BOEL, A., HEINDRYCKX, B., TILLEMAN, K., VAN SOOM, A., GADELLA, B. M., HENDRIX, A. & SMITS, K. 2022. Hatching is modulated by microRNA-378a-3p derived from extracellular vesicles secreted by blastocysts. *Proc Natl Acad Sci U S A,* 119**,** e2122708119.

PEERAER, K., D'HOOGHE, T. M., VANDOREN, C., TRYBOU, J., SPIESSENS, C., DEBROCK, S. & DE NEUBOURG, D. 2017. A 50% reduction in multiple live birth rate is associated with a 13% cost saving: a real-life retrospective cost analysis. *Reprod Biomed Online,* 35**,** 279-286.

PERONA, J. S., VOGLER, O., SANCHEZ-DOMINGUEZ, J. M., MONTERO, E., ESCRIBA, P. V. & RUIZ-GUTIERREZ, V. 2007. Consumption of virgin olive oil influences membrane lipid composition and regulates intracellular signaling in elderly adults with type 2 diabetes mellitus. *J Gerontol A Biol Sci Med Sci,* 62**,** 256-63.

PISARSKA, M. D., CHAN, J. L., LAWRENSON, K., GONZALEZ, T. L. & WANG, E. T. 2019. Genetics and Epigenetics of Infertility and Treatments on Outcomes. *J Clin Endocrinol Metab,* 104**,** 1871-1886.

PORETSKY, L., CATALDO, N. A., ROSENWAKS, Z. & GIUDICE, L. C. 1999. The insulin-related ovarian regulatory system in health and disease. *Endocr Rev,* 20**,** 535-82.

PRIETO, L., QUESADA, J. F., CAMBERO, O., PACHECO, A., PELLICER, A., CODOCEO, R. & GARCIA-VELASCO, J. A. 2012. Analysis of follicular fluid and serum markers of oxidative stress in women with infertility related to endometriosis. *Fertil Steril,* 98**,** 126-30.

PURCELL, S. H. & MOLEY, K. H. 2011. The impact of obesity on egg quality. *J Assist Reprod Genet,* 28**,** 517-24.

PURNELL, J. Q. 2000. Definitions, Classification, and Epidemiology of Obesity. *In:* FEINGOLD, K. R., ANAWALT, B., BLACKMAN, M. R., BOYCE, A., CHROUSOS, G., CORPAS, E., DE HERDER, W. W., DHATARIYA, K., DUNGAN, K., HOFLAND, J., KALRA, S., KALTSAS, G., KAPOOR, N., KOCH, C., KOPP, P., KORBONITS, M., KOVACS, C. S., KUOHUNG, W., LAFERRERE, B., LEVY, M., MCGEE, E. A., MCLACHLAN, R., NEW, M., PURNELL, J., SAHAY, R., SHAH, A. S., SINGER, F., SPERLING, M. A., STRATAKIS, C. A., TRENCE, D. L. & WILSON, D. P. (eds.) *Endotext.* South Dartmouth (MA).

RENAVILLE, B., BACCIU, N., COMIN, A., MOTTA, M., POLI, I., VANINI, G. & PRANDI, A. 2010. Plasma and follicular fluid fatty acid profiles in dairy cows. *Reprod Domest Anim,* 45**,** 118-21.

REUTER, T. Y. 2007. Diet-induced models for obesity and type 2 diabetes. *Drug Discovery Today: Disease Models,* 4**,** 3-8.

REVELLI, A., DELLE PIANE, L., CASANO, S., MOLINARI, E., MASSOBRIO, M. & RINAUDO, P. 2009. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. *Reprod Biol Endocrinol,* 7**,** 40.

REYNIER, P., MAY-PANLOUP, P., CHRETIEN, M. F., MORGAN, C. J., JEAN, M., SAVAGNER, F., BARRIERE, P. & MALTHIERY, Y. 2001. Mitochondrial DNA content affects the fertilizability of human oocytes. *Mol Hum Reprod,* 7**,** 425-9.

REYNOLDS, K. A., BOUDOURES, A. L., CHI, M. M., WANG, Q. & MOLEY, K. H. 2015. Adverse effects of obesity and/or high-fat diet on oocyte quality and metabolism are not reversible with resumption of regular diet in mice. *Reprod Fertil Dev,* 27**,** 716-24.

RHEE, S. G., WOO, H. A., KIL, I. S. & BAE, S. H. 2012. Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides. *J Biol Chem,* 287**,** 4403-10.

RICCHI, M., ODOARDI, M. R., CARULLI, L., ANZIVINO, C., BALLESTRI, S., PINETTI, A., FANTONI, L. I., MARRA, F., BERTOLOTTI, M., BANNI, S., LONARDO, A., CARULLI, N. & LORIA, P. 2009. Differential effect of oleic and palmitic acid on lipid accumulation and apoptosis in cultured hepatocytes. *J Gastroenterol Hepatol,* 24**,** 830-40.

RICHANI, D., DUNNING, K. R., THOMPSON, J. G. & GILCHRIST, R. B. 2021. Metabolic co-dependence of the oocyte and cumulus cells: essential role in determining oocyte developmental competence. *Hum Reprod Update,* 27**,** 27-47.

RIEGER, D. & LOSKUTOFF, N. M. 1994. Changes in the metabolism of glucose, pyruvate, glutamine and glycine during maturation of cattle oocytes in vitro. *J Reprod Fertil,* 100**,** 257-62.

RIVERA, R. M. & ROSS, J. W. 2013. Epigenetics in fertilization and preimplantation embryo development. *Prog Biophys Mol Biol,* 113**,** 423-32.

RIZOS, D., CARTER, F., BESENFELDER, U., HAVLICEK, V. & LONERGAN, P. 2010a. Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows. *J Dairy Sci,* 93**,** 1022-9.

RIZOS, D., CLEMENTE, M., BERMEJO-ALVAREZ, P., DE LA FUENTE, J., LONERGAN, P. & GUTIERREZ-ADAN, A. 2008. Consequences of in vitro culture conditions on embryo development and quality. *Reprod Domest Anim,* 43 Suppl 4**,** 44-50.

RIZOS, D., RAMIREZ, M. A., PINTADO, B., LONERGAN, P. & GUTIERREZ-ADAN, A. 2010b. Culture of bovine embryos in intermediate host oviducts with emphasis on the isolated mouse oviduct. *Theriogenology,* 73**,** 777-85.

RIZOS, D., WARD, F., DUFFY, P., BOLAND, M. P. & LONERGAN, P. 2002. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. *Mol Reprod Dev,* 61**,** 234-48.

ROBERTSON, S. A., CHIN, P. Y., FEMIA, J. G. & BROWN, H. M. 2018. Embryotoxic cytokines-Potential roles in embryo loss and fetal programming. *J Reprod Immunol,* 125**,** 80-88.

ROBKER, R. L. 2008. Evidence that obesity alters the quality of oocytes and embryos. . *Pathophysiology,* 15**,** 115-121.

ROBKER, R. L., AKISON, L. K., BENNETT, B. D., THRUPP, P. N., CHURA, L. R., RUSSELL, D. L., LANE, M. & NORMAN, R. J. 2009a. Obese women exhibit differences in ovarian metabolites, hormones, and gene expression compared with moderate-weight women. *J Clin Endocrinol Metab,* 94**,** 1533-40.

ROBKER, R. L., AKISON, L. K., BENNETT, B. D., THRUPP, P. N., CHURA, L. R., RUSSELL, D. L., LANE, M. & NORMAN, R. J. 2009b. Obese Women Exhibit Differences in Ovarian Metabolites, Hormones, and Gene Expression Compared with Moderate-Weight Women. *Journal of Clinical Endocrinology & Metabolism,* 94**,** 1533-1540.

ROBKER, R. L., WU, L. L. & YANG, X. 2011. Inflammatory pathways linking obesity and ovarian dysfunction. *J Reprod Immunol,* 88**,** 142-8.

RODGERS, R. J. & IRVING-RODGERS, H. F. 2010. Formation of the ovarian follicular antrum and follicular fluid. *Biol Reprod,* 82**,** 1021-9.

RODRIGUEZ-ALONSO, B., HAMDI, M., SANCHEZ, J. M., MAILLO, V., GUTIERREZ-ADAN, A., LONERGAN, P. & RIZOS, D. 2019. An approach to study the local embryo effect on gene expression in the bovine oviduct epithelium in vivo. *Reprod Domest Anim,* 54**,** 1516-1523.

RODRIGUEZ-MARTINEZ, H. 2007. Role of the oviduct in sperm capacitation. *Theriogenology,* 68 Suppl 1**,** S138-46.

ROMERO, L. O., MASSEY, A. E., MATA-DABOIN, A. D., SIERRA-VALDEZ, F. J., CHAUHAN, S. C., CORDERO-MORALES, J. F. & VASQUEZ, V. 2019. Dietary fatty acids fine-tune Piezo1 mechanical response. *Nat Commun,* 10**,** 1200.

RON, D. & WALTER, P. 2007. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol,* 8**,** 519-29.

RUEBEL, M., SHANKAR, K., GADDY, D., LINDSEY, F., BADGER, T. & ANDRES, A. 2016. Maternal obesity is associated with ovarian inflammation and upregulation of early growth response factor 1. *Am J Physiol Endocrinol Metab,* 311**,** E269-77.

RUEBEL, M. L., COTTER, M., SIMS, C. R., MOUTOS, D. M., BADGER, T. M., CLEVES, M. A., SHANKAR, K. & ANDRES, A. 2017. Obesity Modulates Inflammation and Lipid Metabolism Oocyte Gene Expression: A Single-Cell Transcriptome Perspective. *J Clin Endocrinol Metab,* 102**,** 2029-2038.

RUNKEL, E. D., BAUMEISTER, R. & SCHULZE, E. 2014. Mitochondrial stress: balancing friend and foe. *Exp Gerontol,* 56**,** 194-201.

RUTKOWSKI, D. T. & KAUFMAN, R. J. 2004. A trip to the ER: coping with stress. *Trends Cell Biol,* 14**,** 20-8.

SAADELDIN, I. M., OH, H. J. & LEE, B. C. 2015. Embryonic-maternal cross-talk via exosomes: potential implications. *Stem Cells Cloning,* 8**,** 103-7.

SADLER, T. 2009. Gametogenesis: conversion of germ cells into male and female gametes. . *Langman's medical embryology,* 11 ed.**,** 27-30.

SADOWSKA, J., DUDZINSKA, W., SKOTNICKA, E., SIELATYCKA, K. & DANIEL, I. 2019. The Impact of a Diet Containing Sucrose and Systematically Repeated Starvation on the Oxidative Status of the Uterus and Ovary of Rats. *Nutrients,* 11.

SAINT-DIZIER, M., SCHOEN, J., CHEN, S., BANLIAT, C. & MERMILLOD, P. 2019. Composing the Early Embryonic Microenvironment: Physiology and Regulation of Oviductal Secretions. *Int J Mol Sci,* 21.

SAKLAYEN, M. G. 2018. The Global Epidemic of the Metabolic Syndrome. *Curr Hypertens Rep,* 20**,** 12.

SALZANO, A., DE CANDITIIS, C., DELLA RAGIONE, F., PRANDI, A., ZULLO, G., NEGLIA, G., CAMPANILE, G. & GASPARRINI, B. 2018. Evaluation of factors involved in the failure of ovum capture in superovulated buffaloes. *Theriogenology,* 122**,** 102-108.

SANTOS, T. A., EL SHOURBAGY, S. & ST JOHN, J. C. 2006. Mitochondrial content reflects oocyte variability and fertilization outcome. *Fertil Steril,* 85**,** 584-91.

SCARAMUZZI, R. J., BAIRD, D. T., CAMPBELL, B. K., DRIANCOURT, M. A., DUPONT, J., FORTUNE, J. E., GILCHRIST, R. B., MARTIN, G. B., MCNATTY, K. P., MCNEILLY, A. S., MONGET, P., MONNIAUX, D., VINOLES, C. & WEBB, R. 2011. Regulation of folliculogenesis and the determination of ovulation rate in ruminants. *Reprod Fertil Dev,* 23**,** 444-67.

SCHMALTZ-PANNEAU, B., CORDOVA, A., DHORNE-POLLET, S., HENNEQUET-ANTIER, C., UZBEKOVA, S., MARTINOT, E., DORET, S., MARTIN, P., MERMILLOD, P. & LOCATELLI, Y. 2014. Early bovine embryos regulate oviduct epithelial cell gene expression during in vitro co-culture. *Anim Reprod Sci,* 149**,** 103-16.

SCHNEIDER, J. E. 2004. Energy balance and reproduction. *Physiol Behav,* 81**,** 289-317.

SEIF, M. W., DIAMOND, K. & NICKKHO-AMIRY, M. 2015. Obesity and menstrual disorders. *Best Pract Res Clin Obstet Gynaecol,* 29**,** 516-27.

SHAMSI, M. B., GOVINDARAJ, P., CHAWLA, L., MALHOTRA, N., SINGH, N., MITTAL, S., TALWAR, P., THANGARAJ, K. & DADA, R. 2013. Mitochondrial DNA variations in ova and blastocyst: implications in assisted reproduction. *Mitochondrion,* 13**,** 96-105.

SILVESTRIS, E., DE PERGOLA, G., ROSANIA, R. & LOVERRO, G. 2018. Obesity as disruptor of the female fertility. *Reprod Biol Endocrinol,* 16**,** 22.

SIM, K. A., PARTRIDGE, S. R. & SAINSBURY, A. 2014. Does weight loss in overweight or obese women improve fertility treatment outcomes? A systematic review. *Obes Rev,* 15**,** 839-50.

SKAZNIK-WIKIEL, M. E., SWINDLE, D. C., ALLSHOUSE, A. A., POLOTSKY, A. J. & MCMANAMAN, J. L. 2016. High-Fat Diet Causes Subfertility and Compromised Ovarian Function Independent of Obesity in Mice. *Biol Reprod,* 94**,** 108.

SMALL, L., BRANDON, A. E., TURNER, N. & COONEY, G. J. 2018. Modeling insulin resistance in rodents by alterations in diet: what have high-fat and high-calorie diets revealed? *Am J Physiol Endocrinol Metab,* 314**,** E251-E265.

SMITS, A., MAREI, W. F. A., DE NEUBOURG, D. & LEROY, J. 2021. Diet normalization or caloric restriction as a preconception care strategy to improve metabolic health and oocyte quality in obese outbred mice. *Reprod Biol Endocrinol,* 19**,** 166.

SMITS, K., DE CONINCK, D. I., VAN NIEUWERBURGH, F., GOVAERE, J., VAN POUCKE, M., PEELMAN, L., DEFORCE, D. & VAN SOOM, A. 2016. The Equine Embryo Influences Immune-Related Gene Expression in the Oviduct. *Biol Reprod,* 94**,** 36.

SNIDER, A. P. & WOOD, J. R. 2019. Obesity induces ovarian inflammation and reduces oocyte quality. *Reproduction,* 158**,** R79-R90.

SONG, W. H., BALLARD, J. W., YI, Y. J. & SUTOVSKY, P. 2014. Regulation of mitochondrial genome inheritance by autophagy and ubiquitin-proteasome system: implications for health, fitness, and fertility. *Biomed Res Int,* 2014**,** 981867.

SORDILLO, L. M. & RAPHAEL, W. 2013. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. *Vet Clin North Am Food Anim Pract,* 29**,** 267-78.

STOJKOVIC, M., MACHADO, S. A., STOJKOVIC, P., ZAKHARTCHENKO, V., HUTZLER, P., GONCALVES, P. B. & WOLF, E. 2001. Mitochondrial distribution and adenosine triphosphate content of bovine oocytes before and after in vitro maturation:

correlation with morphological criteria and developmental capacity after in vitro fertilization and culture. *Biol Reprod,* 64**,** 904-9.

STURMEY, R. G., REIS, A., LEESE, H. J. & MCEVOY, T. G. 2009. Role of fatty acids in energy provision during oocyte maturation and early embryo development. *Reprod Domest Anim,* 44 Suppl 3**,** 50-8.

SU, L. J., ZHANG, J. H., GOMEZ, H., MURUGAN, R., HONG, X., XU, D., JIANG, F. & PENG, Z. Y. 2019. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. *Oxid Med Cell Longev,* 2019**,** 5080843.

SUAREZ, S. S. 2008. Regulation of sperm storage and movement in the mammalian oviduct. *Int J Dev Biol,* 52**,** 455-62.

SUGIURA, K., SU, Y. Q., DIAZ, F. J., PANGAS, S. A., SHARMA, S., WIGGLESWORTH, K., O'BRIEN, M. J., MATZUK, M. M., SHIMASAKI, S. & EPPIG, J. J. 2007. Oocyte-derived BMP15 and FGFs cooperate to promote glycolysis in cumulus cells. *Development,* 134**,** 2593-603.

SUN, L., YIN, H., LIU, M., XU, G., ZHOU, X., GE, P., YANG, H. & MAO, Y. 2019. Impaired albumin function: a novel potential indicator for liver function damage? *Ann Med,* 51**,** 333-344.

SUN, Q. Y., MIAO, Y. L. & SCHATTEN, H. 2009. Towards a new understanding on the regulation of mammalian oocyte meiosis resumption. *Cell Cycle,* 8**,** 2741-7.

SUTTON, M. L., GILCHRIST, R. B. & THOMPSON, J. G. 2003. Effects of in-vivo and in-vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte developmental capacity. *Hum Reprod Update,* 9**,** 35-48.

SUZUKI, K. 2018. The developing world of DOHaD. *J Dev Orig Health Dis,* 9**,** 266-269.

SZENDROEDI, J. & RODEN, M. 2009. Ectopic lipids and organ function. *Curr Opin Lipidol,* 20**,** 50-6.

TABAS, I. & RON, D. 2011. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nat Cell Biol,* 13**,** 184-90.

TALMOR, A. & DUNPHY, B. 2015. Female obesity and infertility. *Best Pract Res Clin Obstet Gynaecol,* 29**,** 498-506.

TAMURA, S. & SHIMOMURA, I. 2005. Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. *J Clin Invest,* 115**,** 1139-42.

TARTIA, A. P., RUDRARAJU, N., RICHARDS, T., HAMMER, M. A., TALBOT, P. & BALTZ, J. M. 2009. Cell volume regulation is initiated in mouse oocytes after ovulation. *Development,* 136**,** 2247-2254.

TAY, J. I., RUTHERFORD, A. J., KILLICK, S. R., MAGUINESS, S. D., PARTRIDGE, R. J. & LEESE, H. J. 1997. Human tubal fluid: production, nutrient composition and response to adrenergic agents. *Hum Reprod,* 12**,** 2451-6.

TERSIGNI, C., DI NICUOLO, F., D'IPPOLITO, S., VEGLIA, M., CASTELLUCCI, M. & DI SIMONE, N. 2011. Adipokines: new emerging roles in fertility and reproduction. *Obstet Gynecol Surv,* 66**,** 47-63.

THOMPSON, J. G., MCNAUGHTON, C., GASPARRINI, B., MCGOWAN, L. T. & TERVIT, H. R. 2000. Effect of inhibitors and uncouplers of oxidative phosphorylation during compaction and blastulation of bovine embryos cultured in vitro. *J Reprod Fertil,* 118**,** 47-55.

TSUTSUMI, R. & WEBSTER, N. J. 2009. GnRH pulsatility, the pituitary response and reproductive dysfunction. *Endocr J,* 56**,** 729-37.

ULBRICH, S. E., KETTLER, A. & EINSPANIER, R. 2003. Expression and localization of estrogen receptor alpha, estrogen receptor beta and progesterone receptor in the bovine oviduct in vivo and in vitro. *J Steroid Biochem Mol Biol,* 84**,** 279-89.

VAHRATIAN, A. & SMITH, Y. R. 2009. Should access to fertility-related services be conditional on body mass index? *Hum Reprod,* 24**,** 1532-7.

VALCKX, S. D., ARIAS-ALVAREZ, M., DE PAUW, I., FIEVEZ, V., VLAEMINCK, B., FRANSEN, E., BOLS, P. E. & LEROY, J. L. 2014a. Fatty acid composition of the follicular fluid of normal weight, overweight and obese women undergoing assisted reproductive treatment: a descriptive cross-sectional study. *Reprod Biol Endocrinol,* 12**,** 13.

VALCKX, S. D., DE BIE, J., MICHIELS, E. D., GOOVAERTS, I. G., PUNJABI, U., RAMOS-IBEAS, P., GUTIERREZ-ADAN, A., BOLS, P. E. & LEROY, J. L. 2015. The effect of human follicular fluid on bovine oocyte developmental competence and embryo quality. *Reprod Biomed Online,* 30**,** 203-7.

VALCKX, S. D., DE PAUW, I., DE NEUBOURG, D., INION, I., BERTH, M., FRANSEN, E., BOLS, P. E. & LEROY, J. L. 2012. BMI-related metabolic composition of the follicular fluid of women undergoing assisted reproductive treatment and the consequences for oocyte and embryo quality. *Hum Reprod,* 27**,** 3531-9.

VALCKX, S. D., VAN HOECK, V., ARIAS-ALVAREZ, M., MAILLO, V., LOPEZ-CARDONA, A. P., GUTIERREZ-ADAN, A., BERTH, M., CORTVRINDT, R., BOLS, P. E. & LEROY, J. L. 2014b. Elevated non-esterified fatty acid concentrations during in vitro murine follicle growth alter follicular physiology and reduce oocyte developmental competence. *Fertil Steril,* 102**,** 1769-76 e1.

VAN BLERKOM, J. 2004. Mitochondria in human oogenesis and preimplantation embryogenesis: engines of metabolism, ionic regulation and developmental competence. *Reproduction,* 128**,** 269-80.

VAN DER STEEG, J. W., STEURES, P., EIJKEMANS, M. J., HABBEMA, J. D., HOMPES, P. G., BURGGRAAFF, J. M., OOSTERHUIS, G. J., BOSSUYT, P. M., VAN DER VEEN, F. & MOL, B. W. 2008. Obesity affects spontaneous pregnancy chances in subfertile, ovulatory women. *Hum Reprod,* 23**,** 324-8.

VAN HOECK, V., LEROY, J. L., ARIAS ALVAREZ, M., RIZOS, D., GUTIERREZ-ADAN, A., SCHNORBUSCH, K., BOLS, P. E., LEESE, H. J. & STURMEY, R. G. 2013. Oocyte developmental failure in response to elevated nonesterified fatty acid concentrations: mechanistic insights. *Reproduction,* 145**,** 33-44.

VAN HOECK, V., STURMEY, R. G., BERMEJO-ALVAREZ, P., RIZOS, D., GUTIERREZ-ADAN, A., LEESE, H. J., BOLS, P. E. & LEROY, J. L. 2011. Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. *PLoS One,* 6**,** e23183.

VAN WINKLE, L. J. 2001. Amino acid transport regulation and early embryo development. *Biol Reprod,* 64**,** 1-12.

VELAZQUEZ, M. A. 2015. [Impact of maternal overnutrition on the periconceptional period]. *Endocrinol Nutr,* 62**,** 246-53.

VON WALD, T., MONISOVA, Y., HACKER, M. R., YOO, S. W., PENZIAS, A. S., REINDOLLAR, R. R. & USHEVA, A. 2010. Age-related variations in follicular apolipoproteins may influence human oocyte maturation and fertility potential. *Fertility and Sterility,* 93**,** 2354-2361.

WAJCHENBERG, B. L. 2000. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev,* 21**,** 697-738.

WANG, X. H., YIN, S., OU, X. H. & LUO, S. M. 2020. Increase of mitochondria surrounding spindle causes mouse oocytes arrested at metaphase I stage. *Biochem Biophys Res Commun,* 527**,** 1043-1049.

WARZYCH, E., PAWLAK, P., PSZCZOLA, M., CIESLAK, A., MADEJA, Z. E. & LECHNIAK, D. 2017. Interactions of bovine oocytes with follicular elements with respect to lipid metabolism. *Anim Sci J,* 88**,** 1491-1497.

WEISS, R. V. & CLAPAUCH, R. 2014. Female infertility of endocrine origin. *Arq Bras Endocrinol Metabol,* 58**,** 144-52.

WEYDERT, C. J. & CULLEN, J. J. 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. *Nat Protoc,* 5**,** 51-66.

WHO 2017. Prevalence of overweight / obesity among adults, Estimates by WHO region. *Global Health Observatory data repository.*

WHO 2021. Infertility prevalence estimates 1990 - 2021.

WHO. 2022. *Global Health Observatory* [Online]. Our world in data. Available: https://ourworldindata.org/grapher/share-of-females-defined-as-overweight?time=latest [Accessed 2024].

WHO, W. H. O. 2024. *Obesity and overweight fact sheet* [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight#:~:text=Key%20facts,years%20and%20older)%20were%20overweight. [Accessed].

WIENER-MEGNAZI, Z., VARDI, L., LISSAK, A., SHNIZER, S., REZNICK, A. Z., ISHAI, D., LAHAV-BARATZ, S., SHILOH, H., KOIFMAN, M. & DIRNFELD, M. 2004. Oxidative stress indices in follicular fluid as measured by the thermochemiluminescence assay correlate with outcome parameters in in vitro fertilization. *Fertil Steril,* 82 Suppl 3**,** 1171-6.

WIJAYAGUNAWARDANE, M. P., MIYAMOTO, A., CERBITO, W. A., ACOSTA, T. J., TAKAGI, M. & SATO, K. 1998. Local distributions of oviductal estradiol, progesterone,

prostaglandins, oxytocin and endothelin-1 in the cyclic cow. *Theriogenology,* 49**,** 607-18.

WILDING, M., DALE, B., MARINO, M., DI MATTEO, L., ALVIGGI, C., PISATURO, M. L., LOMBARDI, L. & DE PLACIDO, G. 2001. Mitochondrial aggregation patterns and activity in human oocytes and preimplantation embryos. *Hum Reprod,* 16**,** 909-17.

WILLIAMS, L. M., CAMPBELL, F. M., DREW, J. E., KOCH, C., HOGGARD, N., REES, W. D., KAMOLRAT, T., THI NGO, H., STEFFENSEN, I. L., GRAY, S. R. & TUPS, A. 2014. The development of diet-induced obesity and glucose intolerance in C57BL/6 mice on a high-fat diet consists of distinct phases. *PLoS One,* 9**,** e106159.

WILLIS, G. R., REIS, M., GHEINANI, A. H., FERNANDEZ-GONZALEZ, A., TAGLAUER, E. S., YEUNG, V., LIU, X., ERICSSON, M., HAAS, E., MITSIALIS, S. A. & KOUREMBANAS, S. 2021. Extracellular Vesicles Protect the Neonatal Lung from Hyperoxic Injury through the Epigenetic and Transcriptomic Reprogramming of Myeloid Cells. *Am J Respir Crit Care Med,* 204**,** 1418-1432.

WISE, L. A., ROTHMAN, K. J., MIKKELSEN, E. M., SORENSEN, H. T., RIIS, A. & HATCH, E. E. 2010. An internet-based prospective study of body size and time-to-pregnancy. *Hum Reprod,* 25**,** 253-64.

WOLODKO, K., CASTILLO-FERNANDEZ, J., KELSEY, G. & GALVAO, A. 2021. Revisiting the Impact of Local Leptin Signaling in Folliculogenesis and Oocyte Maturation in Obese Mothers. *Int J Mol Sci,* 22.

WOLODKO, K., WALEWSKA, E., ADAMOWSKI, M., CASTILLO-FERNANDEZ, J., KELSEY, G. & GALVAO, A. 2020. Leptin Resistance in the Ovary of Obese Mice is Associated with Profound Changes in the Transcriptome of Cumulus Cells. *Cell Physiol Biochem,* 54**,** 417-437.

WONNACOTT, K. E., KWONG, W. Y., HUGHES, J., SALTER, A. M., LEA, R. G., GARNSWORTHY, P. C. & SINCLAIR, K. D. 2010. Dietary omega-3 and -6 polyunsaturated fatty acids affect the composition and development of sheep granulosa cells, oocytes and embryos. *Reproduction,* 139**,** 57-69.

WU, L. L., DUNNING, K. R., YANG, X., RUSSELL, D. L., LANE, M., NORMAN, R. J. & ROBKER, R. L. 2010. High-fat diet causes lipotoxicity responses in cumulus-oocyte complexes and decreased fertilization rates. *Endocrinology,* 151**,** 5438-45.

WU, L. L., NORMAN, R. J. & ROBKER, R. L. 2011. The impact of obesity on oocytes: evidence for lipotoxicity mechanisms. *Reprod Fertil Dev,* 24**,** 29-34.

WU, L. L., RUSSELL, D. L., WONG, S. L., CHEN, M., TSAI, T. S., ST JOHN, J. C., NORMAN, R. J., FEBBRAIO, M. A., CARROLL, J. & ROBKER, R. L. 2015. Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. *Development,* 142**,** 681-91.

XIE, F., ANDERSON, C. L., TIMME, K. R., KURZ, S. G., FERNANDO, S. C. & WOOD, J. R. 2016. Obesity-Dependent Increases in Oocyte mRNAs Are Associated With

Increases in Proinflammatory Signaling and Gut Microbial Abundance of Lachnospiraceae in Female Mice. *Endocrinology,* 157**,** 1630-43.

YANG, W. J., LIU, F. C., HSIEH, J. S., CHEN, C. H., HSIAO, S. Y. & LIN, C. S. 2015. Matrix metalloproteinase 2 level in human follicular fluid is a reliable marker of human oocyte maturation in in vitro fertilization and intracytoplasmic sperm injection cycles. *Reprod Biol Endocrinol,* 13**,** 102.

YANG, X., SHENG, W., SUN, G. Y. & LEE, J. C. 2011. Effects of fatty acid unsaturation numbers on membrane fluidity and alpha-secretase-dependent amyloid precursor protein processing. *Neurochem Int,* 58**,** 321-9.

YANG, X., WU, L. L., CHURA, L. R., LIANG, X., LANE, M., NORMAN, R. J. & ROBKER, R. L. 2012. Exposure to lipid-rich follicular fluid is associated with endoplasmic reticulum stress and impaired oocyte maturation in cumulus-oocyte complexes. *Fertil Steril,* 97**,** 1438-43.

YOUNG, L. E., SINCLAIR, K. D. & WILMUT, I. 1998. Large offspring syndrome in cattle and sheep. *Rev Reprod,* 3**,** 155-63.

YOUSEF, M. S., MAREY, M. A., HAMBRUCH, N., HAYAKAWA, H., SHIMIZU, T., HUSSIEN, H. A., ABDEL-RAZEK, A. K., PFARRER, C. & MIYAMOTO, A. 2016. Sperm Binding to Oviduct Epithelial Cells Enhances TGFB1 and IL10 Expressions in Epithelial Cells as Well as Neutrophils In Vitro: Prostaglandin E2 As a Main Regulator of Anti-Inflammatory Response in the Bovine Oviduct. *PLoS One,* 11**,** e0162309.

YOUSIF, M. D., CALDER, M. D., DU, J. T., RUETZ, K. N., CROCKER, K., URQUHART, B. L., BETTS, D. H., RAFEA, B. A. & WATSON, A. J. 2020. Oleic Acid Counters Impaired Blastocyst Development Induced by Palmitic Acid During Mouse Preimplantation Development: Understanding Obesity-Related Declines in Fertility. *Reprod Sci,* 27**,** 2038-2051.

YUZEFOVYCH, L. V., MUSIYENKO, S. I., WILSON, G. L. & RACHEK, L. I. 2013. Mitochondrial DNA damage and dysfunction, and oxidative stress are associated with endoplasmic reticulum stress, protein degradation and apoptosis in high fat diet-induced insulin resistance mice. *PLoS One,* 8**,** e54059.

ZACHUT, M., ARIELI, A. & MOALLEM, U. 2011. Incorporation of dietary n-3 fatty acids into ovarian compartments in dairy cows and the effects on hormonal and behavioral patterns around estrus. *Reproduction,* 141**,** 833-40.

ZAIN, M. M. & NORMAN, R. J. 2008. Impact of obesity on female fertility and fertility treatment. *Womens Health (Lond),* 4**,** 183-94.

ZEGERS-HOCHSCHILD, F., ADAMSON, G. D., DE MOUZON, J., ISHIHARA, O., MANSOUR, R., NYGREN, K., SULLIVAN, E., VANDERPOEL, S., INTERNATIONAL COMMITTEE FOR MONITORING ASSISTED REPRODUCTIVE, T. & WORLD HEALTH, O. 2009. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. *Fertil Steril,* 92**,** 1520-4.

ZHANG, X., WU, X. Q., LU, S., GUO, Y. L. & MA, X. 2006. Deficit of mitochondria-derived ATP during oxidative stress impairs mouse MII oocyte spindles. *Cell Res,* 16**,** 841-50.

CHAPTER 2: HYPOTHESIS AND AIMS

Hypothesis and Aims

The increased consumption of an obesogenic diet and the lack of physical activity is leading to a rapidly increasing prevalence of obesity. More and more evidence shows that metabolic disorders linked to obesity can have direct negative effects on fertility. Obesogenic diets are characterized by a high fat content which leads to increased adiposity, hyperlipidemia and inflammation, resulting in cellular dysfunction due to lipotoxicity, reduced insulin sensitivity and oxidative stress. The severity of this metabolic disorder is even further exacerbated by the high sugar content of the obesogenic diets. The direct impact of a high-fat/high-sugar (HF/HS) diet and obesity on cellular functions depends on the duration of the direct exposure to the dietary components. This timeframe during which the detrimental effects may develop after the start of consuming an obesogenic diet has not been previously investigated. However, it is important to know how fast an obesogenic diet starts to affect different physiological functions, including reproduction, as it is not known whether such detrimental effects are chronic and caused by the longer-term feeding of the diet and the impact of the obese phenotype and the associated metabolic disorders, or merely acute and caused by short-term feeding of the diet and through the direct impact of the specific composition of that obesogenic diet (traditionally containing high amounts of saturated fatty acids and sugars). In this PhD thesis we wanted to better understand the potential impact of an obesogenic diet and/or the obese phenotype on embryo development and to estimate the importance of the duration of this exposure Therefore, **we hypothesized that short and long-term feeding of a HF/HS diet leads to acute but also chronic changes in the oocyte quality and in the micro-environment of the newly formed embryo (i.e. the oviduct) of mice. For this we especially focus on local changes in oxidative stress levels, inflammatory responses and the lipid profile.**

To understand and mimic the mechanisms by which an obesogenic diet impacts fertility, HF/HS diet-induced obese mouse models have often been used. The inbred

C57BL/6N strain is most commonly used in such studies, however, an outbred strain (Swiss mice) is more pathophysiologically relevant to the human (outbred) physiology, which might facilitate translation to the human population. The sensitivity to obesogenic diet-induced obesity and its influence on the metabolic profile and oocyte quality have been shown to be dependent on the genetic background of the mouse model. This was recently demonstrated by comparing the outbred Swiss mice with the inbred C57BL/6N mice. Such strain difference may potentially confound our understanding of the impact of an obesogenic diet on early embryo development.

As the oviduct plays a crucial role in providing the optimal micro-environment for oocyte fertilisation and the first steps of embryo development, we aimed to investigate:

1. A. whether short and long-term feeding of an obesogenic (HF/HS) diet results in differential acute and/or chronic changes in the transcriptome of **oviductal epithelial cells** (OEC) in **Swiss compared to C57BL/6N mice**, particularly related to genes involved in **oxidative and cellular stress levels and inflammatory** responses (Chapter 3) and

   B. whether the nature and magnitude of these changes depend on the **duration** of feeding.
   Hereto, we wished to investigate OEC samples collected from Swiss and C57BL/6N mice that were fed a HF/HS diet for different periods of time, namely 3 days, 1 week, 4 weeks, 8 weeks, 12 weeks and 16 weeks. (Chapter 3)

The dynamics and the nature of changes in **the lipid composition of the oviduct** upon and during HF/HS diet feeding has not been described. However, as described above, fatty acids play an important role as structural membrane components, in cell signalling and cell-to-cell interactions. Thus, alterations to the lipid composition of the oviduct

may reduce its ability to support early embryo development, which may have long-lasting consequences. Therefore, it is important to understand how diet and obesity can influence the oviductal lipidomic profile to better estimate changes in the embryo supportive capacity of the oviduct. For that reason, we aimed to:

2. investigate whether a HF/HS diet can alter **the lipidomic profile of the oviduct** in an outbred **Swiss** mouse model and to study the patterns of these changes in the oviductal epithelial layer over time as the mice continue to consume the obesogenic diet for a longer period and develop obesity.
   Hereto, oviductal cross-sections were analysed using the matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI) technique for the analysis of the spatial distribution of lipids in the oviducts after 3 days, 1 week, 4 weeks, 8 weeks, 12 weeks and 16 weeks of feeding the HF/HS diet. (Chapter 4)

Whereas the effects of maternal metabolic stress on the oviduct were much less well characterized, the direct impact of an obesogenic diet and obesity on the ovarian follicular fluid micro-environment and oocyte quality is relatively more documented in animal models and women. It is known that the impact of lipotoxicity, caused by an obesogenic diet, on oocyte quality is mediated by oxidative stress, endoplasmic reticulum stress and mitochondrial dysfunction. However, also here the nature and magnitude of these changes may be dependent on the duration of feeding (and thus the development of the obesogenic phenotype), which is still to be investigated. Therefore, we aimed to investigate:

3. whether feeding a HF/HS diet to **Swiss mice** for different short and long-term periods of time, namely 24 hours, 3 days, 1 week, 4 weeks, 8 weeks, 12 weeks

and 16 weeks, leads to acute and/or chronic changes in **the ovary, oocyte quality and the cellular pathways in the granulosa cells**. (Chapter 5)

As previously described, the absence of a specific marker or consensus to assess the presence or absence of obesity in mice, makes defining a specific timepoint for the onset of obesity after the start of feeding an obesogenic diet difficult. Therefore, in this PhD thesis, the findings were described as acute and chronic changes that were defined based on the duration of exposure, being short and long-term feeding of the obesogenic diet. The effects detected after short-term exposure to the obesogenic diet of 24 hours, 3 days and up to 1 week, are considered acute effects. Based on previous research, continued feeding was expected to show an adaptive phase in between short and long-term exposure (from 1 week up to 12 weeks) to the diet. Effects that were detected after long-term exposure to the diet of 12 and 16 weeks were considered chronic effects. A general overview of the experimental design of the research conducted in this PhD thesis is presented in Figure 1.

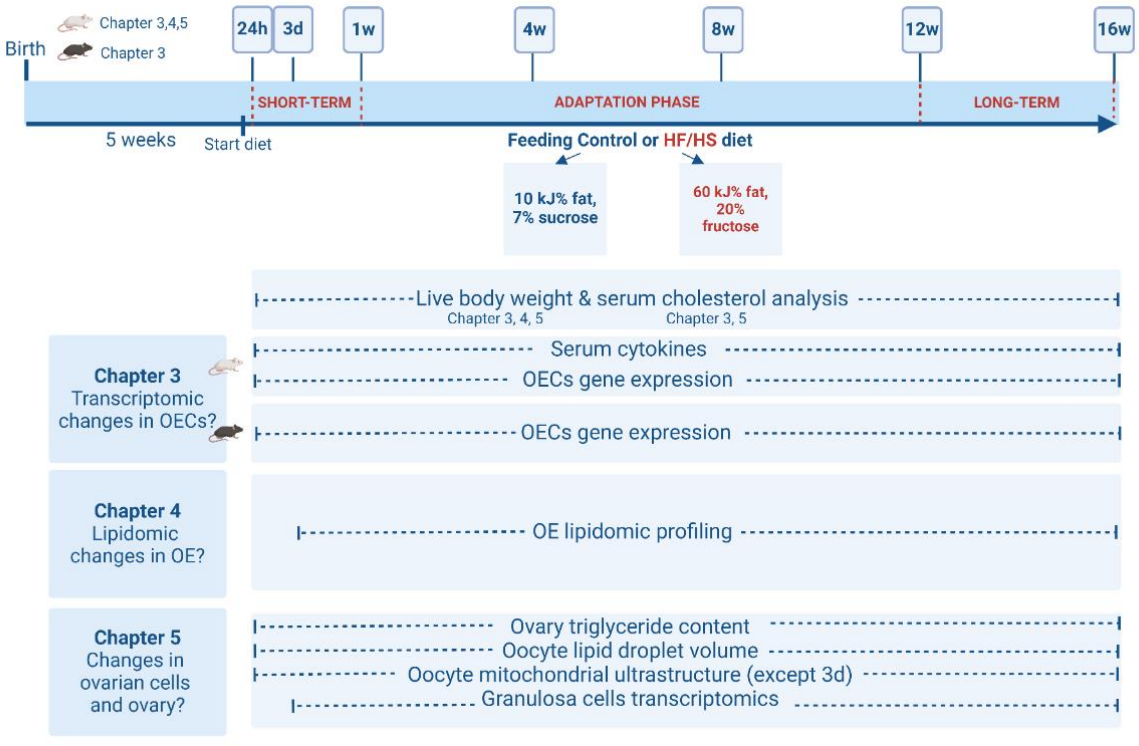

Figure 1. General experimental design of the experiments conducted in this PhD thesis with a presentation of the different outcome parameters and their assessed time points (made with Biorender). (OEC = Oviductal Epithelial Cells; OE = Oviductal Epithelium; HF/HS = high-fat/high-sugar)

CHAPTER 3:
EFFECTS OF A WESTERN TYPE DIET ON CELLULAR STRESS MARKERS IN OVIDUCTAL EPITHELIAL CELLS OVER TIME: A GENE EXPRESSION ANALYSIS

Effects of an obesogenic diet on the oviduct depend on the duration of feeding

Short title: Obesogenic diet alters the oviductal microenvironment

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1. Abstract

Research question: How long does it take for an obesogenic (high-fat/high-sugar, HF/HS) diet to influence the oviductal microenvironment? What are the affected cellular pathways and are they dependent on the genetic background of the mouse model?

Design: Female Swiss (outbred) and C57BL/6N (B6, inbred) mice were fed either a control (10% fat) or HF/HS (60% fat, 20% fructose) diet. Body weight was measured weekly. Mice were sacrificed at 3 days (3d), 1 week (1w), 4w, 8w, 12w and 16w on the diet (n=5 per treatment per time point). Total cholesterol concentrations and inflammatory cytokines were measured in serum. Oviductal epithelial cells (OECs) were used to study the expression of genes involved in (mitochondrial) oxidative stress (OS), endoplasmic reticulum (ER) stress and inflammation using qPCR.

Results: Body weight and blood cholesterol increased significantly in the HF/HS mice in both strains compared to controls. In Swiss mice, HF/HS diet acutely increased ER-stress and OS-related genes in the OECs already after 3d. Subsequently, mitochondrial and cytoplasmic antioxidants were upregulated and ER-stress was alleviated at 1w. After 4-8w (mid-phase), the expression of ER-stress and OS-related genes was increased again and persisted throughout the late-phase (12-16w). Serum inflammatory cytokines and inflammatory marker-gene expression in the OECs were increased only in the late-phase. Some of the OEC stress responses were stronger or earlier in the B6.

Conclusions: OECs are sensitive to an obesogenic diet and may exhibit acute stress responses already after a few days of feeding. This may impact the oviductal microenvironment and contribute to diet-induced subfertility.

Keywords: Cellular stress; High-fat/high-sugar diet; Inflammation; Metabolic stress; Oviductal epithelial cells

2. Introduction

The prevalence of obesity and type-II diabetes is rapidly increasing worldwide (Oestreich and Moley, 2017; WHO, 2021) due to the increased consumption of the obesogenic diet and the lack of physical activity (Saklayen, 2018; Bluher, 2019; Haley et al., 2019). Metabolic syndrome has become a major health hazard of the modern world with an average prevalence of 31% (Engin, 2017; Saklayen, 2018). Recently there is increasing evidence that metabolic disorders linked to obesity can have direct negative effects on fertility (Silvestris et al., 2018). Obese or overweight women are less likely to achieve spontaneous pregnancy and have lower reproductive outcomes (Klenov and Jungheim, 2014). The underlying mechanisms are progressively unravelled but are not fully elucidated.

Obesogenic diets are characterized by a high fat content (Panchal and Brown, 2011), especially saturated fats, which are known to lead to increased adiposity, hyperlipidemia and inflammation. This results in cellular dysfunction due to lipotoxicity, reduced insulin sensitivity and oxidative stress (**OS**) (Engin; Lin et al., 2005; Fernandez-Sanchez et al., 2011; Herdt, 2013; Engin, 2017). The high sugar content in these diets (Panchal and Brown, 2011) further exacerbates the severity of this metabolic disorder (Stanhope, 2016).

High-fat/high-sugar (**HF/HS**) diet-induced obesity in mice has been used in many studies to mimic and understand the mechanisms by which an obesogenic diet impacts fertility. Beside possible systemic endocrine disruption, the impact of HF/HS diet and obesity has been shown to be mainly mediated through direct detrimental effects on the reproductive organs during oocyte and embryo development and during pregnancy (Jungheim and Moley, 2010). The direct impact of obesity on the ovarian follicular fluid (**FF**) microenvironment and oocyte quality is relatively more documented and clearly elucidated in animal models (Robker, 2008; Igosheva et al., 2010a; Jungheim et al., 2010; Wu et al., 2011; Jungheim et al., 2012; Valckx et al., 2014; Marei et al., 2017; Marei et al., 2020) and women (Wittemer et al., 2000; Valckx et al., 2012; Gonzalez et

al., 2018). Oocytes matured under lipotoxic conditions exhibit altered mitochondrial functions and OS, an aberrant proteomic profile and metabolic activity, ultimately leading to lower oocyte developmental competence and lower embryo quality (Robker, 2008; Igosheva et al., 2010b; Van Hoeck et al., 2013; Marei et al., 2017; Marei et al., 2020; Smits et al., 2021).

The effects of maternal metabolic stress on the oviduct are much less well characterized. This is however important as several crucial reproductive events take place in the oviduct such as oocyte and sperm capacitation and fertilization, syngamy, embryo genome activation and epigenetic (re)programming (Aviles et al., 2010; Besenfelder et al., 2012; Maillo et al., 2016; Li and Winuthayanon, 2017). The oviductal secretory epithelial cells and the oviductal fluid provide nutrients, growth factors and antioxidants (AO) to support early embryo development (which is in most mammalian species during the first 3-5 days post-fertilization) and changes in this micro-environment may lead to lower pregnancy success or even to offspring health defects (Velazquez, 2015; Robertson et al., 2018).

Only a few studies have previously expanded on that. For example it has been shown that cows suffering from severe negative energy balance and systemic hyperlipidemia and lipotoxicity due to fat mobilization exhibited reduced oviductal ability to support embryo development after embryo transfer (Rizos et al., 2010; Yousif et al., 2020). Additionally, Jordaens et al. (2020) showed that *in vitro* exposure of bovine oviductal cells to elevated non-esterified fatty acid (NEFA) concentrations leads to direct embryo toxicity and to a reduced oviductal ability to support and protect early embryo development (Jordaens et al., 2020). Nevertheless, potential direct effects of HF/HS diet-induced obesity on the oviductal microenvironment have not been investigated in humans or in relevant animal models.

The direct impact of a HF/HS diet and obesity on cellular functions depends on the duration of the direct exposure to the dietary components. Systemic inflammation was reported in mice as early as 3 days (3d) after the start of the high-fat diet (HFD) and

was shown to be limited to distinct phases (acute and late periods) with an adaptive period in between (Williams et al., 2014). In contrast, other studies demonstrated a gradual increase (Kleemann et al., 2010) or even no inflammatory responses (Haley et al., 2019) in mice fed a HFD. Local inflammatory responses are also expected at the cellular level and may contribute to reproductive dysfunction (Ohtsu et al., 2017; Chankeaw et al., 2018). On the other hand, diet-induced hyperlipidemia is also known to induce lipotoxicity in non-adipose tissues, alter cellular metabolism due to mitochondrial dysfunction, and increase OS levels (Engin; Lin et al., 2005; Herdt, 2013). Nevertheless, it is not known if the microenvironment of the oviduct exhibits such pathophysiological changes in response to an obesogenic diet. Also the timeframe during which these alterations may develop after consuming an obesogenic diet has not been previously investigated. Fundamental insight in this area is necessary to determine the potential impact on embryo development and the timeframe at which the early embryo might be at risk.

We have recently shown that the sensitivity to HFD-induced obesity and its influence on the metabolic profile and oocyte quality was dependent on the mitochondrial and/or genetic background of the mouse model. This was demonstrated by comparing the outbred Swiss mice with the inbred C57BL/6N mice (Marei et al., 2020). Such difference may also influence the metabolic impact on the oviductal microenvironment.

In this study, we hypothesized that feeding a HF/HS diet can lead to acute and/or long-term changes in the oviduct of mice, particularly related to oxidative and cellular stress levels and inflammatory responses and that the nature and magnitude of these changes depend on the duration of feeding. This may also be dependent on the mouse strain used in the model. To test this hypothesis, we aimed to analyze serum and oviductal epithelial cell (**OEC**) samples collected from Swiss and C57BL/6N mice that were exposed to a HF/HS diet for different periods of time, namely 24 hours, 3 days, 1 week,

4 weeks, 8 weeks, 12 weeks and 16 weeks. Outcome parameters were focused on serum total cholesterol and cytokine concentrations and on the expression of genes involved in response to OS, endoplasmic reticulum (ER) stress and inflammation in OECs.

3. Material and methods

3.1. Ethical approval

All procedures in this study were approved by the ethical committee of the University of Antwerp and performed according (ECD approval number nr. 2014-57). All methods were performed in accordance with the relevant guidelines and regulations. This study complies with the ARRIVE guidelines. Euthanasia was done by decapitation.

3.2. Experimental animals, diet and experimental design

Five-week-old, non-pregnant, sexually mature female outbred Rj:Orl Swiss mice (hereafter referred to as “Swiss” mice, n=70, Janvier labs), and inbred C57BL/6N mice (hereafter referred to as “Black 6” (B6) mice, n=70, Janvier labs) were used.

Mice of each strain were randomly divided into two groups with ad libitum access to either a control diet (CTRL) or a high-fat/high-sugar diet (HF/HS). A HF/HS diet is more representative for the typical Western style diet, compared to high-fat diet only. The HF/HS group was fed with 60 kJ% fat (beef tallow) and 9.4% sucrose diet (E15741-34, Sniff diets, Soest, Germany; Supplementary file 1) in combination with supplementing drinking water with fructose at a final concentration of 20% (Merck, 102109450). Beef tallow was chosen over lard because beef tallow contains more saturated fatty acids. Fructose was chosen over glucose or sucrose since a high consumption of fructose has proven to lead to insulin resistance and obesity in rodents (Tappy and Le, 2010). A high-fat/high-fructose diet is known to induce metabolic syndrome and type 2 diabetes in mice (Panchal and Brown, 2011; Dissard et al., 2013). Fructose will also stimulate higher

food consumption because it is more palatable and has a reduced capacity to stimulate satiety. Furthermore, fructose appears to be a better inducer of metabolic syndrome compared to glucose (Pereira et al., 2017).

Mice in the control group had ad libitum access to water and were exposed to a matched, purified (not a grain-based chow diet) CTRL diet (E157453-04, Sniff diets, Soest, Germany; Supplementary file 2), containing 10 kJ% fat and 7% sucrose. This diet is also lower in saturated fatty acids. Food intake of all mice was monitored and mice were weighed weekly during the whole experiment.

Mice were euthanized by decapitation at 7 time points (n=5 per treatment per time point): 24 hours, 3 days, 1 week (1w), 4w, 8w, 12w and 16w after the start of feeding of the CTRL and the HF/HS diets. Mice were not fasted before euthanasia to avoid counteracting the dietary effects especially in the early timepoints. Serum and OECs were collected as explained hereafter to study different outcome parameters. Female mice were exposed to bedding from male cages 24 hours before euthanasia to synchronize their estrous cycles (Whitten effect) and to collect the samples during the follicular phase (before ovulation), ensuring no follicular cells or oocytes are present in the oviduct at sample collection. The time points at which samples were collected are based on the results of previous studies where multiphasic acute and long-term effects of a HFD on general metabolic features have been reported (Williams et al., 2014).

3.3 Live body weight

The weight of each mouse was recorded weekly. The weight gain data is derived from 35 mice per dietary group per strain at the first time point. The number of mice were reduced after each time point due to culling of a subset of animals (n=5 per strain) at each time point for sample collection.

3.4 Serum collection and analysis

Mice were decapitated, and blood was collected immediately after decapitation and centrifuged after 30 min at 6000xg for 2 min at 4°C. Serum was then stored at -80°C. Serum samples of both Swiss and B6 mice of all time points were analyzed for total cholesterol concentrations in a commercial certified laboratory (Algemeen Medisch Labo, AML, Antwerp, Belgium) using automated facilities. Total cholesterol concentrations were measured on an Abbott Architect c16000 (Abbott, Illinois, U.S.A). Serum cytokine concentration was determined using a multiplex bead-based immunoassay according to the manufacturer's guidelines (LEGENDplex™ mouse cytokine panel 2 kit, BioLegend) and flowcytometry (FACSCantoII). The kit allows simultaneous quantification of 13 mouse cytokines (IL-1α, IL-1β, IL-6, IL-10, IL12p70, IL-17A, IL-23, IL-27, MCP-1, IFN-β, IFN-γ, TNF-α and GM-CSF). This was measured in the Swiss serum samples but not in the B6 mice due to insufficient volume of the collected serum. An overview of the different cytokines and their function can be found in S1 Table. The setup of the flow cytometer was performed according to the setup procedure provided by the manufacturer. As a validation step, a template for data acquisition was obtained using the raw beads that were provided in the kit. The Median Fluorescence Intensity (MFI) of each cytokine was measured in duplicate. The individual cytokine concentrations were calculated by linear equation using a 7 point standard curve.

3.5 Oviductal epithelial cells collection and analysis

At each time point, mice were dissected and the oviducts were collected in L15 medium (Thermo Fisher Scientific, Belgium) supplemented with 50 IU/mL penicillin G sodium salt (Merck, Belgium). The oviducts were straightened and milked with a bent needle for cell collection (OECs) in L15 medium, which was done in a DNAse and RNAse-free environment. The medium containing cells was transferred to a vial which was then centrifuged at 600xg for 10 minutes at 25°C and the supernatant was removed. This centrifugation step was repeated after adding RNAse-free phosphate buffered saline

(PBS; Life Technologies, Belgium) for pellet washing. The supernatant was removed and the pellets were snap frozen in liquid nitrogen (LN_2) and stored at -80°C.

3.6 Quantification of gene expression using real time qPCR

3.6.1 RNA extraction

Total RNA was extracted from OECs of both Swiss mice and B6 mice (5 mice/ strain/ treatment/ time point) using a TRIzol® (Invitrogen) based protocol according to the manufacturer's instructions. RNA samples were stored at -80°C until cDNA synthesis. RNA purity and concentrations were assessed using a NanoDrop™. All RNA samples had an acceptable 260/280 ratio. RNA integrity was also checked using an Agilent RNA 6000 nano kit and a Bioanalyzer (Agilent, Santa Clara, California, U.S.A) and all samples had acceptable RIN values (≥7) except a few samples collected at 24h which were excluded from the analysis. A few other samples were not included in the analysis due to a very low RNA yield. Therefore, gene expression data is reported based on at least three samples per treatment per time point.

3.6.2 cDNA synthesis

cDNA was synthesized from 1750 ng RNA/sample using Omniscript RT kit (Qiagen). First, RNA samples were treated with DNase (Promega RQ1 RNase-free DNase kit) to eliminate genomic DNA contamination.

3.6.3 Quantification of gene expression using qPCR

Transcripts of the target genes of interest were quantified by quantitative Polymerase Chain Reaction (qPCR) (QuantStudio™ 3 Real-Time PCR System (Thermofisher Sci)) using SYBR green (SsoAdvanced Universal SYBR Green supermix, Bio-Rad). RT-negative control samples and no template control samples (lacking cDNA template) were included, and all samples were analysed in duplicates in the same run per gene. Genes of interest were related to OS (*SOD2, PRDX1, PRDX3, PRDX6, NRF1, NRF2*), ER-stress

(BiP, ATF4), mitochondrial stress (*HSPE1, HSPD1*), chaperons (*HSPA8*) and inflammation (*IL-1β, IL-6, NFkB, TNF-α*) (an overview of the genes of interest and their function can be found in S2 Table). Quantification (Cq values) was normalized using the geometric mean of 3 housekeeping genes (*ACTB, B2M, H2AFZ*) for the Swiss samples and 2 housekeeping genes (*ACTB, PPIA*) for the B6 samples, according to their stability scores calculated by the NormFinder add-in in Excel (MOMA, Aarhus University Hospital, Denmark). The relative expression of each gene was calculated using the comparative quantification cycle method ($2^{-\Delta\Delta CT}$), as described by Livak and Schmittgen (2001) (Livak and Schmittgen, 2001). Forward and reverse primers for all genes of interest were designed using the Primer-BLAST tool of the U.S. National Center for Biotechnolgy Information (NCBI) and ordered at Sigma-Aldrich. The annealing and reading temperature of all assays were optimized using a gradient qPCR on a QuantStudio™ 3 Real-Time PCR System (Thermofisher Sci). An overview of all primer sequences, expected PCR product lengths, GeneBank accession numbers and temperatures can be found in Table 1.

Table 1. Primer sequences, expected PCR product lengths, GeneBank accession numbers and temperatures of the primers used for qPCR

GENE	PRIMER SEQUENCE (5'-3')	FRAGMENT SIZE (BP)	GENEBANK ACCESSION NO.	EXON SPANNING / INTRON FLANKING	ANNEALING T°C	READIN G T°C
	Oxidative stress					
SOD2	CTGGACAAACCTGAGCCCTA GCAGCAATCTGTAAGCGACCT	186	NM_013671.3	Intron	63	≤77
NRF1	CTCATCCAGGTTGGTACAGG	164	NM_010938.4	Intron	59	≤80

	GTCGTCTGGATGGTCATTTC					
PRDX1	TGTCCCACGGAGATCATTGC GGGTGTGTTAATCCATGCCAG	120	NM_011034.4	Exon	63	≤76
PRDX3	TCGTCAAGCACCTGAGTGTC TTGGCTTGATCGTAGGGGAC	147	NM_007452.2	Exon	63	≤81
PRDX6	GTTGACTGGAAGAAGGGAGAGA GCCACGATCTTTCTACGGAC	246	NM_007453.4	Exon	63	≤81
NRF2	CTCCCAGGTTGCCCACATTC GAGCTATTGAGGGACTGGGC	177	NM_010902.4	Exon	63	≤80
	Endoplasmic reticulum stress					
BiP (HSPA5)	AGGTGGGCAAACCAAGACAT CTTTGGTTGCTTGTCGCTGG	158	NM_001163434.1	Intron	63	76-78
ATF4	CGGCTATGGATGATGGCTTG AGAGCTCATCTGGCATGGTTT	156	NM_001287180.1	Intron	59	≤80
	Mitochondrial stress					
HSPE1	GGAGGGAAAGGAAAGAGTGGAG TAGTTCAGACATCAGTGGAATGGC	211	NM_008303.4	Intron	63	≤77
HSPD1	GCCAATAACACAAACGAAGAGG GCATCCACAGCCAACATCAC	146	NM_010477.4	Intron	59	≤80
	Protein folding (chaperons)					
HSPA8	CCTCGGAAAGACCGTTACCA AGCCGTAAGCAATAGCAGCA	152	NM_031165.5	Exon	63	≤80

	Inflammation					
IL-1β	TGTCTTTCCCGTGGACCTTC AGCTCATGGAGAATATCACTTGTTG	255	NM_008361.4	Exon	59 (can also be 61 or 63)	≤80
IL-6	CGTGGAAATGAGAAAAGAGTTGTG TCTGAAGGACTCTGGCTTTGTC	251	NM_031168.2	Exon	61	78
***NF*kB**	CCTGCAACAGATGGGCTACA TTGCGGAAGGATGTCTCCAC	201	NM_008689.2	Intron	59	84
***TNF-* α**	GTCCCCAAAGGGATGAGAAGT TTGCTACGACGTGGGCTACA	123	NM_013693.3	Exon	63	80
	Housekeeping genes					
B2M	GGTCTTTCTATATCCTGGCTCACA TTGATCACATGTCTCGATCCCA	126	NM_009735.3	Exon	61	≤79
ACTB	GCAAGTACTCTGTGTGGATCGG AACGCAGCTCAGTAACAGTCC	148	NM_007393.5	Exon	61	≤82
H2AFZ	CCTCACCGCAGAGGTACTTGA CCACGTATAGCAAGCTGCAAG	96	NM_016750.3	Exon	63	≤76
PPIA	ATGGCAAGCATGTGGTCTTTGG GGGTAGGGACGCTCTCCTGA	198	NM_008907.2	Exon	61	≤80

3.7 Statistical analysis

Each individual mouse is considered as an experimental unit. Power analysis was performed with the PS program for Power and Sample Size calculations (version 3.1.2, 2014 (from Vanderbilt University)) to achieve a power (1-β) of 90% to detect statistical

differences at P-value (*P*) <0.05 based on expected mean differences from similar previous experiments performed in our laboratory. All statistical analyses were carried out using IBM Statistics SPSS (IBM SPSS statistics version 26). All the outcome measures generated numerical data and were checked for normality of distribution and homogeneity of variance.

Live body weight was analysed using repeated measures ANOVA to study the main effects and interaction of treatment and time. In addition, two-tailed independent sample T-tests were performed to study the effect of treatment on body weight within each time point. Serum cholesterol data were analysed using a univariate ANOVA test on the control groups of different time points to check for potential aging effects. In addition, two-tailed independent sample T-tests were performed to study treatment effects within each time point.

Serum cytokine and gene expression data were not normally distributed. Aging effects were tested by comparing the controls of different timepoints using a Kruskal Wallis test with Bonferroni correction. Since no significant age effects could be detected, and based on preliminary comparisons between the HF/HS and control at each time point, and patterns of gene expression, it was decided to merge the data into biologically relevant distinct phases. Differences at 3d were considered as “acute phase” changes and those at 1w are referred to as an “early phase”. Data from 4w and 8w were merged as a “mid phase” and those from 12w and 16w were presented as a “late phase”. At each phase, cytokine data and gene expression data were analysed using two-tailed independent sample T-tests on log-transformed data to study the treatment effect.
All data are expressed as mean +/- S.E.M. (standard error of mean). Data were considered significantly different at $P \leq 0.05$ and higher P-values up to 0.1 are described as tendencies.

Pearson correlation was performed to assess the relationship between serum cholesterol concentrations, body weight and gene expression patterns in Swiss mice and B6 mice.

4. Results

4.1 The impact of the HF/HS diet on body weight

Repeated measures ANOVA showed a significant treatment x time effect for both Swiss mice and B6 mice ($P < 0.001$). HF/HS diet progressively increased body weight of both Swiss and B6 mice. The difference in body weight between the HF/HS and the control group was already significant from 1w onwards in the Swiss mice, and from 2w onwards in B6 mice. Bigger error bars, and thus more variance, was observed in the Swiss strain compared to the B6 strain. Body weight continued to increase in the B6 mice until 16w while it reached a plateau at 12w after the onset of the dietary treatment in the Swiss mice (Fig 1).

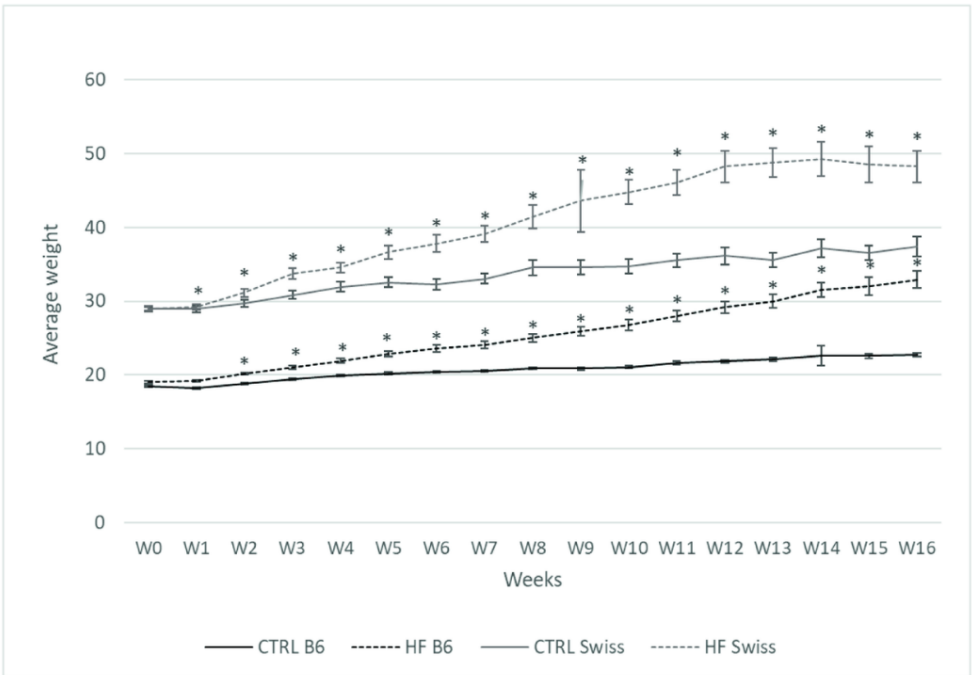


Fig 1. The effect of HF/HS diet on body weight in Swiss mice and B6 mice. Experimental feeding was started at W0. Body weight was weekly monitored. Data are shown as mean ± SEM. Significant difference ($P \leq 0.05$) between treatment groups within each strain per time point are indicated by an asterisk (*).

4.2 Serum total cholesterol concentrations

Based on the two way ANOVA, no effects of age on serum total cholesterol concentrations were detected for both the Swiss ($P = 0.544$) and the B6 ($P = 0.303$) strain. In Swiss mice, there was no effect of diet on blood cholesterol concentration at 24h and 3d ($P > 0.1$). Feeding the HF/HS diet for 1 week significantly increased blood total cholesterol concentrations ($P = 0.004$). This difference could not be detected after 4 weeks ($P = 0.136$) but was more distinct again at 8w, 12w and 16 weeks ($P = 0.006$, $P = 0.068$ and $P = 0.085$ respectively) (Fig 2A). Less variation was observed in the B6 strain. Exposure to the diet for only 3d already resulted in a significant increase in blood total cholesterol concentrations ($P = 0.001$). This significant increase in cholesterol

concentration remained present at all time points thereafter (1w to 12w: $P < 0.001$; 16w: $P = 0.003$) (Fig 2B).

In addition, Pearson correlation showed that serum cholesterol concentration correlated positively with body weight for both Swiss mice (Pearson correlation factor (r) = 0.322; $P = 0.008$) and B6 mice (r = 0.536; $P = 0.000$) (S3 and S4 Tables).

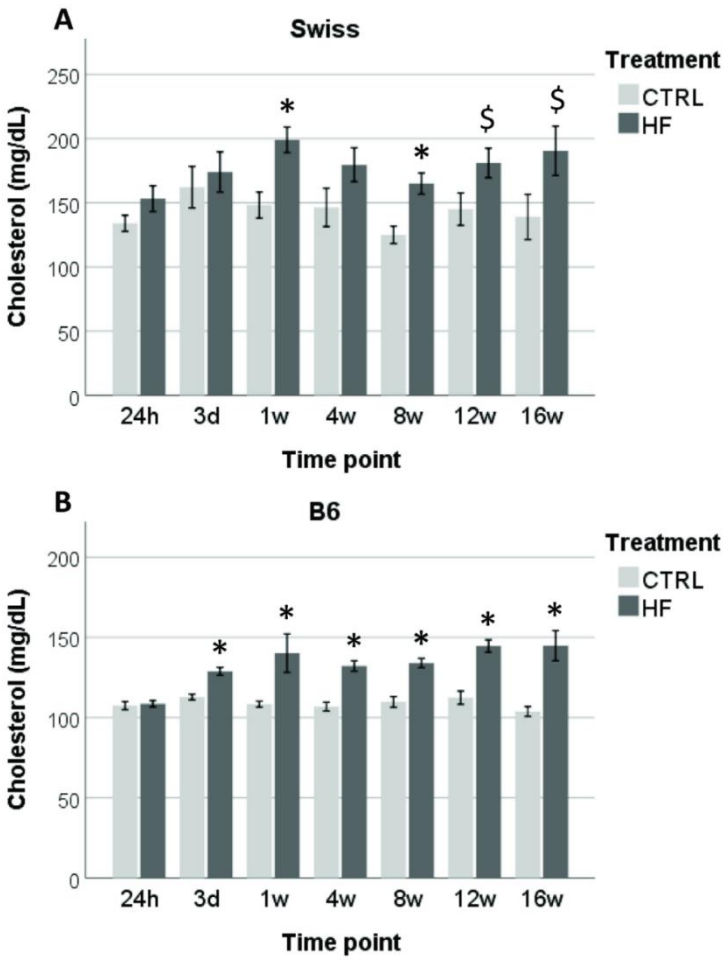


Fig 2. The effect of HF/HS diet on total serum cholesterol concentrations in both Swiss (A) and B6 (B) mice. Data are shown as mean ± SEM. Significant differences ($P \leq 0.05$) between HF/HS and control groups within each strain per time point are indicated by an asterisk (*). Tendencies ($P < 0.1$ and > 0.05) are indicated by a dollar sign ($).

4.3 Serum cytokine concentrations of Swiss mice

Based on the Kruskal Wallis test, no effects of age could be detected ($P > 0.1$) for any of the tested cytokines. The concentrations of GM-CSF, IFN-β, IL-1α , IFN-γ, MCP-1, TNF-α and IL-17A were not affected by diet ($P > 0.1$) at any of the time points (Figs 3A, 3B, 3C, 3D, 3E, 3F, 3G). Whereas, the HF/HS group exhibited higher serum concentrations of IL-23 ($P = 0.017$), IL-10 ($P = 0.046$), IL-27 ($P = 0.049$), IL12p70 ($P = 0.050$) and IL-1β ($P = 0.011$) and a tendency for a higher IL-6 ($P = 0.070$) only at the late phase compared with the control mice (Figs 3H, 3I, 3K, 3L, 3M, 3J).

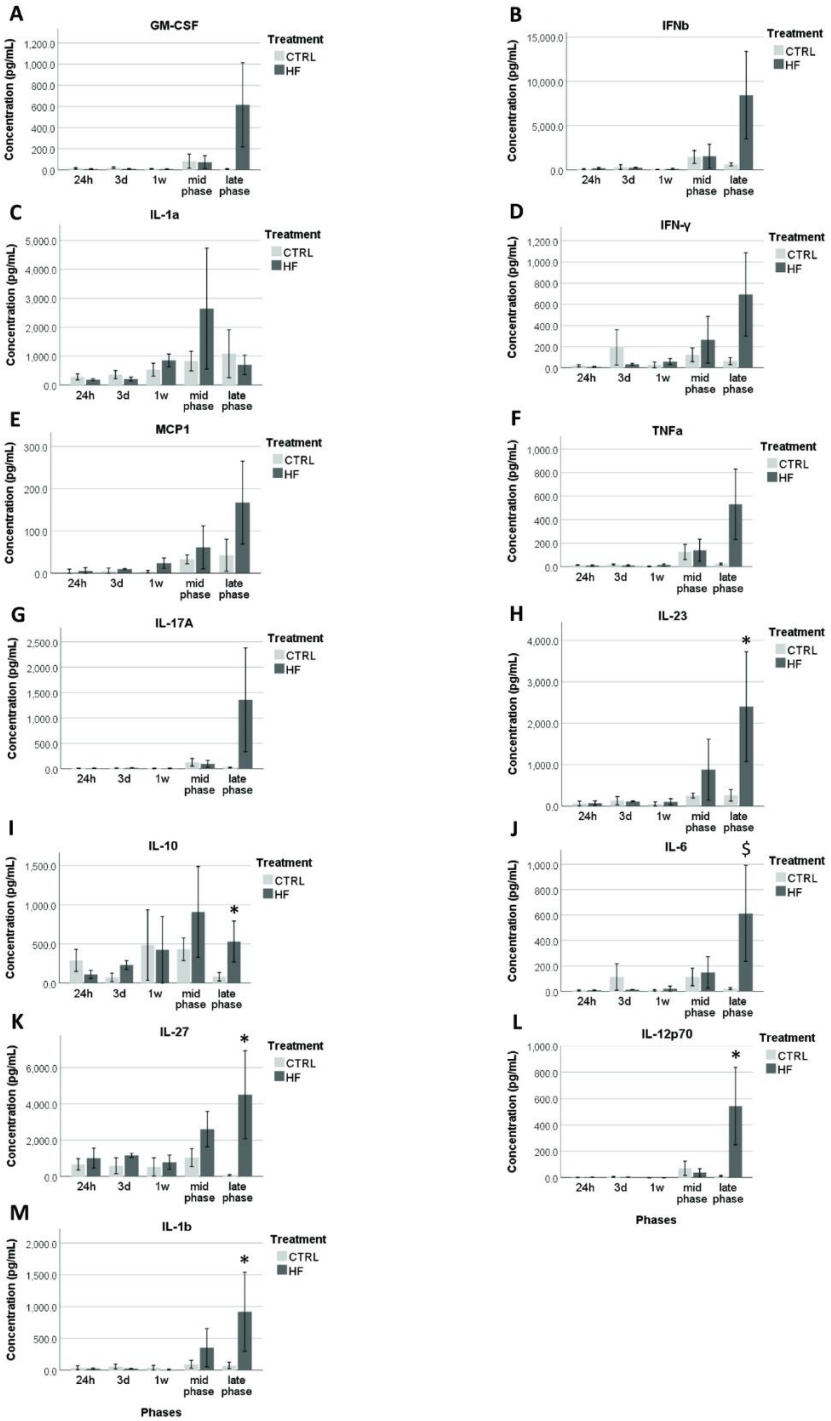

A
GM-CSF
B
IFNb
C
IL-1a
D
IFN-γ
E
MCP1
F
TNFa
G
IL-17A
H
IL-23
I
IL-10
J
IL-6
K
IL-27
L
IL-12p70
M
IL-1b
Concentration (pg/mL)
Treatment
CTRL
HF
24h
3d
1w
mid phase
late phase
Phases
*
$

Fig 3. HF/HS diet effects on serum concentrations (pg/mL) of different cytokines in Swiss mice. GM-CSF (A), IFN-β (B), IL-1α (C), IFN-γ (D), MCP-1 (E), TNF-α (F), IL-17A (G), IL-23 (H), IL-10 (I), IL-6 (J), IL-27 (K), IL12p70 (L), IL-1β (M). Data are shown as mean ± SEM. Significant differences ($P \leq 0.05$) between HF/HS and control groups per time point are indicated by an asterisk (*). Tendencies ($P < 0.1$ and > 0.05) are indicated by a dollar sign ($).

4.4 Gene expression patterns in OECs of Swiss mice

Kruskal Wallis comparison between the controls of different time points showed that aging did not affect gene expression patterns for any of the tested genes ($P > 0.1$). *PRDX1*, *PRDX3* and *PRDX6* are all genes involved in OS. *PRDX3* mRNA expression in HF/HS OECs was already significantly increased ($P = 0.049$) after 3d compared to the controls. No differences in expression were seen during the early and mid-phase. During the late phase, *PRDX3* expression tended to be upregulated ($P = 0.101$) in the HF/HS group. *PRDX1* only showed a tendency for upregulation ($P = 0.086$) during the early phase. *PRDX6* expression was only significantly increased during the late phase ($P = 0.030$) (Figs 4A, 4B, 4C). *NRF1*, a gene involved in OS pathways, did not show any differences at any time point between controls and HF/HS (Fig 4D). *NRF2*, another gene related to OS , tended to be upregulated 3d after a HF/HS diet ($P = 0.077$) and during the mid-phase ($P = 0.057$). No differences in expression between HF/HS group and control group were observed during the early phase and late phase (Fig 4E). *SOD2*, also related to OS, showed a continuous increase in expression in the HF/HS group starting at 1w. However, during the mid-phase this increase was not significant ($P = 0.064$) (Fig 4F). An upregulation of *BiP*, a marker gene related to ER-stress, was apparent in the acute phase ($P = 0.042$), mid-phase ($P = 0.014$) and late phase ($P = 0.029$). Nevertheless, *ATF4*, the downstream transcription factor of *BiP*, showed no significant differences between controls and HF/HS ($P > 0.1$) (Figs 4G and 4H). *IL-1*β was significantly upregulated ($P = 0.050$) during the late phase in the HF/HS group (Fig 4I). Finally, markers of mitochondrial stress (*HSPE1*, *HSPD1*) and protein folding (*HSPA8*) showed no different expression levels ($P > 0.1$) at any of the time points (Figs 4J, 4K, 4L). Pearson correlation showed a positive correlation between *IL-1*β and *PRDX6* ($r = 0.492$; $P = 0.011$) and also *NRF1* and *NRF2* correlated positively ($r = 0.918$; $P = 0.000$), all irrespective of time point (S3 Table).

4.5 Gene expression patterns in OECs of B6 mice

Similar to the Swiss mice, no age effects could be detected for any of the tested genes ($P > 0.1$). *PRDX1*, a marker of OS, was significantly increased in the HF/HS group 3d after the start of the diet ($P = 0.019$). No differences in expression levels of *PRDX1* were measured at later time points. *PRDX3* and *PRDX6* did not show a significant difference between HF/HS and control group at any of the time points (Figs 5A, 5B, 5C). No significant differences in mRNA expression were found for *NRF1* (Fig 5D). As was observed for *PRDX1*, *NRF2*, which is also related to OS, only showed a significantly increased expression level after 3d ($P = 0.021$) (Fig 5E). *SOD2* expression was significantly increased in the HF/HS group during the mid-phase ($P = 0.042$). No significant differences were observed at other time points (Fig 5F). *BiP* and *ATF4* were analyzed to evaluate ER-stress, and both were significantly higher in the HF/HS group 3d after the start of experimental feeding with P-values of 0.006 and 0.008 respectively (Figs 5G and 5H). *IL-1*β was significantly higher expressed in the HF/HS group during the mid-phase ($P = 0.002$) (Fig 5I). After 3d, *HSPD1*, a marker gene for mitochondrial stress, was significantly upregulated in the HF/HS group ($P = 0.008$). *HSPE1* never showed significant different expression levels (Fig 5J, 5K). Finally, *HSPA8*, which is related to protein folding, was significantly increased in the HF/HS group after 3d with a P-value of 0.002 (Fig 5L). The graphs (Fig 5) show that, all genes (except *HSPD1, NRF2* and *ATF4*) were downregulated in the HF/HS compared to the controls at 1w and during the late phase. However, these differences were not significant. A positive correlation was found between *BiP* and *ATF4* ($r = 0.665$; $P = 0.000$) and between *NRF1* and *NRF2* ($r = 0.741$; $P = 0.000$) (S4 Table).

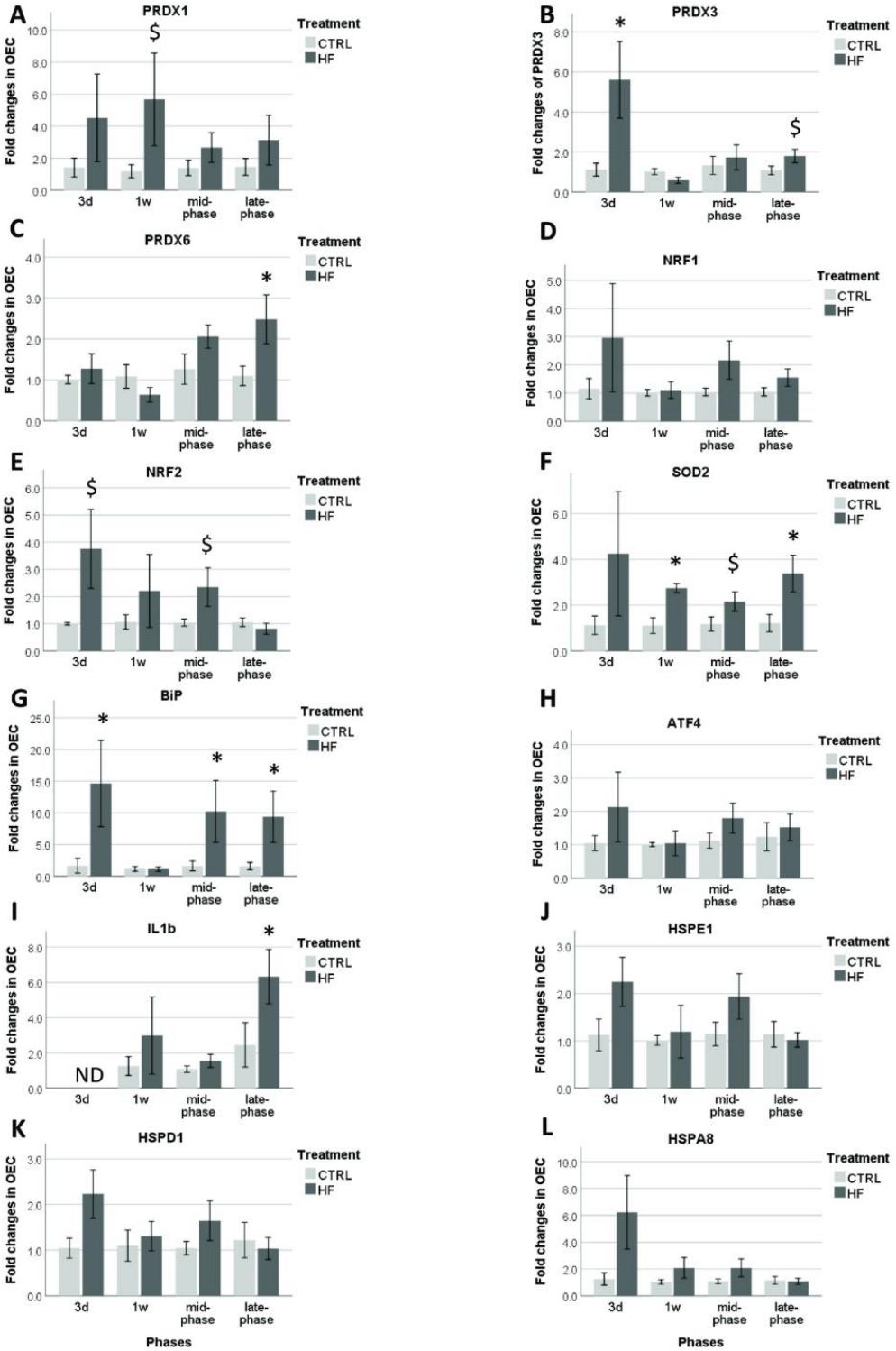

A
PRDX1
B
PRDX3
C
PRDX6
D
NRF1
E
NRF2
F
SOD2
G
BiP
H
ATF4
I
IL1b
J
HSPE1
K
HSPD1
L
HSPA8
Fold changes in OEC
Fold changes of PRDX3
Treatment
CTRL
HF
3d
1w
mid-phase
late-phase
Phases
ND

Fig 4. HF/HS diet effects on transcription markers of oxidative stress (A,B,C,D,E,F), ER-stress (G,H), inflammation (I), mitochondrial stress (J,K) and protein folding (L) in Swiss OECs. Columns display mean ± SEM of fold changes relative to housekeeping genes. Significant changes ($P \leq 0.05$) between HF/HS and control groups per time point are indicated with an asterisk (*). Tendencies ($P < 0.1$ and > 0.05) are indicated by a dollar sign ($). ND stands for 'not detected'.

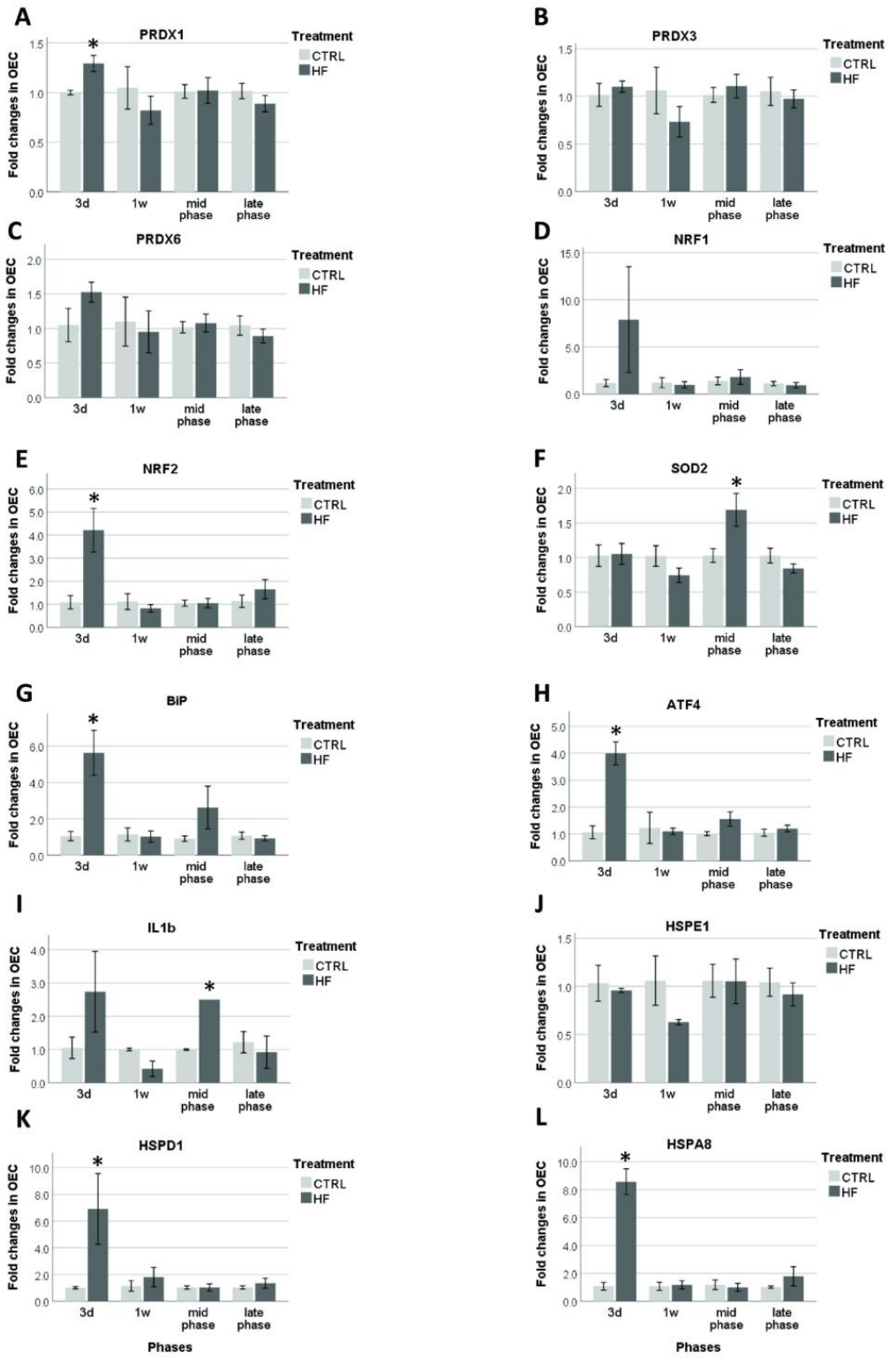

A
PRDX1
B
PRDX3
C
PRDX6
D
NRF1
E
NRF2
F
SOD2
G
BiP
H
ATF4
I
IL1b
J
HSPE1
K
HSPD1
L
HSPA8
Fold changes in OEC
Treatment
CTRL
HF
3d
1w
mid phase
late phase
Phases
*

Fig 5. HF/HS diet effects on transcription markers of oxidative stress (A,B,C,D,E,F), ER-stress (G,H), inflammation (I), mitochondrial stress (J,K) and protein folding (L) in B6 OECs. Columns display means ± SEM of fold changes relative to housekeeping genes. Significant changes ($P \leq 0.05$) between HF/HS and control groups per time point are indicated with an asterisk (*).

5. Discussion

The aim of this study was to investigate the effect of feeding a HF/HS diet for different durations (from 24h to 16 weeks) on the oviductal epithelial cell (OECs) physiology in both B6 (inbred) and Swiss (outbred) mice. We chose to focus this investigation at the transcriptomic level because changing gene expression is the first cellular response to stress. We focused the analysis on a strategically selected list of genes that are known to be involved in cellular lipotoxicity and oxidative stress. This maximises the chance of detecting specific changes compared to wide screening techniques such as RNAseq where such differences might be overlooked. Feeding a HF/HS diet resulted in a significant increase in body weight and blood cholesterol concentrations compared to the controls very early after starting the HF/HS diet feeding. These effects progressively increased over time and were later, in the Swiss mice, associated with a significant increase in circulating inflammatory cytokines after 12w compared with mice fed a control diet. Feeding a HF/HS diet altered the expression of genes related to oxidative stress (OS) in the OECs as early as 3d, which was subsequently followed by a cascade of transcriptomic changes related to mitochondrial reactive oxygen species (ROS) production and ER-stress, and ultimately transcriptomic changes showing a local inflammatory response during the late phase (12-16w). The magnitude and timing of these transcriptomic changes were strain dependent.

5.1 Temporal systemic metabolic changes after the introduction of a HF/HS diet

The difference in weight gain between HF/HS and controls mice was significant starting from 1w of feeding in the Swiss mice and from 2w in the B6 mice, which is in line with our previous report (Marei et al., 2020). The response of Swiss mice in weight gain was more variable compared to B6 mice due to their outbred nature. Swiss mice reached a maximum weight (plateau) at 12w whereas the B6 continued to increase in weight until week 16. This might be due to a metabolic adaptation to the increased adiposity in older Swiss mice (Krishna et al., 2016) while the B6 mice appear to maintain their ability to store fat in their adipocytes (Kleinert et al., 2018). Feeding a HF/HS diet also led to a significant increase in serum total cholesterol concentration both in Swiss (from 1w onwards) and B6 (from 3d onwards) mice. The significant increase in body weight and serum total cholesterol concentrations confirm that not only the B6 (Eisinger et al., 2014), but also the Swiss strain is metabolically sensitive to the high dietary saturated fat and fructose content (Marei et al., 2020). Such acute increase in serum cholesterol in B6, already from 3d after feeding, was also reported by Williams et al. (2014). Cholesterol concentrations continued to increase over time in both HF/HS groups and were also more variable in the Swiss mice. Total cholesterol concentrations were relatively lower in the B6 compared to the Swiss mice regardless of the diet, which is obviously due to the genetic background (Carter et al., 1997). Different factors might influence the difference in total cholesterol concentrations, such as increased hepatic synthesis rates of cholesterol due to obesity (Stahlberg et al., 1997) or intestinal cholesterol absorption which showed to have a direct correlation to serum cholesterol concentrations in mice (Schwarz et al., 2001). These factors may suggest a lower capacity of B6 mice to synthesize cholesterol in the liver or absorb cholesterol. Our data also show a positive correlation between body weight and total serum cholesterol concentrations, which was expected since increased weight gain has been shown to be associated with a higher rate of cholesterol synthesis (Nestel et al., 1973).

To investigate if a HF/HS diet can also induce a systemic inflammatory response, and to study the time by which such response is detected after feeding, we analyzed the 13 most commonly investigated inflammatory cytokines in serum using a multiplex immunoassay. We found that six of the tested cytokines, namely IL-6, IL-10, IL12p70, IL-23, IL-27 and IL-1β, were significantly higher during the late phase (12-16w) in the HF/HS mice serum compared to the controls, whereas no signs of inflammation could be detected earlier. Williams et al. (2014) reported an increased systemic inflammation in B6 mice (increased serum IL-6) only after 12w of HFD feeding, but an acute inflammatory response was also observed at 3d. IL-6 is produced by adipocytes and therefore its increased concentration might be due to obesity (Wueest and Konrad, 2018; Snider and Wood, 2019). On the other hand, our data show that the other measured cytokines including TNF-α and MCP-1 were not changed at any time point, which is also in line with previous studies (Williams et al., 2014; Haley et al., 2019; Li et al., 2020). Surprisingly, we found that IL-10, a cytokine with an anti-inflammatory capacity that limits the production of IL-1α, IL-1β, IL-6, IL-12, TNF-α and MCP-1 (Couper et al., 2008) was increased during the late phase in the HF/HS group. This increase was not detected in the study of Li et al. (2020) in Kunming, C57BL/6, BALB/c and ICR mouse strains and may be compensatory. IL-6, a pro-inflammatory cytokine which is stimulated by IL-17A (Zenobia and Hajishengallis, 2015) and IL12p70, a pro-inflammatory cytokine related to IL-27 and IL-23 (Gee et al., 2009) were still significantly higher or tended to be higher in the HF/HS group during late phase. It is probably not a coincidence that also IL-27, a cytokine with both pro-inflammatory and anti-inflammatory capacities and IL-23, which is considered a pro-inflammatory cytokine, were significantly increased during this phase (Carl and Bai, 2008; Tang et al., 2012). Besides IL-6, IL-17A is also known to stimulate the production of IL-1β, which was also increased during late phase in the serum of HF/HS mice (Zenobia and Hajishengallis, 2015).

5.2 Acute and chronic effects after short and long-term feeding of the obesogenic diet and the dietary induced metabolic stress on the oviduct

As mentioned in the introduction, the impact of HF/HS diet-induced metabolic alterations on the oviductal cell functions are not clearly defined. As described in other cell types, the mechanisms by which hyperlipidaemia induces lipotoxicity are known to be mainly mediated through OS, mitochondrial dysfunction, ER-stress and local cellular inflammatory responses (Engin; Lin et al., 2005; Herdt, 2013). Therefore we focused on these pathways in our OEC gene expression analysis. Studies for example showed an increase in proinflammatory genes and genes related to OS in ovaries of 4w HF-fed Sprague Dawley (SD) rats (Ruebel et al., 2016).

We could detect changes in the expression of some of the target genes, an effect which was dependent both on the duration of feeding and on the mouse strain. **In Swiss mice**, we observed that **3d** of feeding a HF/HS diet already resulted in increased expression of genes related to ER-stress and OS in the OECs; since both *BiP* and *PRDX3* were significantly upregulated. BiP is an ER chaperon protein that binds to misfolded proteins in the ER lumen and helps stabilizing and restoring the efficiency of protein folding by initiating UPRs (Wang et al., 2017). Nevertheless, *ATF4*, the downstream transcription factor of *BiP,* was not upregulated at any of the time points, meaning that the ATF4-mediated UPR in ER was not initiated. PRDX3 is a mitochondrial AO (Jeong et al., 2018) and hence its upregulation indicates an increased cellular OS level particularly within the mitochondria (Sadowska et al., 2019). These changes were accompanied by a tendency for a higher *NRF2* expression also at 3d, a protein that reacts to OS and ER-stress and act as a transcription factor that upregulates the expression of genes harbouring the antioxidant response element (ARE) sequence in their promotor, like the mitochondrial *SOD2* (Ohtsuji et al., 2008; Ruperez et al., 2014; Ashtekar et al., 2018). Altogether, this suggests an acute increase in mitochondrial OS and (mild) ER-stress in response to introducing a HF/HS diet, even before a significant change in body

weight or serum cholesterol could be detected. On the other hand, the expression of NRF1, which is a transcription factor with a similar binding specificity and expression profile as NRF2 (Ohtsuji et al., 2008) was not significantly affected by diet. Nevertheless, the level of expression of NRF1 and NRF2 were significantly positively correlated in the Swiss OEC data. A few days later, the increase in *NRF2* expression observed at 3d was indeed followed by an upregulation of *SOD2* at 1w (early phase), showing that the downstream effect of *NRF2* on *SOD2* was provoked (Ma et al., 2021). An increase in *SOD2* expression was also previously recorded in the white adipose tissue of obese mice and in the uterus, but not in the ovaries, of Wistar rats that were fed a HFD for 8w (Ruperez et al., 2014; Sadowska et al., 2019). In addition, Jordaens at al. (2017) reported a significant increase in *SOD1* expression in bovine OECs that were exposed to lipotoxic concentrations of free fatty acids *in vitro* (Jordaens et al., 2017). Nevertheless, according to our knowledge, our data here is the first to report such early NRF2-SOD2 OS response in the oviduct. The increase in *SOD2* reported here was also accompanied by a tendency for a higher *PRDX1* (AO) expression, which is also considered an NRF2-regulated gene (Ma, 2013). At the ER level, and unlike the response at 3d, *BiP* expression in the HF/HS group at 1w was similar to the controls indicating that protein folding in the ER was temporarily stabilized, and confirming that the ER-stress exhibited at 3d was mild. During the mid-phase (4-8w) a second wave of *NRF2/SOD2* AO response and ER-stress (*BiP*) was apparent. But again, no significant change in the *ATF4* expression was detected. Finally, in the late phase (12-16w), in addition to the *SOD2* and *BiP* which remained upregulated in the HF/HS OECs, a significantly higher expression of both *PRDX6* and *IL-1*β was detected. This timing of PRDX6 upregulation is similar to that reported in Swiss oocytes after 13w of feeding a high fat diet (Marei et al., 2020). PRDX6 is an AO enzyme modulated by the inflammatory cytokines IFN-γ and TNF-α. It regulates TNF or IFN-induced apoptosis through IL-1β production, by its phospholipase A2 (PLA2) activity (Burke and Dennis, 2009; Paula et al., 2013; Vazquez-Medina et al., 2013; Arevalo and Vazquez-Medina, 2018). Hence, the simultaneous

upregulation of *PRDX6* and *IL-1β* strongly suggests a chronic diet-induced inflammatory response in the oviduct during the late phase. PRDX6 does not only play a protective AO role, but is also considered as an activator of inflammatory pathways (Arevalo and Vazquez-Medina, 2018).

It is important to note the dynamics of the different PRDX antioxidants (PRDX1, 3 and 6) in the OECs in this study in response to the HF/HS diet. Unlike PRDX6, both PRDX1 and PRDX3 are not modulated by cytokines. And while PRDX1 and PRDX6 are localized in the cytosol, PRDX3 is expressed in the mitochondria (Paula et al., 2013; Vazquez-Medina et al., 2013). Therefore, the early upregulation of *PRDX3* at 3d may indicate that mitochondria are more sensitive to lipotoxicity compared to the ER in the OECs. Nevertheless, markers of mitochondrial stress (*HSPD1, HSPE1*) were not affected by the HF/HS diet. However, it has been previously shown that feeding a high-fat diet for 16 weeks induces mtDNA damage which correlates with mitochondrial dysfunction and increased OS in skeletal muscle and liver of mice (Yuzefovych et al., 2013). Again, in our study this indicates that the level of mitochondrial OS was not strong enough to induce mitochondrial UPRs in the OECs.

On the other hand, **in B6 mice**, the effects of the HF/HS diet on OECs gene expression were similar but not identical to the responses seen in the Swiss mice. Likewise, the changes in gene expression suggested an increase in OS and ER-stress already at 3d after the start of the HF/HS diet feeding. *NRF2* (AO), *PRDX1* (AO) and *BiP* (ER-stress) were significantly upregulated at 3d in the HF/HS B6 OECs. The earlier upregulation of NRF2 downstream gene *PRDX1* compared to the Swiss OECs (where it was upregulated at 1w) may be explained by the fact that NRF2 is not only activated by increasing levels of ROS, but also by increased circulating cholesterol as observed by Ma (2013), since serum cholesterol concentrations were increased earlier in B6 mice compared to Swiss mice. In contrast to the Swiss OECs, *ATF4* (the downstream transcription factor of BiP) was also significantly increased after 3d. This indicates an early activation of the ATF4-dependent UPRs in the ER and a higher level of cellular stress compared to that

experienced in the Swiss OECs (Ron and Walter, 2007). *BiP* and *ATF4* expression levels were positively correlated in the B6 OEC samples (r = 0.665; $P < 0.001$) but not in Swiss. *HSPA8*, which is important for the correct folding of proteins, was also significantly increased during the acute phase (3d). This is different from the Swiss mice where no changes in *HSPA8* expression were recorded, again suggesting a higher degree of stress in the B6 mice. As observed in the Swiss strain, *NRF1* expression was positively correlated with *NRF2* but was not significantly upregulated. It is remarkable that *HSPD1*, which is a marker gene for mitochondrial stress and responsible for refolding of misfolded proteins in the mitochondria (Klebl et al., 2021) was also significantly upregulated at 3d. This indicates that, unlike in Swiss mice, the HF/HS diet induced a very acute mitochondrial stress in B6 OECs. These cells failed to upregulate PRDX3 expression which may have contributed to increased mitochondrial stress levels. Marei et al. (2020) also reported an increase in mitochondrial stress in cumulus cells of HFD fed B6 mice that was not found in oocytes or cumulus cells of HFD fed Swiss mice. However, in their study it was *HSPE1* that was significantly differentially expressed (Marei et al., 2020). Such higher mitochondrial sensitivity in B6 has been previously described to be genetic in origin due to inbreeding. Cross insertion of mtDNA from B6 mice to the outbred C3H/HeN mice *in vivo* resulted in higher mitochondrial inner membrane potential and the generation of more ROS in cardiomyocytes (Fetterman et al., 2013). A higher proportion of abnormal mitochondria was also detected in the oocytes of the B6 compared to the Swiss oocytes even in mice fed a normal diet (Marei et al., 2020). Subsequently, and in contrast to the changes observed at 3d, none of the tested genes was significantly affected by the diet during the early phase (at 1w), suggesting that the stress levels were temporarily normalized or stabilized by the earlier adaptations in gene transcription. After longer exposure to the diet, during the mid-phase, a significant increase in mitochondrial *SOD2* was detected, showing a second wave of mitochondrial OS and the presence of a *NRF2/SOD2* AO response. In addition, *IL-1β* was also significantly increased during this phase, indicating a relatively

earlier inflammatory response compared to that seen in the Swiss OECs at the late phase. However, this change was not accompanied with changes in *PRDX6* expression in B6. Upregulation of *IL-1β* was also reported in the muscles of B6 mice after 12w of HFD feeding (Williams et al., 2014). This relatively earlier sign of cellular inflammation in B6 compared to Swiss might also be due to the relatively higher cellular stress levels. Finally, during the late phase, none of the tested genes was significantly affected by diet despite the continued increase in weight gain and hypercholesterolemia. This could be due to the advanced age of the mice at this time point and the relatively shorter reproductive lifespan of the B6 compared to Swiss mice (Czajkowska et al., 2020).

Our gene expression data shows that the sequence of pathophysiological changes that are known to occur at the cellular level in response to diet-induced lipotoxicity are evident in the oviduct of both Swiss and B6 mice. In other words, the OECs appear to be sensitive to the maternal metabolic stress induced by the HF/HS diet. These cells are in direct contact with the embryo and create its micro-environment (Aviles et al., 2010). Based on our in-depth analysis of the strategically-selected target genes, we can state that the sequence of changes in Swiss mice start with an acute increase in OS and protein misfolding in the ER followed by the initiation of a cascade of transcriptomic changes to control mitochondrial ROS production and ER-stress. The specific metabolic changes in B6 mice are mainly observed during the acute phase (3d) and involve higher levels of mitochondrial OS. The level of cellular stress appears to be higher in B6 to the level that stimulates the expression of UPR genes in the ER and mitochondria. Despite the activation of some endogenous AO mechanisms, chronic exposure to such stress results in local inflammatory responses that were initiated earlier in B6 mice compared to the Swiss mice.

It is important to notice that these very interesting and biologically relevant differences that we could detect between the dietary groups at different timepoints validated one

another as they formed a very logical network of molecular interaction and cellular events. The fact that similar pathways are affected in a similar sequence (with a few differences in severity) in both mouse strains can also be seen as a strong validation of the described responses.

We are the first to show that the impact of the HF/HS diet on OECs can lead to changes in the oviductal microenvironment that may occur as a very acute oxidative stress response to the diet even before the development of an obese phenotype. These changes can put the developing embryo at risk and may lead to reduced fertility. This illustrates that the mechanism by which the obesogenic diet impacts fertility is not only mediated through reduced oocyte quality, but might also directly impact early embryo development in the oviduct, leading to long-term effects on fetal development, pregnancy success, and postnatal health through epigenetic alterations. Therefore, our findings might explain why subtle nutritional challenges exerted exclusively during the preimplantation period, resulted in offspring with a higher risk of developing deleterious phenotypes in adulthood (Velazquez, 2015). However, such impact on the developing zygote within the oviduct of HF/HS diet-fed mothers is practically and technically very difficult to investigate.

In **conclusion**, administration of a HF/HS diet for a short period (as shown here at 3d) results in acute systemic changes and acute local OS or mitochondrial stress effects on OECs, evident already after short-term obesogenic feeding and before the development of an obese phenotype. The acute effects in the OECs initiate a cascade of transcriptomic changes to control mitochondrial ROS production and ER-stress. However, in the mid and late phase a persistent upregulation of (mitochondrial) OS and ER-stress is observed, with ultimate signs of local and systemic inflammation in the late phase.

To the best of our knowledge, this is the first study that describes the effect of metabolic stress on the oviductal microenvironment *in vivo* and illustrates that

oviductal cells can sense (or can react on) systemic diet-induced hyperlipidaemia. Further studies in our laboratory currently focus on the impact of such increased OEC stress levels on early embryo development. This study also shows different responses to a HF/HS diet between Swiss and B6 mice. The acute responses showed a higher mitochondrial sensitivity in B6 mice compared to the Swiss mice, which were showing acute OS responses. B6 mice showed earlier local inflammatory responses compared to the Swiss mice. These differences indicate that the effects of a HF/HS diet are dependent on the genetic background which should aid in designing further research.

6. Supplementary material

S1 Table. Functions and full names of cytokines measured in the serum

Cytokine	Full name	Function
GM-CSF	Granulocyte macrophage colony-stimulating factor	Monomeric glycoprotein that functions as a cytokine. Stimulates stem cells to produce granulocytes (neutrophils, eosinophils and basophils) (Becher et al., 2016).
IFN-β	Interferon β	Type I interferon. Induces the transcription of genes encoding inflammatory cytokines and chemokines (GeneCards).
IL-1α	Interleukin 1α	Inflammatory cytokine that activates the inflammatory process (Di Paolo and Shayakhmetov, 2016). Cytokine required for activating the innate immune response (Ott et al., 2007).
IFN-γ	Interferon γ	Type II interferon with an antiviral activity (Tau and Rothman, 1999).
MCP-1	Monocyte chemoattractant protein-1	Chemokine that regulates the migration and infiltration of monocytes/macrophages (GeneCards).
TNF-α	Tumor necrosis factor α	Inflammatory cytokine produced by macrophages/monocytes during acute inflammation. Responsible for a diverse range of signalling events within cells, leading to necrosis or apoptosis (Idriss and Naismith, 2000). Cytokine required for activating the innate immune response (Ott et al., 2007).
IL-17A	Interleukin 17A	Proinflammatory cytokine produced by activated T cells. Stimulates the expression of IL6 and COX-2 (Zenobia and Hajishengallis, 2015).

IL-23	Interleukin 23	Pro-inflammatory cytokine that stimulates the production of IL-17 (Tang et al., 2012).
IL-10	Interleukin 10	Anti-inflammatory cytokine that limits the production of proinflammatory cytokines and chemokines (Couper et al., 2008).
IL-6	Interleukin 6	Both a pro-inflammatory and an anti-inflammatory cytokine. Induces different acute phase proteins and IL-10 but has also inhibitory effects in TNF-α and IL-1 (Tanaka et al., 2014).
IL-27	Interleukin 27	Has both pro-inflammatory and anti-inflammatory properties. Considerable conflicting data exists about the role of IL-27 (Carl and Bai, 2008).
IL12p70	Interleukin 12p70	Pro-inflammatory cytokine. Induces IFN- γ and TNF-α production (Gee et al., 2009).
IL-1β	Interleukin 1β	Pro-inflammatory cytokine. Synergizes with TNF to produce IL-6. Synergize with IL-23 to induce IL-17 production (Zenobia and Hajishengallis, 2015).

1. Becher B, Tugues S, Greter M. GM-CSF: From Growth Factor to Central Mediator of Tissue Inflammation. Immunity. 2016;45(5):963-73.
2. GeneCards. [Available from: https://www.genecards.org/.
3. Di Paolo NC, Shayakhmetov DM. Interleukin 1alpha and the inflammatory process. Nat Immunol. 2016;17(8):906-13.
4. Ott LW, Resing KA, Sizemore AW, Heyen JW, Cocklin RR, Pedrick NM, et al. Tumor Necrosis Factor-alpha- and interleukin-1-induced cellular responses: coupling proteomic and genomic information. J Proteome Res. 2007;6(6):2176-85.
5. Tau G, Rothman P. Biologic functions of the IFN-gamma receptors. Allergy. 1999;54(12):1233-51.
6. Idriss HT, Naismith JH. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). Microsc Res Tech. 2000;50(3):184-95.
7. Zenobia C, Hajishengallis G. Basic biology and role of interleukin-17 in immunity and inflammation. Periodontol 2000. 2015;69(1):142-59.
8. Tang C, Chen S, Qian H, Huang W. Interleukin-23: as a drug target for autoimmune inflammatory diseases. Immunology. 2012;135(2):112-24.
9. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. J Immunol. 2008;180(9):5771-7.
10. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6(10):a016295.
11. Carl JW, Bai XF. IL27: its roles in the induction and inhibition of inflammation. Int J Clin Exp Pathol. 2008;1(2):117-23.
12. Gee K, Guzzo C, Che Mat NF, Ma W, Kumar A. The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. Inflamm Allergy Drug Targets. 2009;8(1):40-52.

S2 Table. Functions and full names of genes of interest used for qPCR

Gene	Full name	Domain	Function
SOD2	Superoxide dismutase 2	Oxidative stress	Mitochondrial enzyme that helps maintain the redox balance by converting superoxide radicals to hydrogen peroxide (Ashtekar et al., 2018).
NRF1	Nuclear respiratory factor 1	Oxidative stress	Binds to ARE (antioxidant response element) and regulates the expression of a number of genes involved in oxidative stress (Biswas and Chan, 2010).
PRDX1	Peroxiredoxin-1	Oxidative stress	Antioxidant protein, constitute a potent defence system for maintaining redox balance by converting hydrogen peroxide to water (Jeong et al., 2018).
PRDX3	Peroxiredoxin-3 / Thioredoxin-dependent peroxide reductase	Oxidative stress	Antioxidant protein, constitute a potent defence system for maintaining redox balance by converting hydrogen peroxide to water (Jeong et al., 2018).
PRDX6	Peroxiredoxin-6	Oxidative stress	Antioxidant protein, constitute a potent defence system for maintaining redox balance by converting hydrogen peroxide to water (Jeong et al., 2018).
NRF2	Nuclear factor erythroid 2-related factor 2	Oxidative stress	Binds to ARE (antioxidant response element) and regulates the expression of a number of genes involved in oxidative stress (Biswas and Chan, 2010).

BiP (HSPA5)	Binding immunoglobulin protein (Heat Shock Protein Family A Member 5)	Endoplasmic reticulum stress	Involved in maintaining proper protein folding and assembly in the ER (Wang et al., 2014).
ATF4	Activating transcription factor 4	Endoplasmic reticulum stress	Coordinates the response to oxidative stress and ER-stress by promoting expression of genes linked to resistance to oxidative stress (Lange et al., 2008).
HSPE1	Heat shock protein family E member 1	Mitochondrial stress	Assists folding of proteins in the mitochondrial matrix space (Bie et al., 2016).
HSPD1	Heat shock protein family D member 1	Mitochondrial stress	Responsible for (re)-folding of nuclear-encoded proteins that are imported into the mitochondria (Klebl et al., 2021).
HSPA8	Heat shock protein family A member 8	Protein folding (chaperons)	Plays a role in protein quality control, ensuring the correct folding and re-folding of selected proteins (Bonam et al., 2019).
IL-1β	Interleukin-1beta	Inflammation	Inflammatory cytokine (Madan et al., 2009).

S3 Table. Pearson correlation Swiss mice

		Weight	Cholesterol	*BiP*	*PRDX1*	*HSPE1*	*SOD2*	*PRDX6*	*PRDX3*	*NRF2*	*HSPD1*	*HSPA8*	*ATF4*	*NRF1*	*IL-1 β*
Weight	Pearson Correlation	1													
	Sig. (2-tailed)														
Cholesterol	Pearson Correlation	.322**	1												
	Sig. (2-tailed)	0,008													
BiP	Pearson Correlation	0,205	.330*	1											
	Sig. (2-tailed)	0,193	0,035												
PRDX1	Pearson Correlation	-0,027	.361*	0,117	1										
	Sig. (2-tailed)	0,864	0,02	0,46											
HSPE1	Pearson Correlation	0,01	0,052	.597**	-0,135	1									
	Sig. (2-tailed)	0,952	0,748	0	0,393										
SOD2	Pearson Correlation	0,163	.414**	0,289	.683**	0,033	1								
	Sig. (2-tailed)	0,302	0,007	0,063	0	0,835									
PRDX6	Pearson Correlation	.563**	.359*	0,186	.330*	0,07	.349*	1							
	Sig. (2-tailed)	0	0,025	0,25	0,038	0,669	0,027								
PRDX3	Pearson Correlation	-0,165	0,123	0,282	.551**	0,012	.526**	0,105	1						
	Sig. (2-tailed)	0,297	0,442	0,07	0	0,938	0	0,521							
NRF2	Pearson Correlation	-0,211	-0,216	-0,074	0,01	-0,122	0,028	-0,141	.325*	1					
	Sig. (2-tailed)	0,179	0,176	0,639	0,948	0,442	0,858	0,385	0,036						
HSPD1	Pearson Correlation	-0,243	-0,209	-0,032	0,195	-0,072	-0,014	-0,177	.628**	.580**	1				
	Sig. (2-tailed)	0,121	0,189	0,842	0,216	0,651	0,928	0,275	0	0					
HSPA8	Pearson Correlation	-0,258	-0,205	0,024	0,114	-0,025	0,138	-0,193	.565**	.924**	.750**	1			
	Sig. (2-tailed)	0,099	0,2	0,878	0,47	0,874	0,382	0,234	0	0	0				
ATF4	Pearson Correlation	-0,122	-0,251	-0,136	0,193	-0,218	0,099	-0,106	.560**	.720**	.832**	.795**	1		
	Sig. (2-tailed)	0,44	0,114	0,389	0,221	0,165	0,531	0,513	0	0	0	0			
NRF1	Pearson Correlation	-0,204	-0,199	-0,026	0,072	-0,135	-0,025	0,029	.448**	.918**	.801**	.916**	.842**	1	
	Sig. (2-tailed)	0,194	0,213	0,872	0,649	0,393	0,873	0,857	0,003	0	0	0	0		
IL-1 β	Pearson Correlation	.684**	.479*	0,07	0,381	-0,043	.540**	.492*	0,13	-0,17	-0,203	0,04	0,236	0,348	1
	Sig. (2-tailed)	0	0,015	0,733	0,055	0,833	0,004	0,011	0,525	0,407	0,32	0,844	0,247	0,081	

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

S4 Table. Pearson correlation B6 mice

		Weight	Cholesterol	*BiP*	*PRDX1*	*HSPE1*	*SOD2*	*PRDX6*	*PRDX3*	*NRF2*	*HSPD1*	*HSPA8*	*ATF4*	*NRF1*	*IL-1 β*
Weight	Pearson Correlation	1													
	Sig. (2-tailed)														
Cholesterol	Pearson Correlation	.536**	1												
	Sig. (2-tailed)	0													
BiP	Pearson Correlation	-0,047	0,22	1											
	Sig. (2-tailed)	0,766	0,162												
PRDX1	Pearson Correlation	-0,215	-0,16	0,04	1										
	Sig. (2-tailed)	0,171	0,312	0,802											
HSPE1	Pearson Correlation	-0,031	-0,099	-0,194	.409**	1									
	Sig. (2-tailed)	0,845	0,533	0,218	0,007										
SOD2	Pearson Correlation	-0,173	0,009	0,186	.443**	0,106	1								
	Sig. (2-tailed)	0,273	0,955	0,237	0,003	0,502									
PRDX6	Pearson Correlation	-0,045	0,005	0,103	.322*	0,052	0,208	1							
	Sig. (2-tailed)	0,779	0,974	0,515	0,037	0,743	0,187								
PRDX3	Pearson Correlation	-0,068	-0,105	0,085	.560**	0,032	.601**	.470**	1						
	Sig. (2-tailed)	0,669	0,509	0,59	0	0,841	0	0,002							
NRF2	Pearson Correlation	0,09	0,199	.478**	0,259	-0,079	0,118	.439**	.308*	1					
	Sig. (2-tailed)	0,571	0,206	0,001	0,097	0,618	0,455	0,004	0,047						
HSPD1	Pearson Correlation	-0,039	0,14	.462**	0,11	-0,045	0,027	.499**	0,118	.826**	1				
	Sig. (2-tailed)	0,806	0,377	0,002	0,487	0,776	0,864	0,001	0,455	0					
HSPA8	Pearson Correlation	-0,01	0,188	.649**	0,125	-0,038	-0,048	.417**	0,15	.772**	.840**	1			
	Sig. (2-tailed)	0,948	0,234	0	0,429	0,813	0,764	0,006	0,345	0	0				
ATF4	Pearson Correlation	-0,071	0,194	.665**	0,172	-0,248	0,221	.472**	0,226	.748**	.775**	.808**	1		
	Sig. (2-tailed)	0,655	0,219	0	0,276	0,113	0,161	0,002	0,151	0	0	0			
NRF1	Pearson Correlation	-0,069	0,056	.425**	-0,033	-0,104	0,122	.335*	0,09	.741**	.882**	.627**	.696**	1	
	Sig. (2-tailed)	0,664	0,727	0,005	0,837	0,513	0,44	0,03	0,569	0	0	0	0		
IL-1 β	Pearson Correlation	-0,175	-0,089	-0,003	.644**	0,109	0,307	0,133	.558**	.398*	0,19	0,093	0,169	0,174	1
	Sig. (2-tailed)	0,392	0,666	0,989	0	0,595	0,127	0,517	0,003	0,044	0,353	0,65	0,409	0,394	

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

DIO – 60 kJ% fat (beef tallow)

ssniff

HF diet for rodents with tallow (& soybean oil) ¦ corresponds to D12492 (II) mod.

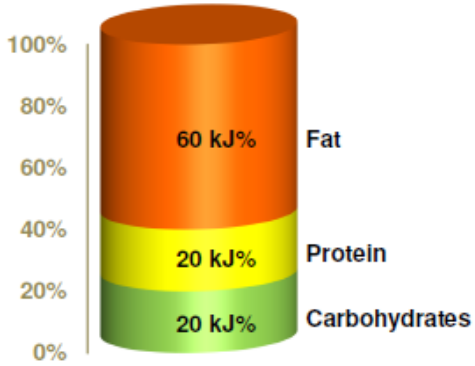
Description

This purified diet of the DIO series has very high fat contents (tallow). It is therefore used to induce obesity and metabolic syndrome / diabetes (NIDDM) in mice. Beef tallow is characterized by a more saturated fatty acid composition (C16:0 & C18:0) compared to lard.

The diet is intended for ad libitum feeding. The animals should have free access to fresh water.

Gross Energy (GE)	**25.0**	**MJ/kg**
Metabolizable Energy (ME) [1)]	**21.6**	**MJ/kg**

[1)] = *Physiological fuel value (Atwater); correspond to 5,150 kcal/kg*



Crude Nutrients	[%]
Crude protein (N x 6.25)	24.4
Crude fat	34.6
Crude fibre	6.0
Crude ash	5.3
Starch	0.1
Sugar	9.4
N free extracts	26.3

Minerals	[%]
Calcium	0.92
Phosphorus	0.64
Ca / P	1.44: 1
Sodium	0.20
Magnesium	0.23
Potassium	0.97

Fatty acids	[%]
C 12:0	0.04
C 14:0	1.18
C 16:0	8.27
C 17:0	0.38
C 18:0	6.06
C 20:0	0.04
C 16:1	1.33
C 18:1	12.29
C 18:2	2.53
C 18:3	0.34

Amino acids	[%]
Lysine	2.02
Methionine	0.86
Cystine	0.45
Met+Cys	1.31
Threonine	1.07
Tryptophan	0.33
Arginine	0.95
Histidine	0.74
Valine	1.70
Isoleucine	1.38
Leucine	2.42
Phenylalanine	1.27
Phe+Tyr	2.56
Glycine	0.52
Glutamic acid	5.50
Aspartic acid	1.82
Proline	2.80
Serine	1.46
Alanine	0.81

Vitamins	per kg	
Vitamin A	15,000	IU
Vitamin D_3	1,500	IU
Vitamin E	150	mg
Vitamin K (as MNB)	20	mg
Thiamine (B_1)	25	mg
Riboflavin (B_2)	16	mg
Pyridoxine (B_6)	16	mg
Cobalamin (B_{12})	30	µg
Nicotinic acid	47	mg
Pantothenic acid	55	mg
Folic acid	16	mg
Biotin	300	µg
Choline	1,140	mg

Trace elements	per kg	
Iron	168	mg
Manganese	95	mg
Zinc	65	mg
Copper	13	mg
Iodine	1.2	mg
Selenium	0.2	mg

Main products

E15741-30 Meal / powder
E15741-34 10 mm pellets
E15741-347 10 mm pellets, γ-irradiated 25 kGy

Extra cholesterol addition on request (customized)

DIO – 10 kJ% fat, ~7 % sucrose

ssniff

LF Control diet for rodents with lard (& soybean oil) ¦ corresponds to D12450J

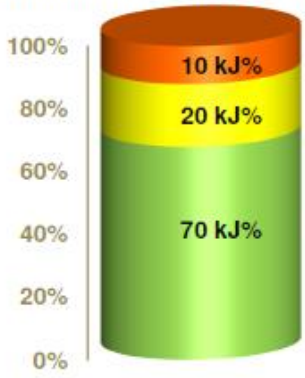
Description

This purified diet of the DIO series is one of three control diets with low fat and energy contents. The sucrose content of this diet has been further reduced 6.8 %.

The diet is intended for ad libitum feeding. The animals should have free access to fresh water.

Gross Energy (GE)	**17.9**	**MJ/kg**
Metabolizable Energy (ME) [1)]	**15.2**	**MJ/kg**

[1)] *= Physiological fuel value (Atwater); correspond to 3,630 kcal/kg*



Crude Nutrients	[%]
Crude protein (N x 6.25)	18.2
Crude fat	4.1
Crude fibre	5.0
Crude ash	5.3
Starch	43.5
Sugar	6.8
N free extracts	63.0

Minerals	[%]
Calcium	0.92
Phosphorus	0.63
Ca / P	1.46: 1
Sodium	0.20
Magnesium	0.23
Potassium	0.97

Fatty acids	[%]
C 12:0	0.01
C 14:0	0.04
C 16:0	0.69
C 18:0	0.31
C 20:0	0.02
C 16:1	0.05
C 18:1	1.30
C 18:2	1.43
C 18:3	0.15

Amino acids	[%]
Lysine	1.52
Methionine	0.66
Cystine	0.33
Met+Cys	0.99
Threonine	0.80
Tryptophan	0.24
Arginine	0.71
Histidine	0.55
Valine	1.27
Isoleucine	1.02
Leucine	1.80
Phenylalanine	0.95
Phe+Tyr	1.90
Glycine	0.39
Glutamic acid	4.10
Aspartic acid	1.35
Proline	2.09
Serine	1.09
Alanine	0.55

Vitamins	per kg	
Vitamin A	15,000	IU
Vitamin D_3	1,500	IU
Vitamin E	150	mg
Vitamin K (as MNB)	20	mg
Thiamine (B_1)	25	mg
Riboflavin (B_2)	16	mg
Pyridoxine (B_6)	16	mg
Cobalamin (B_{12})	30	µg
Nicotinic acid	47	mg
Pantothenic acid	55	mg
Folic acid	16	mg
Biotin	300	µg
Choline	920	mg

Trace elements	per kg	
Iron	168	mg
Manganese	95	mg
Zinc	65	mg
Copper	13	mg
Iodine	1.2	mg
Selenium	0.2	mg

Main products

E157453-00	Meal / powder
E157453-04	10 mm pellets
E157453-047	10 mm pellets, γ-irradiated 25 kGy

7. References

AREVALO, J. A. & VAZQUEZ-MEDINA, J. P. 2018. The Role of Peroxiredoxin 6 in Cell Signaling. *Antioxidants (Basel),* 7.

ASHTEKAR, A., HUK, D., MAGNER, A., LA PERLE, K. M. D., BOUCAI, L. & KIRSCHNER, L. S. 2018. Alterations in Sod2-Induced Oxidative Stress Affect Endocrine Cancer Progression. *J Clin Endocrinol Metab,* 103**,** 4135-4145.

AVILES, M., GUTIERREZ-ADAN, A. & COY, P. 2010. Oviductal secretions: will they be key factors for the future ARTs? *Mol Hum Reprod,* 16**,** 896-906.

BECHER, B., TUGUES, S. & GRETER, M. 2016. GM-CSF: From Growth Factor to Central Mediator of Tissue Inflammation. *Immunity,* 45**,** 963-973.

BESENFELDER, U., HAVLICEK, V. & BREM, G. 2012. Role of the oviduct in early embryo development. *Reprod Domest Anim,* 47 Suppl 4**,** 156-63.

BIE, A. S., FERNANDEZ-GUERRA, P., BIRKLER, R. I., NISEMBLAT, S., PELNENA, D., LU, X., DEIGNAN, J. L., LEE, H., DORRANI, N., CORYDON, T. J., PALMFELDT, J., BIVINA, L., AZEM, A., HERMAN, K. & BROSS, P. 2016. Effects of a Mutation in the HSPE1 Gene Encoding the Mitochondrial Co-chaperonin HSP10 and Its Potential Association with a Neurological and Developmental Disorder. *Front Mol Biosci,* 3**,** 65.

BISWAS, M. & CHAN, J. Y. 2010. Role of Nrf1 in antioxidant response element-mediated gene expression and beyond. *Toxicol Appl Pharmacol,* 244**,** 16-20.

BLUHER, M. 2019. Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol,* 15**,** 288-298.

BONAM, S. R., RUFF, M. & MULLER, S. 2019. HSPA8/HSC70 in Immune Disorders: A Molecular Rheostat that Adjusts Chaperone-Mediated Autophagy Substrates. *Cells,* 8.

BURKE, J. E. & DENNIS, E. A. 2009. Phospholipase A2 structure/function, mechanism, and signaling. *J Lipid Res,* 50 Suppl**,** S237-42.

CARL, J. W. & BAI, X. F. 2008. IL27: its roles in the induction and inhibition of inflammation. *Int J Clin Exp Pathol,* 1**,** 117-23.

CARTER, C. P., HOWLES, P. N. & HUI, D. Y. 1997. Genetic variation in cholesterol absorption efficiency among inbred strains of mice. *J Nutr,* 127**,** 1344-8.

CHANKEAW, W., GUO, Y. Z., BAGE, R., SVENSSON, A., ANDERSSON, G. & HUMBLOT, P. 2018. Elevated non-esterified fatty acids impair survival and promote lipid accumulation and pro-inflammatory cytokine production in bovine endometrial epithelial cells. *Reprod Fertil Dev,* 30**,** 1770-1784.

COUPER, K. N., BLOUNT, D. G. & RILEY, E. M. 2008. IL-10: the master regulator of immunity to infection. *J Immunol,* 180**,** 5771-7.

CZAJKOWSKA, K., WALEWSKA, A., ISHIKAWA, T., SZCZEPANSKA, K. & AJDUK, A. 2020. Age-related alterations in fertilization-induced Ca2+ oscillations depend on the genetic background of mouse oocytesdagger. *Biol Reprod,* 103**,** 986-999.

DI PAOLO, N. C. & SHAYAKHMETOV, D. M. 2016. Interleukin 1alpha and the inflammatory process. *Nat Immunol,* 17**,** 906-13.

DISSARD, R., KLEIN, J., CAUBET, C., BREUIL, B., SIWY, J., HOFFMAN, J., SICARD, L., DUCASSE, L., RASCALOU, S., PAYRE, B., BULEON, M., MULLEN, W., MISCHAK, H., TACK, I., BASCANDS, J. L., BUFFIN-MEYER, B. & SCHANSTRA, J. P. 2013. Long term metabolic syndrome induced by a high fat high fructose diet leads to minimal renal injury in C57BL/6 mice. *PLoS One,* 8**,** e76703.

EISINGER, K., LIEBISCH, G., SCHMITZ, G., ASLANIDIS, C., KRAUTBAUER, S. & BUECHLER, C. 2014. Lipidomic analysis of serum from high fat diet induced obese mice. *Int J Mol Sci,* 15**,** 2991-3002.

ENGIN, A. 2017. The Definition and Prevalence of Obesity and Metabolic Syndrome. *Adv Exp Med Biol,* 960**,** 1-17.

ENGIN, A. B. *Obesity and Lipotoxicity*.

ENGIN, A. B. Obesity and Lipotoxicity. 960**,** 624.

FERNANDEZ-SANCHEZ, A., MADRIGAL-SANTILLAN, E., BAUTISTA, M., ESQUIVEL-SOTO, J., MORALES-GONZALEZ, A., ESQUIVEL-CHIRINO, C., DURANTE-MONTIEL, I., SANCHEZ-RIVERA, G., VALADEZ-VEGA, C. & MORALES-GONZALEZ, J. A. 2011. Inflammation, oxidative stress, and obesity. *Int J Mol Sci,* 12**,** 3117-32.

FETTERMAN, J. L., ZELICKSON, B. R., JOHNSON, L. W., MOELLERING, D. R., WESTBROOK, D. G., POMPILIUS, M., SAMMY, M. J., JOHNSON, M., DUNHAM-SNARY, K. J., CAO, X., BRADLEY, W. E., ZHANG, J., WEI, C. C., CHACKO, B., SCHURR, T. G., KESTERSON, R. A., DELL'ITALIA, L. J., DARLEY-USMAR, V. M., WELCH, D. R. & BALLINGER, S. W. 2013. Mitochondrial genetic background modulates bioenergetics and susceptibility to acute cardiac volume overload. *Biochem J,* 455**,** 157-67.

GEE, K., GUZZO, C., CHE MAT, N. F., MA, W. & KUMAR, A. 2009. The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. *Inflamm Allergy Drug Targets,* 8**,** 40-52.

GENECARDS. Available: https://www.genecards.org/ [Accessed].

GONZALEZ, M. B., LANE, M., KNIGHT, E. J. & ROBKER, R. L. 2018. Inflammatory markers in human follicular fluid correlate with lipid levels and Body Mass Index. *J Reprod Immunol,* 130**,** 25-29.

HALEY, M. J., KRISHNAN, S., BURROWS, D., DE HOOG, L., THAKRAR, J., SCHIESSL, I., ALLAN, S. M. & LAWRENCE, C. B. 2019. Acute high-fat feeding leads to disruptions in glucose homeostasis and worsens stroke outcome. *J Cereb Blood Flow Metab,* 39**,** 1026-1037.

HERDT, T. H. 2013. Metabolic diseases of dairy cattle. *Vet Clin North Am Food Anim Pract,* 29**,** xi-xii.

IDRISS, H. T. & NAISMITH, J. H. 2000. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). *Microsc Res Tech,* 50**,** 184-95.

IGOSHEVA, N., ABRAMOV, A. Y., POSTON, L., ECKERT, J. J., FLEMING, T. P., DUCHEN, M. R. & MCCONNELL, J. 2010a. Maternal Diet-Induced Obesity Alters

Mitochondrial Activity and Redox Status in Mouse Oocytes and Zygotes. *Plos One,* 5.

IGOSHEVA, N., ABRAMOV, A. Y., POSTON, L., ECKERT, J. J., FLEMING, T. P., DUCHEN, M. R. & MCCONNELL, J. 2010b. Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. *PLoS One,* 5**,** e10074.

JEONG, S. J., KIM, S., PARK, J. G., JUNG, I. H., LEE, M. N., JEON, S., KWEON, H. Y., YU, D. Y., LEE, S. H., JANG, Y., KANG, S. W., HAN, K. H., MILLER, Y. I., PARK, Y. M., CHEONG, C., CHOI, J. H. & OH, G. T. 2018. Prdx1 (peroxiredoxin 1) deficiency reduces cholesterol efflux via impaired macrophage lipophagic flux. *Autophagy,* 14**,** 120-133.

JORDAENS, L., VAN HOECK, V., DE BIE, J., BERTH, M., MAREI, W. F. A., DESMET, K. L. J., BOLS, P. E. J. & LEROY, J. 2017. Non-esterified fatty acids in early luteal bovine oviduct fluid mirror plasma concentrations: An ex vivo approach. *Reprod Biol,* 17**,** 281-284.

JORDAENS, L., VAN HOECK, V., PINTELON, I., THYS, S., BOLS, P. E. J., MAREI, W. F. A. & LEROY, J. 2020. Altered embryotrophic capacities of the bovine oviduct under elevated free fatty acid conditions: an in vitro embryo--oviduct co-culture model. *Reprod Fertil Dev,* 32**,** 553-563.

JUNGHEIM, E. S. & MOLEY, K. H. 2010. Current knowledge of obesity's effects in the pre- and periconceptional periods and avenues for future research. *Am J Obstet Gynecol,* 203**,** 525-30.

JUNGHEIM, E. S., SCHOELLER, E. L., MARQUARD, K. L., LOUDEN, E. D., SCHAFFER, J. E. & MOLEY, K. H. 2010. Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. *Endocrinology,* 151**,** 4039-46.

JUNGHEIM, E. S., TRAVIESO, J. L., CARSON, K. R. & MOLEY, K. H. 2012. Obesity and reproductive function. *Obstet Gynecol Clin North Am,* 39**,** 479-93.

KLEBL, D. P., FEASEY, M. C., HESKETH, E. L., RANSON, N. A., WURDAK, H., SOBOTT, F., BON, R. S. & MUENCH, S. P. 2021. Cryo-EM structure of human mitochondrial HSPD1. *iScience,* 24**,** 102022.

KLEEMANN, R., VAN ERK, M., VERSCHUREN, L., VAN DEN HOEK, A. M., KOEK, M., WIELINGA, P. Y., JIE, A., PELLIS, L., BOBELDIJK-PASTOROVA, I., KELDER, T., TOET, K., WOPEREIS, S., CNUBBEN, N., EVELO, C., VAN OMMEN, B. & KOOISTRA, T. 2010. Time-resolved and tissue-specific systems analysis of the pathogenesis of insulin resistance. *PLoS One,* 5**,** e8817.

KLEINERT, M., CLEMMENSEN, C., HOFMANN, S. M., MOORE, M. C., RENNER, S., WOODS, S. C., HUYPENS, P., BECKERS, J., DE ANGELIS, M. H., SCHURMANN, A., BAKHTI, M., KLINGENSPOR, M., HEIMAN, M., CHERRINGTON, A. D., RISTOW, M., LICKERT, H., WOLF, E., HAVEL, P. J., MULLER, T. D. & TSCHOP, M. H. 2018. Animal models of obesity and diabetes mellitus. *Nat Rev Endocrinol,* 14**,** 140-162.

KLENOV, V. E. & JUNGHEIM, E. S. 2014. Obesity and reproductive function: a review of the evidence. *Curr Opin Obstet Gynecol,* 26**,** 455-60.

KRISHNA, K. B., STEFANOVIC-RACIC, M., DEDOUSIS, N., SIPULA, I. & O'DOHERTY, R. M. 2016. Similar degrees of obesity induced by diet or aging cause strikingly different immunologic and metabolic outcomes. *Physiol Rep,* 4.

LANGE, P. S., CHAVEZ, J. C., PINTO, J. T., COPPOLA, G., SUN, C. W., TOWNES, T. M., GESCHWIND, D. H. & RATAN, R. R. 2008. ATF4 is an oxidative stress-inducible, prodeath transcription factor in neurons in vitro and in vivo. *J Exp Med,* 205**,** 1227-42.

LI, J., WU, H., LIU, Y. & YANG, L. 2020. High fat diet induced obesity model using four strainsof mice: Kunming, C57BL/6, BALB/c and ICR. *Exp Anim,* 69**,** 326-335.

LI, S. & WINUTHAYANON, W. 2017. Oviduct: roles in fertilization and early embryo development. *J Endocrinol,* 232**,** R1-R26.

LIN, J., YANG, R., TARR, P. T., WU, P. H., HANDSCHIN, C., LI, S., YANG, W., PEI, L., ULDRY, M., TONTONOZ, P., NEWGARD, C. B. & SPIEGELMAN, B. M. 2005. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. *Cell,* 120**,** 261-73.

LIVAK, K. J. & SCHMITTGEN, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods,* 25**,** 402-8.

MA, C. S., LV, Q. M., ZHANG, K. R., TANG, Y. B., ZHANG, Y. F., SHEN, Y., LEI, H. M. & ZHU, L. 2021. NRF2-GPX4/SOD2 axis imparts resistance to EGFR-tyrosine kinase inhibitors in non-small-cell lung cancer cells. *Acta Pharmacol Sin,* 42**,** 613-623.

MA, Q. 2013. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol,* 53**,** 401-26.

MADAN, J. C., DAVIS, J. M., CRAIG, W. Y., COLLINS, M., ALLAN, W., QUINN, R. & DAMMANN, O. 2009. Maternal obesity and markers of inflammation in pregnancy. *Cytokine,* 47**,** 61-4.

MAILLO, V., SANCHEZ-CALABUIG, M. J., LOPERA-VASQUEZ, R., HAMDI, M., GUTIERREZ-ADAN, A., LONERGAN, P. & RIZOS, D. 2016. Oviductal response to gametes and early embryos in mammals. *Reproduction,* 152**,** R127-41.

MAREI, W. F. A., ALVAREZ, M. A., VAN HOECK, V., GUTIERREZ-ADAN, A., BOLS, P. E. J. & LEROY, J. 2017. Effect of nutritionally induced hyperlipidaemia on in vitro bovine embryo quality depends on the type of major fatty acid in the diet. *Reprod Fertil Dev,* 29**,** 1856-1867.

MAREI, W. F. A., SMITS, A., MOHEY-ELSAEED, O., PINTELON, I., GINNEBERGE, D., BOLS, P. E. J., MOERLOOSE, K. & LEROY, J. 2020. Differential effects of high fat diet-induced obesity on oocyte mitochondrial functions in inbred and outbred mice. *Sci Rep,* 10**,** 9806.

NESTEL, P. J., SCHREIBMAN, P. H. & AHRENS, E. H., JR. 1973. Cholesterol metabolism in human obesity. *J Clin Invest,* 52**,** 2389-97.

OESTREICH, A. K. & MOLEY, K. H. 2017. Developmental and Transmittable Origins of Obesity-Associated Health Disorders. *Trends Genet,* 33**,** 399-407.

OHTSU, A., TANAKA, H., SENO, K., IWATA, H., KUWAYAMA, T. & SHIRASUNA, K. 2017. Palmitic acid stimulates interleukin-8 via the TLR4/NF-kappaB/ROS pathway and induces mitochondrial dysfunction in bovine oviduct epithelial cells. *Am J Reprod Immunol,* 77.

OHTSUJI, M., KATSUOKA, F., KOBAYASHI, A., ABURATANI, H., HAYES, J. D. & YAMAMOTO, M. 2008. Nrf1 and Nrf2 play distinct roles in activation of antioxidant response element-dependent genes. *J Biol Chem,* 283**,** 33554-62.

OTT, L. W., RESING, K. A., SIZEMORE, A. W., HEYEN, J. W., COCKLIN, R. R., PEDRICK, N. M., WOODS, H. C., CHEN, J. Y., GOEBL, M. G., WITZMANN, F. A. & HARRINGTON, M. A. 2007. Tumor Necrosis Factor-alpha- and interleukin-1-induced cellular responses: coupling proteomic and genomic information. *J Proteome Res,* 6**,** 2176-85.

PANCHAL, S. K. & BROWN, L. 2011. Rodent models for metabolic syndrome research. *J Biomed Biotechnol,* 2011**,** 351982.

PAULA, F. M., FERREIRA, S. M., BOSCHERO, A. C. & SOUZA, K. L. 2013. Modulation of the peroxiredoxin system by cytokines in insulin-producing RINm5F cells: down-regulation of PRDX6 increases susceptibility of beta cells to oxidative stress. *Mol Cell Endocrinol,* 374**,** 56-64.

PEREIRA, R. M., BOTEZELLI, J. D., DA CRUZ RODRIGUES, K. C., MEKARY, R. A., CINTRA, D. E., PAULI, J. R., DA SILVA, A. S. R., ROPELLE, E. R. & DE MOURA, L. P. 2017. Fructose Consumption in the Development of Obesity and the Effects of Different Protocols of Physical Exercise on the Hepatic Metabolism. *Nutrients,* 9.

RIZOS, D., CARTER, F., BESENFELDER, U., HAVLICEK, V. & LONERGAN, P. 2010. Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows. *J Dairy Sci,* 93**,** 1022-9.

ROBERTSON, S. A., CHIN, P. Y., FEMIA, J. G. & BROWN, H. M. 2018. Embryotoxic cytokines-Potential roles in embryo loss and fetal programming. *J Reprod Immunol,* 125**,** 80-88.

ROBKER, R. L. 2008. Evidence that obesity alters the quality of oocytes and embryos. *Pathophysiology,* 15**,** 115-21.

RON, D. & WALTER, P. 2007. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol,* 8**,** 519-29.

RUEBEL, M., SHANKAR, K., GADDY, D., LINDSEY, F., BADGER, T. & ANDRES, A. 2016. Maternal obesity is associated with ovarian inflammation and upregulation of early growth response factor 1. *Am J Physiol Endocrinol Metab,* 311**,** E269-77.

RUPEREZ, A. I., GIL, A. & AGUILERA, C. M. 2014. Genetics of oxidative stress in obesity. *Int J Mol Sci,* 15**,** 3118-44.

SADOWSKA, J., DUDZINSKA, W., SKOTNICKA, E., SIELATYCKA, K. & DANIEL, I. 2019. The Impact of a Diet Containing Sucrose and Systematically Repeated Starvation on the Oxidative Status of the Uterus and Ovary of Rats. *Nutrients,* 11.

SAKLAYEN, M. G. 2018. The Global Epidemic of the Metabolic Syndrome. *Curr Hypertens Rep,* 20**,** 12.

SCHWARZ, M., DAVIS, D. L., VICK, B. R. & RUSSELL, D. W. 2001. Genetic analysis of cholesterol accumulation in inbred mice. *J Lipid Res,* 42**,** 1812-9.

SILVESTRIS, E., DE PERGOLA, G., ROSANIA, R. & LOVERRO, G. 2018. Obesity as disruptor of the female fertility. *Reprod Biol Endocrinol,* 16**,** 22.

SMITS, A., MAREI, W. F. A., DE NEUBOURG, D. & LEROY, J. 2021. Diet normalization or caloric restriction as a preconception care strategy to improve metabolic health and oocyte quality in obese outbred mice. *Reprod Biol Endocrinol,* 19**,** 166.

SNIDER, A. P. & WOOD, J. R. 2019. Obesity induces ovarian inflammation and reduces oocyte quality. *Reproduction,* 158**,** R79-R90.

STAHLBERG, D., RUDLING, M., ANGELIN, B., BJORKHEM, I., FORSELL, P., NILSELL, K. & EINARSSON, K. 1997. Hepatic cholesterol metabolism in human obesity. *Hepatology,* 25**,** 1447-50.

STANHOPE, K. L. 2016. Sugar consumption, metabolic disease and obesity: The state of the controversy. *Crit Rev Clin Lab Sci,* 53**,** 52-67.

TANAKA, T., NARAZAKI, M. & KISHIMOTO, T. 2014. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol,* 6**,** a016295.

TANG, C., CHEN, S., QIAN, H. & HUANG, W. 2012. Interleukin-23: as a drug target for autoimmune inflammatory diseases. *Immunology,* 135**,** 112-24.

TAPPY, L. & LE, K. A. 2010. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev,* 90**,** 23-46.

TAU, G. & ROTHMAN, P. 1999. Biologic functions of the IFN-gamma receptors. *Allergy,* 54**,** 1233-51.

VALCKX, S. D., DE PAUW, I., DE NEUBOURG, D., INION, I., BERTH, M., FRANSEN, E., BOLS, P. E. & LEROY, J. L. 2012. BMI-related metabolic composition of the follicular fluid of women undergoing assisted reproductive treatment and the consequences for oocyte and embryo quality. *Hum Reprod,* 27**,** 3531-9.

VALCKX, S. D., VAN HOECK, V., ARIAS-ALVAREZ, M., MAILLO, V., LOPEZ-CARDONA, A. P., GUTIERREZ-ADAN, A., BERTH, M., CORTVRINDT, R., BOLS, P. E. & LEROY, J. L. 2014. Elevated non-esterified fatty acid concentrations during in vitro murine follicle growth alter follicular physiology and reduce oocyte developmental competence. *Fertil Steril,* 102**,** 1769-76 e1.

VAN HOECK, V., LEROY, J. L., ARIAS ALVAREZ, M., RIZOS, D., GUTIERREZ-ADAN, A., SCHNORBUSCH, K., BOLS, P. E., LEESE, H. J. & STURMEY, R. G. 2013. Oocyte developmental failure in response to elevated nonesterified fatty acid concentrations: mechanistic insights. *Reproduction,* 145**,** 33-44.

VAZQUEZ-MEDINA, J. P., POPOVICH, I., THORWALD, M. A., VISCARRA, J. A., RODRIGUEZ, R., SONANEZ-ORGANIS, J. G., LAM, L., PETI-PETERDI, J., NAKANO, D., NISHIYAMA, A. & ORTIZ, R. M. 2013. Angiotensin receptor-mediated oxidative stress is associated with impaired cardiac redox signaling and mitochondrial function in insulin-resistant rats. *Am J Physiol Heart Circ Physiol,* 305**,** H599-607.

VELAZQUEZ, M. A. 2015. Impact of maternal malnutrition during the periconceptional period on mammalian preimplantation embryo development. *Domest Anim Endocrinol,* 51**,** 27-45.

WANG, J., LEE, J., LIEM, D. & PING, P. 2017. HSPA5 Gene encoding Hsp70 chaperone BiP in the endoplasmic reticulum. *Gene,* 618**,** 14-23.

WANG, J., PAREJA, K. A., KAISER, C. A. & SEVIER, C. S. 2014. Redox signaling via the molecular chaperone BiP protects cells against endoplasmic reticulum-derived oxidative stress. *Elife,* 3**,** e03496.

WHO 2021. Facts about Obesity and Overweight.

WILLIAMS, L. M., CAMPBELL, F. M., DREW, J. E., KOCH, C., HOGGARD, N., REES, W. D., KAMOLRAT, T., THI NGO, H., STEFFENSEN, I. L., GRAY, S. R. & TUPS, A. 2014. The development of diet-induced obesity and glucose intolerance in C57BL/6 mice on a high-fat diet consists of distinct phases. *PLoS One,* 9**,** e106159.

WITTEMER, C., OHL, J., BAILLY, M., BETTAHAR-LEBUGLE, K. & NISAND, I. 2000. Does body mass index of infertile women have an impact on IVF procedure and outcome? *J Assist Reprod Genet,* 17**,** 547-52.

WU, L. L., NORMAN, R. J. & ROBKER, R. L. 2011. The impact of obesity on oocytes: evidence for lipotoxicity mechanisms. *Reprod Fertil Dev,* 24**,** 29-34.

WUEEST, S. & KONRAD, D. 2018. The role of adipocyte-specific IL-6-type cytokine signaling in FFA and leptin release. *Adipocyte,* 7**,** 226-228.

YOUSIF, M. D., CALDER, M. D., DU, J. T., RUETZ, K. N., CROCKER, K., URQUHART, B. L., BETTS, D. H., RAFEA, B. A. & WATSON, A. J. 2020. Oleic Acid Counters Impaired Blastocyst Development Induced by Palmitic Acid During Mouse Preimplantation Development: Understanding Obesity-Related Declines in Fertility. *Reprod Sci,* 27**,** 2038-2051.

YUZEFOVYCH, L. V., MUSIYENKO, S. I., WILSON, G. L. & RACHEK, L. I. 2013. Mitochondrial DNA damage and dysfunction, and oxidative stress are associated with endoplasmic reticulum stress, protein degradation and apoptosis in high fat diet-induced insulin resistance mice. *PLoS One,* 8**,** e54059.

ZENOBIA, C. & HAJISHENGALLIS, G. 2015. Basic biology and role of interleukin-17 in immunity and inflammation. *Periodontol 2000,* 69**,** 142-59.

CHAPTER 4:

EFFECTS OF A WESTERN TYPE DIET ON THE OVIDUCT LIPIDOMIC PROFILE OVER TIME

How the oviduct lipidomic profile changes over time after the start of an obesogenic diet in an outbred mouse model

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Simple Summary: This study investigated the lipidomic changes in the oviduct at different time points (from 3 days to 16 weeks) during feeding an obesogenic diet, in an outbred mouse model. We used MALDI mass spectrometry imaging focusing on changes in the oviductal epithelium (OE). The obesogenic diet resulted in an overall higher average peak intensity of all detected lipids, and we could identify differentially regulated lipids (DRLs) already after 3 days. The number of DRLs progressively increased and became more persistent after long-term obesogenic diet feeding. Functional annotation revealed that the alterations were mainly in phospholipids, sphingomyelins and lysophospholipids.

1. Abstract

We investigated whether a high-fat/high-sugar (HF/HS) diet alters the lipidomic profile of the oviductal epithelium (OE) and studied the patterns of these changes over time. Female outbred Swiss mice were fed either a control (10% fat) or HF/HS (60% fat, 20% fructose) diet. Mice (n = 3 per treatment per time point) were sacrificed and oviducts were collected at 3 days and 1, 4, 8, 12 and 16 weeks on the diet. Lipids in the OE were imaged using matrix-assisted laser desorption ionisation mass spectrometry imaging. Discriminative m/z values and differentially regulated lipids were determined in the HF/HS versus control OEs at each time point. Feeding the obesogenic diet resulted in acute changes in the lipid profile in the OE already after 3 days, and thus even before the development of an obese phenotype. The changes in the lipid profile of the OE progressively increased and became more persistent after long-term HF/HS diet feeding. Functional annotation revealed a differential abundance of phospholipids, sphingomyelins and lysophospholipids in particular. These alterations appear to be not only caused by the direct accumulation of the excess circulating dietary fat but also a reduction in the de novo synthesis of several lipid classes, due to oxidative stress and endoplasmic reticulum dysfunction. The described diet-induced lipidomic changes suggest alterations in the OE functions and the oviductal microenvironment which may

impact crucial reproductive events that take place in the oviduct, such as fertilization and early embryo development.

Keywords: obesity; infertility; high-fat/high-sugar diet; oviductal epithelium; lipidomics; MALDI-MSI

2. Introduction

The prevalence of obesity among women of reproductive age has been significantly increasing worldwide, mainly due to the overconsumption of obesogenic (high-fat/high-sugar, HF/HS) diets and a sedentary lifestyle. This is strongly linked with reduced fertility (Silvestris et al., 2018). Overweight and obese women are more likely to have lower fertilization rates, poor oocyte and embryo quality, and higher rates of miscarriage compared to normal-weight women (Pandey et al., 2010; Dag and Dilbaz, 2015). Several studies have demonstrated that most pregnancy losses occur very early, in the first 2 weeks after fertilization, suggesting early preimplantation embryo mortality and failure of implantation (Fedorcsak et al., 2004; Tremellen et al., 2017). This is usually attributed to reduced oocyte quality (Pandey et al., 2010; Gonzalez et al., 2022; Leroy et al., 2022). However, alterations in the oviductal microenvironment may also impact early embryo development and increase the risk of early embryonic losses (Li and Winuthayanon, 2017). Several crucial reproductive events take place in the oviduct, such as oocyte and sperm capacitation, fertilization, early embryo development, embryo genome activation and epigenetic (re)programming (Aviles et al., 2010; Besenfelder et al., 2012; Maillo et al., 2016; Li and Winuthayanon, 2017). Nevertheless, very little is known about the potential effects of an obesogenic diet and obesity on the oviduct and its microenvironment.

High-fat/high-sugar (HF/HS)-diet-induced obese mouse models are frequently used to study the pathogenesis of obesity and its complications (de Moura et al., 2021). The consumption of obesogenic diets has been shown to induce acute responses, as early as after three days of feeding, where mice exhibit reduced glucose tolerance, and increased blood cholesterol and inflammatory cytokines (Williams et al., 2014; Haley et al., 2019). Prolonged feeding of an obesogenic diet leads to insulin resistance and increases hyperlipidaemia, which increases adiposity and accumulation of lipids in non-adipose tissues, causing lipotoxicity and oxidative stress which eventually results in

cellular dysfunctions (Lin et al., 2005; Fernandez-Sanchez et al., 2011; Herdt, 2013; Engin, 2017a; Engin). More specifically, diet-induced changes in the lipid content of different tissues such as the liver, heart and muscles have been described, which is associated with alterations in cellular metabolism and functions and an increased risk of various metabolic disorders (Liisberg et al., 2016; Valsesia et al., 2016; Pakiet et al., 2020; Spooner et al., 2021). These tissues are metabolically highly active and might be more sensitive to changes in circulating lipids. Whether the same changes also occur in the reproductive tissues is not known. Some studies have shown evidence of increased lipid content in the ovarian follicular fluid microenvironment and the oocytes in diet-induced obese mouse models (Robker, 2008; Igosheva et al., 2010; Jungheim et al., 2010; Wu et al., 2011; Jungheim et al., 2012; Valckx et al., 2014; Marei et al., 2017; Marei et al., 2020; Smits et al., 2022) and in women (Wittemer et al., 2000; Valckx et al., 2012; Gonzalez et al., 2018). Many of these studies have demonstrated a direct lipotoxic impact on oocyte developmental competence. Furthermore, the effect on ovarian functions appears to be mainly induced by the obesogenic diet per se regardless of the development of the obese phenotype (Skaznik-Wikiel et al., 2016). On the other hand, the potential effects of HF/HS-diet-induced obesity on the oviductal microenvironment and its lipid content have not been elucidated in humans or in relevant animal models.

The sensitivity of the oviduct to hyperlipidemia has been examined only in a few studies. Free fatty acid (FFA) concentrations in the blood were shown to be correlated to those in the oviductal fluid in cattle (Jordaens et al., 2017a) and exposure of the oviductal cells in vitro to high FFA concentrations altered the oviductal cell proliferation and integrity, and sperm binding capacity (Jordaens et al., 2015). Data from studies in which bovine embryos, in vitro produced under standard conditions, were transferred to the oviducts of cows suffering from high blood FFA concentrations due to negative energy balance, indicate that the oviducts of these cows may be compromised in their

ability to support early embryo development, which may contribute to lower survival rates of the embryos compared to the embryos transferred to healthy cows (Rizos et al., 2010; Maillo et al., 2012). In addition, we have recently shown that feeding an HF/HS diet in mice upregulated genes involved in endoplasmic reticulum stress responses and in oxidative stress in the oviductal epithelial cells only after 3 days of feeding (Moorkens et al., 2022). These responses reoccurred and were aggravated during later phases of HF/HS feeding and were associated with marks of inflammation after long-term feeding and the development of obesity (Moorkens et al., 2022). Whether such impact is linked to alterations in the lipid profile of the oviduct is not known yet.

Importantly, feeding an obesogenic diet is not only expected to increase neutral lipid accumulation in somatic cells, but may also change the composition of lipids that play very important roles in the structure and functions of the plasma membrane, membranous organelles, and in cell signalling and cell-to-cell interaction (Cockcroft, 2021). Alterations in the dietary fat content and in the ratio between saturated and unsaturated fatty acids could influence the lipid profile of the ovarian follicular fluid, follicular cells and endometrial epithelial cells in cows and sheep (Childs et al., 2008) (Childs et al., 2008; Wonnacott et al., 2010; Zachut et al., 2011; Freret et al., 2019). This has not been illustrated in the oviduct while it is now generally accepted that biochemical alterations in the oviduct can be critical for early embryo development (Niemann and Wrenzycki, 2000; Desmet et al., 2016). The oviductal cells and fluid are rich in lipids, such as cholesterol; high- (HDLs) and low-density lipoproteins (LDLs), triglycerides and fatty acids, (Banliat et al., 2019) as well as phospholipids and sphingolipids (Henault and Killian, 1993; Skotland et al., 2019), which can significantly influence the oviductal and embryonic cell signalling and molecular functions (Saint-Dizier et al., 2019).

Taken together, a profound understanding of how diet and obesity can influence the oviductal lipidomic profile is important for increasing awareness and implementing

preventative measures during the periconceptional period to enhance fertility. Advances in the matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI MSI) technique have enabled the analysis of the spatial distribution of small molecules such as lipids in tissues of interest (Meding and Walch, 2013), and the technique was successfully used to describe the spatial distribution of different lipid classes in the ovarian follicles (Uzbekova et al., 2015). The aim of this study was to investigate whether an HF/HS diet can alter the lipidomic profile of the oviductal epithelium and to study the patterns of these changes in the oviductal epithelial layer over time as the mice continue to consume such an obesogenic diet and develop an obese phenotype. This was examined in an outbred mouse model.

3. Material and Methods

3.1. Ethical Approval

All procedures in this study were approved by the ethical committee of the University of Antwerp and performed accordingly (ECD approval number no. 2014-57). All methods were performed in accordance with the relevant ethical guidelines and regulations. This study complies with the ARRIVE guidelines (Percie du Sert et al., 2020).

3.2. Experimental Animals, Diet and Experimental Design

Five-week-old, non-pregnant, sexually mature female outbred Rj:Orl Swiss mice (hereafter referred to as “Swiss” mice, *n* = 42, Janvier labs) were used. The mice were randomly divided into two groups with ad libitum access to either a control diet (CTRL) or a high-fat/high-sugar diet (HF/HS). An HF/HS diet is more representative of the typical Western-style diet, compared to a high-fat diet (HFD) (Panchal and Brown, 2011). The HF/HS group was fed with a 60 kJ% fat (beef tallow) and 9.4% sucrose diet (E15741-34, Sniff diets, Soest, Germany) in combination with drinking water containing 20% fructose (Merck, 102109450). Beef tallow was chosen since it contains more saturated fat compared to lard. Fructose was chosen over glucose and sucrose because high

consumption of fructose is known to cause insulin resistance and obesity in rodents (Tappy and Le, 2010). A high-fat/high-fructose diet can thus induce metabolic syndrome and type 2 diabetes in mice (Panchal and Brown, 2011). Furthermore, it has been shown that fructose has a reduced capacity to stimulate satiety and it also appears to be a better inducer of metabolic syndrome compared to glucose (Pereira et al., 2017).

Mice in the control group were exposed to a matched, purified (not a grain-based chow diet) CTRL diet (E157453-04, Sniff diets, Soest, Germany), containing 10 kJ% fat and 7% sucrose, and had ad libitum access to fructose-free water. This CTRL diet is also lower in saturated fatty acids. Experimental feeding continued for up to 16 weeks. The food intake of all mice was monitored and the mice were weighed weekly during the whole experiment.

Mice were euthanized by decapitation at 6 different time points (n = 3 per treatment per time point): 3 days, 1 week (1 w), 4 w, 8 w, 12 w and 16 w after the start of feeding the CTRL or the HF/HS diet. Oviducts were collected as explained below to study different outcome parameters. Female mice were exposed to bedding from male cages 24 h before euthanasia to synchronize their oestrous cycles (Whitten effect) (Whitten, 1956) and to collect the samples during the follicular phase (before ovulation), ensuring no follicular cells or oocytes were present in the oviduct at sample collection. The time points at which samples were collected are based on the results of previous studies where multiphasic acute and long-term effects of an HFD on general metabolic features in mice have been reported (Williams et al., 2014).

3.3. Assessment of Live Body Weight

The weight of each mouse was recorded weekly. The weight gain data are derived from 21 mice per dietary group at the first time point. The number of mice was reduced after each time point due to the culling of a subset of animals (n = 3) at each time point for sample collection.

3.4. Oviduct Collection

At each time point, mice were dissected and the oviducts were collected in L15 medium (Thermo Fisher Scientific, Belgium) supplemented with 50 IU/mL penicillin G sodium salt (Merck, Belgium). Fat and surrounding tissue were removed under a stereomicroscope. The oviducts were straightened by dissecting the surrounding connective tissue and ligaments, and trimmed from both sides to remove the infundibulum and isthmus regions (Behringer, 2012). From each mouse, one oviduct was used for lipidomic analysis, while the other oviduct was used to collect oviductal epithelial cells (OECs) for gene expression analysis, the data of which is already published in Moorkens et al. (2022) (Moorkens et al., 2022). The ampullary parts of oviducts that were used for lipidomic analysis were then washed two times in phosphate-buffered saline (PBS; Life Technologies, Belgium), wrapped straight in a piece of aluminium foil, snap frozen in liquid nitrogen and stored at −80 °C.

3.5. Assessment of the Lipid Distribution in the OE Using MALDI Mass Spectrometry Imaging (MALDI MSI)

Matrix-assisted laser desorption ionisation (MALDI) imaging was used for the imaging of lipids that are present in the mouse oviductal tissue.

3.6. Reagents

Carboxymethylcellulose (CMC), norharmane, red phosphorus, ammonium acetate (NH_4OAc 7.5M solution) and formic acid (FA) were obtained from Sigma (Merck Life Science B.V., Overijse, Belgium, molecular grade). LC-MS-grade acetone, isopropanol (IPA), methanol (MeOH) and water were obtained from Biosolve B.V. (Valkenswaard, Netherlands), while *n*-hexane was obtained from VWR International BVBA (Leuven, Belgium).

3.7. Sample Preparation and Processing and Imaging Data Acquisition

The straightened oviduct segments were first embedded in 2% carboxymethyl cellulose (CMC) after which they were stored at −80 °C. The embedded oviduct segments were then thawed from −80 °C to −20 °C for 30 min, after which 10 µm oviductal ampullary sections were obtained using a cryostat (Leica Microsystems, Belgium BVBA). The sections were mounted on indium tin oxide (ITO)-coated slides (LaserBio Labs, Sophia-Antipolis, Valbonne France) (n = 3 sections per animal per treatment group) and dried under vacuum in a desiccator for 15 min. Norharmane was used as a matrix as it is suitable for both negative and positive reflectron modes (Scott et al., 2016). Sublimation of the slide with matrix was necessary for the successful generation of ions by the absorption of laser irradiation. The matrix was sublimated for 13 min using a sublimation setup assembled in-house, composed of a sublimation apparatus (Merck) that was heated at 140 °C with an oil bath (Filter Service NV, Eupen, Belgium).

Lipid imaging was performed using a RapifleX MALDI Tissue-typer TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The instrument was equipped with a Smartbeam 3D Nd:YAG (355 mm) laser capable of firing up to 10 kHz and was controlled using FlexControl 4.0 (Build 46) software (Bruker Daltonics). The single smartbeam parameter was set to a 6-µm × 6-µm scan range with a resulting field size of 10 µm. Spectra at the scan range of *m*/*z* 400–2000 were obtained in positive and negative reflectron mode at a laser repetition rate of 10 kHz. Both acquisition modes were used to detect all possible lipids that ionise better in either the positive or the negative reflectron mode. The sampling rate was set to 1.25 GS/s. The laser power was adjusted using test shots at random positions on the sample in order to reach the optimal ionization threshold. A total of 500 laser shots were accumulated for each raster point. The deflection of masses below *m*/*z* 400 was activated by default. Spectra were acquired at a lateral resolution of 10 µm. External calibration was performed using

the PepMix 6 standard (LaserBio Labs) and red phosphorus adducts in positive and negative modes, respectively.

3.8. Assessment of the Different Histological Regions in the Oviductal Cross-Section Using Histological Staining

A bright field image was obtained for each section used for MALDI MSI after hematoxylin and eosin (H&E) staining. For that, the matrix was first removed by submerging the slide in 70% then 100% isopropanol (Carl-Roth, Karlsruhe, Germany). The slides were placed in an acetic acid formaldehyde (VWR, Leuven, Belgium) solution for 2 min (min), after which they were dipped five times in tap water and then AquaDest. H&E staining was then performed following standard procedures (Cardiff et al., 2014). Finally, the sections were covered with DPX mount medium and a cover slip and stored at room temperature. Images were made of the H&E-stained sections and these images were used to align (co-register) the MALDI MSI images to identify the epithelial cell layer of the oviduct as described below.

3.9. Lipidomic Data Acquisition and Analysis of the Oviductal Epithelial Cell Layer

The MSI data sets were analysed with SCiLS Lab 3D, version 2016b (Bruker Daltonics) (Trede et al., 2012). SCiLS reduces the impact of systematic and technical variations to improve the reproducibility of MALDI MSI experiments. The baseline was corrected using the Tophat morphological algorithm and the data were normalized based on the total ion count (TIC) method (Deininger et al., 2011). Orthogonal matching pursuit (OMP) was used for peak detection and the peaks were aligned to the mean spectrum by centroid matching (Alexandrov and Kobarg, 2011).

A first overview of the spatial regions of the luminal epithelial cells and stroma in the cross sections of the oviduct along the ampulla was achieved through spatial segmentation by applying the bisecting k-means algorithm using the Manhattan

distance metric. The depth of clustering was selected interactively. The cluster corresponding to the epithelial cell regions was identified by co-registering the MS images with the optical scans of the same section after it had been subjected to H&E staining. Spectra from this cluster were subjected to receiver operating characteristic (ROC) analysis at each time point to detect *m/z* values that were discriminative between the HF/HS and CTRL groups. The ROC curve, which plots sensitivity versus 1-specificity (Zou et al., 2007) is a commonly used measure to assess how well a test or model can discriminate individuals, such as the detected masses, into two classes (Cook, 2008), such as HF/HS and CTRL in the case of our study. To avoid the technical bias that may occur towards the detection of more abundant lipid masses, the ROC analysis was performed in two directions: HF/HS versus CTRL and CTRL versus HF/HS. The analysis was also followed by MS/MS and database search to identify the detected differentially regulated lipids (DRLs) as described by Bertevello et al. (2018) (Bertevello et al., 2018).

3.10. Statistical Analysis

Each individual mouse is considered an experimental unit. Statistical analysis of live body weight was carried out using IBM Statistics SPSS (IBM SPSS statistics version 26). The numerical data were checked for homogeneity of variance using Levene's test. Live body weight was analysed using repeated measures ANOVA to study the main effects and interaction of treatment and time. In addition, two-tailed independent sample t-tests were performed to study the effect of treatment on body weight at each time point. As previously mentioned, MSI data sets were analysed with SCiLS Lab 3D, version 2016b (Bruker Daltonics).

The receiver operating characteristic (ROC) analysis was used to evaluate whether a detected lipid signal was discriminative between the CTRL and HF/HS OE. For this, two directions of change were tested: HF/HS vs. CTRL and CTRL vs. HF/HS. A discrimination threshold of 0.7 was used for the area under the curve (AUC) of the ROC plot, above which a mass is considered discriminative or differentially regulated between the two

treatment groups. A representative ROC plot can be found in the Supplementary Materials (Figure S1). Masses with an AUC ≥ 0.7 in the HF/HS vs. CTRL analysis were higher in abundance in the HF/HS group compared to the CTRLs, and vice versa.

4. Results

4.1. The Impact of the HF/HS Diet on Live Body Weight

A significant treatment x time effect of the HF/HS diet consumption on body weight was shown ($p < 0.001$) using repeated measures ANOVA. The difference in body weight between the HF/HS and the control group was already significant from 1 w onwards. The increase in body weight in the HF/HS group reached a plateau at 12 w of feeding where the mice were about 33.6% heavier than those in the control group (48.30 g ± 2.14 vs. 36.16 g ± 1.17) (Figure 1).

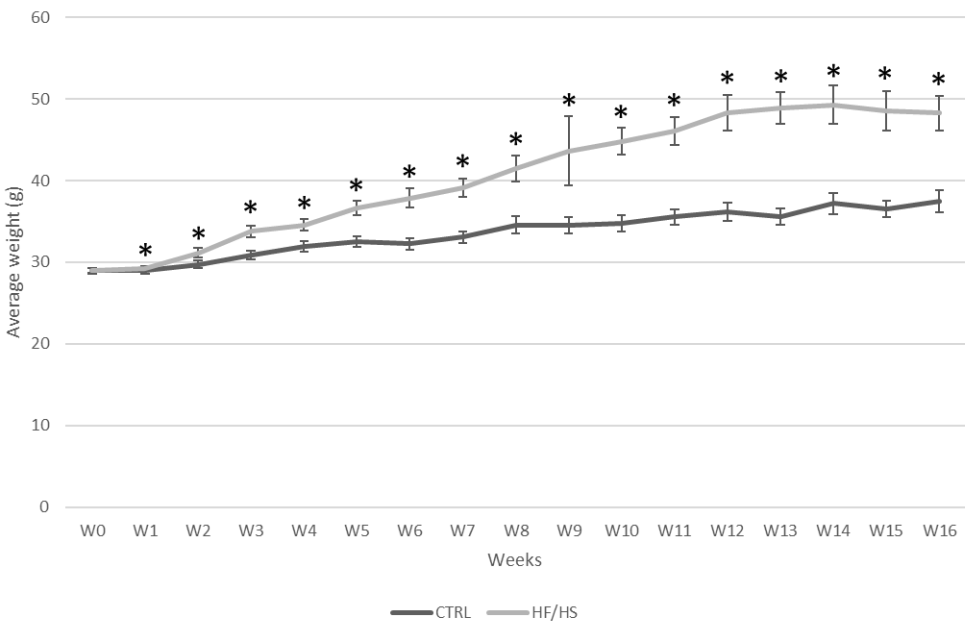


Figure 1. The effect of HF/HS diet on body weight in Swiss mice. Experimental feeding was started at 5 weeks of age (W0) for 21 mice per dietary group at the first time point. Body weight was monitored weekly. The number of mice was reduced after each time point due to the culling of a subset of animals ($n = 3$) at each time point for

sample collection. Data are shown as mean ± SEM. Significant differences ($p \leq 0.05$) between treatment groups per time point are indicated by an asterisk (*).

4.2. Distinct Lipid Distribution in the OE

The OE was highlighted as the region of interest on H&E staining images (Figure 2A) as well as on the optical scans (Figure 2B). Spatial segmentation, a method that generates a segmentation map by grouping spectra acquired in an imaging experiment by similarity using a clustering algorithm (bisecting k-means), was applied to the imaging datasets (Figure 2C). This method showed that spectra acquired from regions associated with the oviductal epithelia (brown) are distinct from those acquired from regions associated with the stromal (green) regions, and can be isolated for further statistical analyses. An overlay of the H&E image and the spatial segmentation maps was used for alignment and to focus the subsequent analysis on the epithelial layer (Figure 2D).

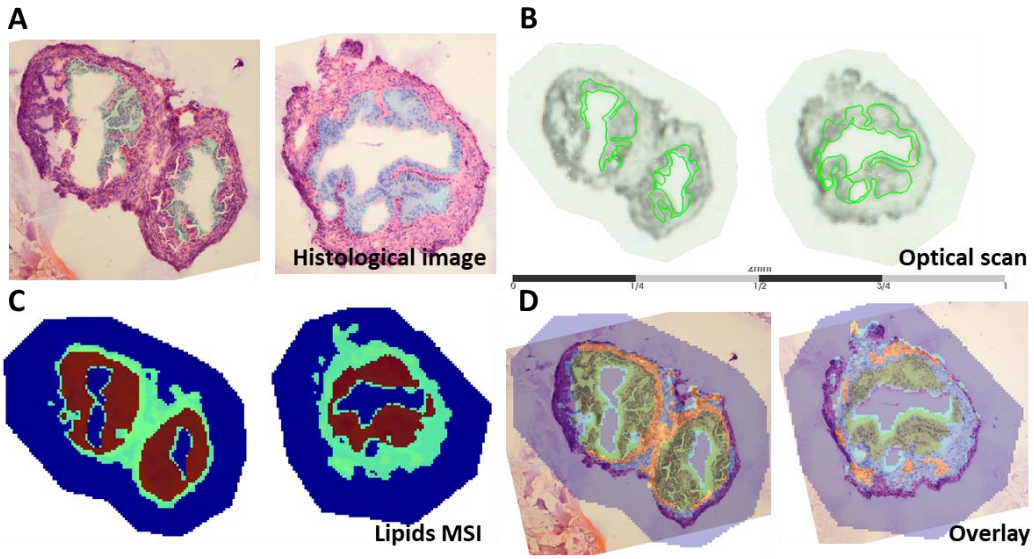


Figure 2. MALDI MSI analysis of two representative oviductal cross-sections at the 16-week time point. (**A**) Histological images of the oviductal cross-sections with the region of interest (ROI; the epithelium) highlighted in blue and (**B**) the co-registration of the MS images with the optical scans with the ROI indicated in green. (**C**) Spatial segmentation maps of the whole acquired spectrum with each ROI arbitrarily assigned a particular colour to show specific clusters associated with the OE (highlighted in brown) and oviductal stroma (highlighted in green). (**D**) Histological image overlaid (overlay) on spatial segmentation maps**.**

Ion density maps indeed demonstrate the preferential localization and variable abundance of specific lipid species in either the OE or oviductal stroma. For example, Figure 3 demonstrates that *m/z* 863.6 is more abundant in the mucosa (represented by an orange-red coloured OE) compared to the oviductal stroma (which is green-blue coloured), whereas *m/z* 810.5 is more abundant in other parts of the stroma (represented by orange-red colour in this region) and less abundant in the oviductal mucosa (displayed by the light blue colour).

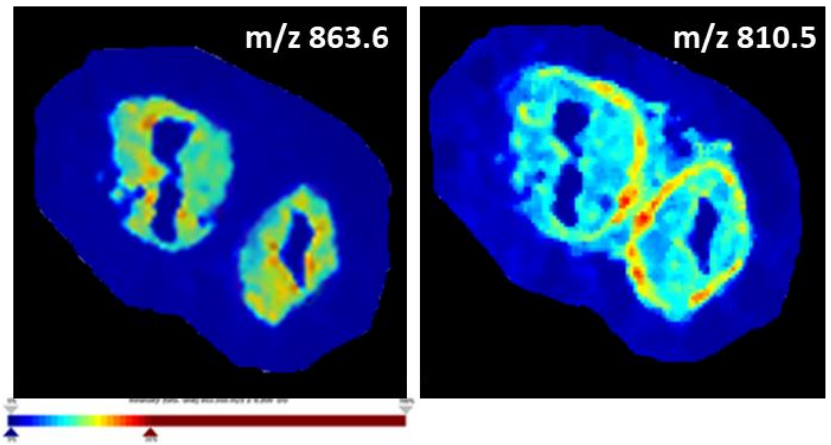


Figure 3. Ion density maps plotting the distribution of specific lipid masses, *m*/*z* 863.6 and *m*/*z* 810.5, based on their intensity across the entire tissue section showing their preferential localization to the OE (*m*/*z* 863.6) and the oviductal stroma (*m*/*z* 810.5). The intensity levels of the *m*/*z* values are presented by a colour gradient going from dark blue, corresponding to the lowest intensity (i.e., low abundance of that specific lipid mass in a particular spot), to dark red, showing the highest intensity.

4.3. The effect of the HF/HS Diet on the Average MALDI MSI Peak Intensity at Different Time Points

MALDI imaging analysis of the oviductal sections was performed in both positive and negative reflectron modes. Skyline projection spectra were generated from all detected ion signals in the scan range of *m*/*z* 400–2000 coming from all CTRL or all HF/HS oviductal epithelium sections at each time point. The skyline projection spectra show numerous peaks with variable intensity in both negative (Figure 4A) and positive (Figure 4B) modes. The *m*/*z* peak coming from matrix clusters is represented in both spectra at the *m*/*z* interval of *m*/*z* 500 and was excluded (interval size of 14 *m*/*z*) from the analysis.

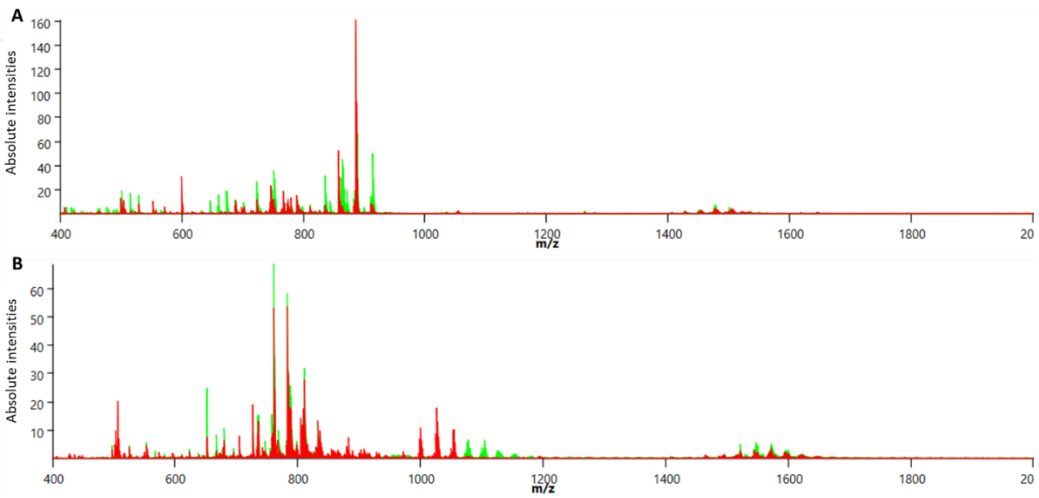


Figure 4. Representative skyline projection spectra of MALDI MSI analysis of the oviductal epithelium sections in negative (**A**) and positive (**B**) reflectron mode. CTRL samples are represented by green peaks; HF/HS samples are represented by red peaks. Y-axis shows the absolute intensity of the detected peaks or *m/z* (mass-to-charge ratio) signals that are shown on the X-axis.

The overall average peak intensity of all detected signals on the spectra varied from one time point to another. This could be due to technical reasons such as matrix deposition variation and detector sensitivity variation which are difficult to avoid in lipidomic analysis. All control and HF/HS samples of each time point were always processed, imaged and analysed together in one batch. Therefore, we focus on the relative comparison between the control and the HF/HS samples at each time point. The results show that the HF/HS OE exhibit a higher overall average peak intensity at all time points compared to the CTRL OE (Figure 5). At 3 days of feeding, the average peak intensity of the HF/HS OE cells was more than twofold higher than that of the CTRLs. In both acquisition modes, this difference was less pronounced from 1 to 12 weeks and then increased again at 16 weeks.

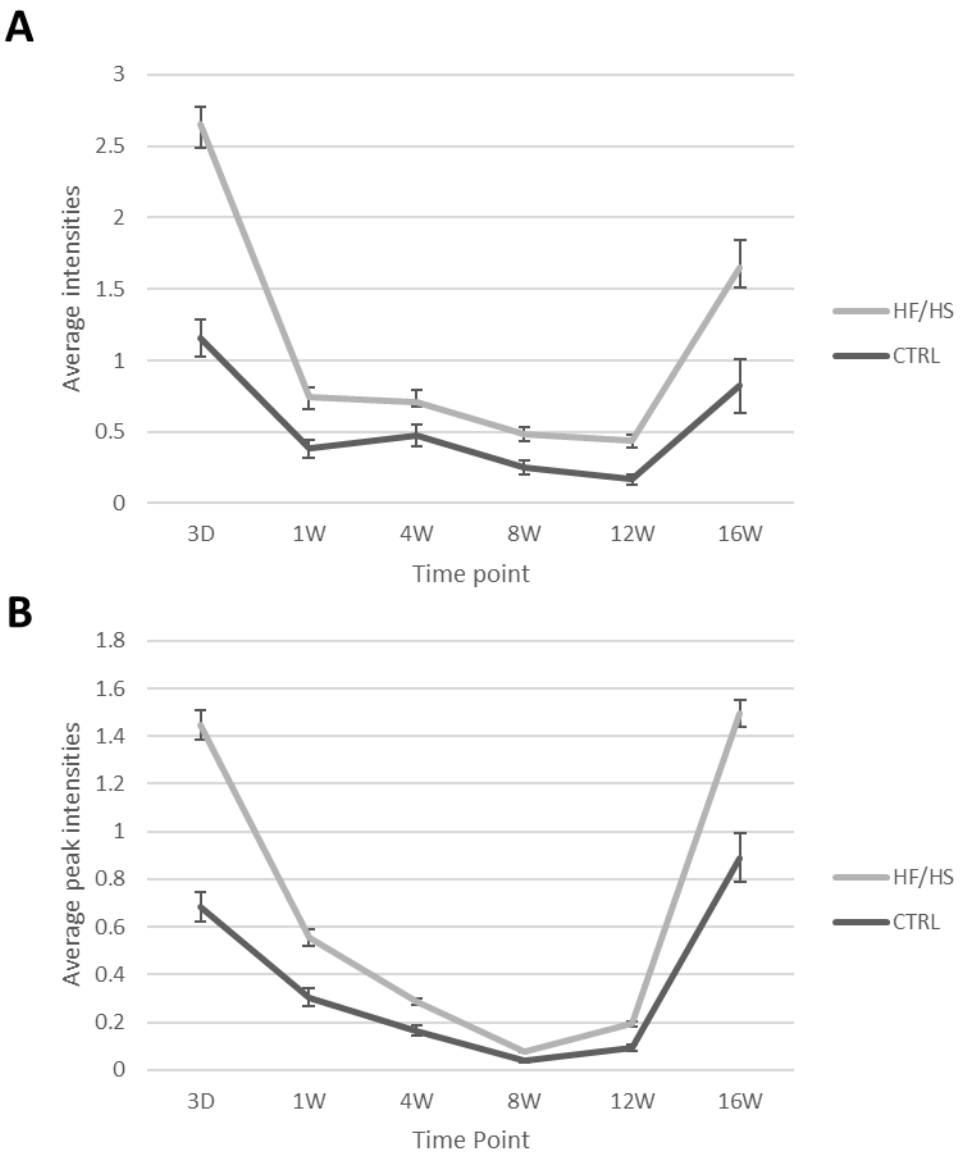


Figure 5. The average peak intensity of all signals coming from CTRL and HF/HS oviductal epithelia over the different time points in negative (**A**) and positive (**B**) reflectron mode. Data are shown as mean ± SEM.

4.4 DRLs Induced by the HF/HS Diet at Each Time Point

4.4.1 ROC Analysis of Spectra

Receiver operating characteristic (ROC) analysis on the spectra of the OE revealed a progressive change in the lipid profile of the OE in response to HF/HS diet feeding over time, compared to the CTRLs. This was shown by an increase in the total number of detected discriminative masses (DMs) over the different time points for both acquisition modes and in both directions of the ROC analysis (HF/HS vs. CTRL and CTRL vs. HF/HS) (Table 1). A total of 11 masses were discriminative already after 3 days of feeding. This increased to 74 masses at 1 week and stayed in this range until week 12. A marked increase in the number of DMs (227) was then detected at 16 w of feeding. The majority of these DMs were more abundant in the CTRL OE compared to the HF/HS OE (i.e., were detected in the CTRL vs. HF/HS comparison).

Table 1. Number of discriminative masses (DMs) at each time point for both acquisition modes and both comparisons. Both positive and negative acquisition modes were used to maximize the detection of different lipids.

Time Point	**Negative Mode**		**Positive Mode**		**Total**	**Total**	**Total DMs**
	HF/HS vs. CTRL	**CTRL vs. HF/HS**	**HF/HS vs. CTRL**	**CTRL vs. HF/HS**	**HF/HS vs. CTRL**	**CTRL vs. HF/HS**	
3 d	none	10	none	1	none	11	11
1 w	28	12	1	33	29	45	74
4 w	5	39	17	16	22	55	77
8 w	23	21	24	29	47	50	97
12 w	37	18	7	2	44	20	64
16 w	27	83	18	99	45	182	227

In order to illustrate and confirm this difference, the overall average intensity of all DMs detected at 16 w (as a representative example) in the HF/HS vs. CTRL comparison (45 DMs) and in the CTRL vs. HF/HS comparison (182 DMs) are plotted in Figure 6 to show the differences in their intensities between the HF/HS and CTRL groups. HF/HS vs. CTRL DMs indeed exhibit higher intensities in the HF/HS group, and vice versa.

In both acquisition modes, the detected DRLs across all time points were focused in specific mass ranges (Figure 7). Most peaks were detected at around *m*/*z* 700–900 and a few lipid ions were detected around *m*/*z* 1400–1600. A few more peaks were detected in a lower mass range below *m*/*z* 700. The lipid classes corresponding to these mass ranges are specified in the Supplementary Materials (Tables S3 and S4).

Furthermore, we noticed that the majority of the DMs detected at 16 weeks were also discriminative at other time points as shown in the Supplementary Materials (Tables S1 and S2).

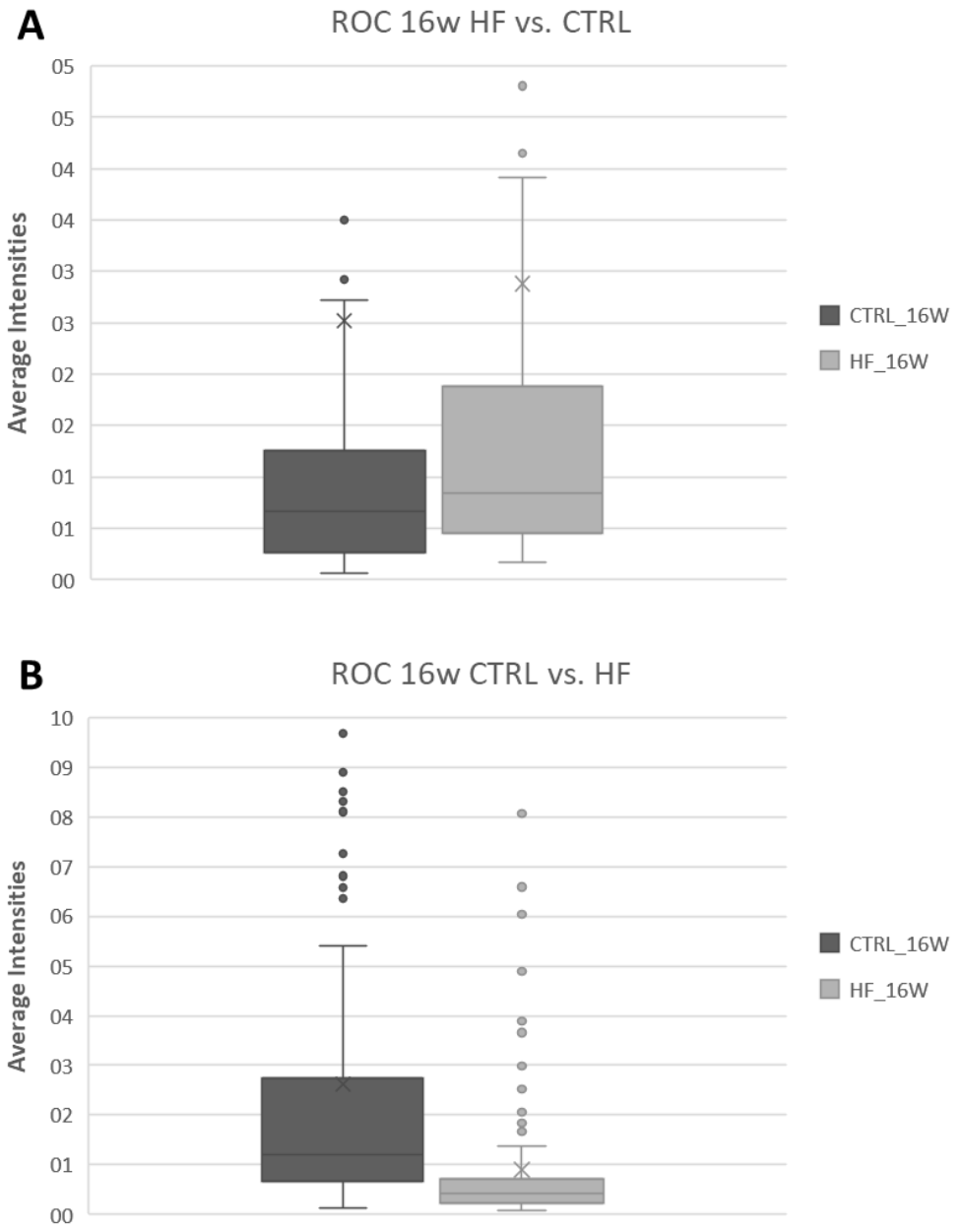


Figure 6. Representative boxplots showing the distribution of the average intensity of the peaks of ROC-detected DRL in the OE in either the HF/HS vs. CTRL (**A**) or CTRL vs. HF/HS (**B**) comparison after 16 w of feeding. (Legend: HF = HF/HS.)

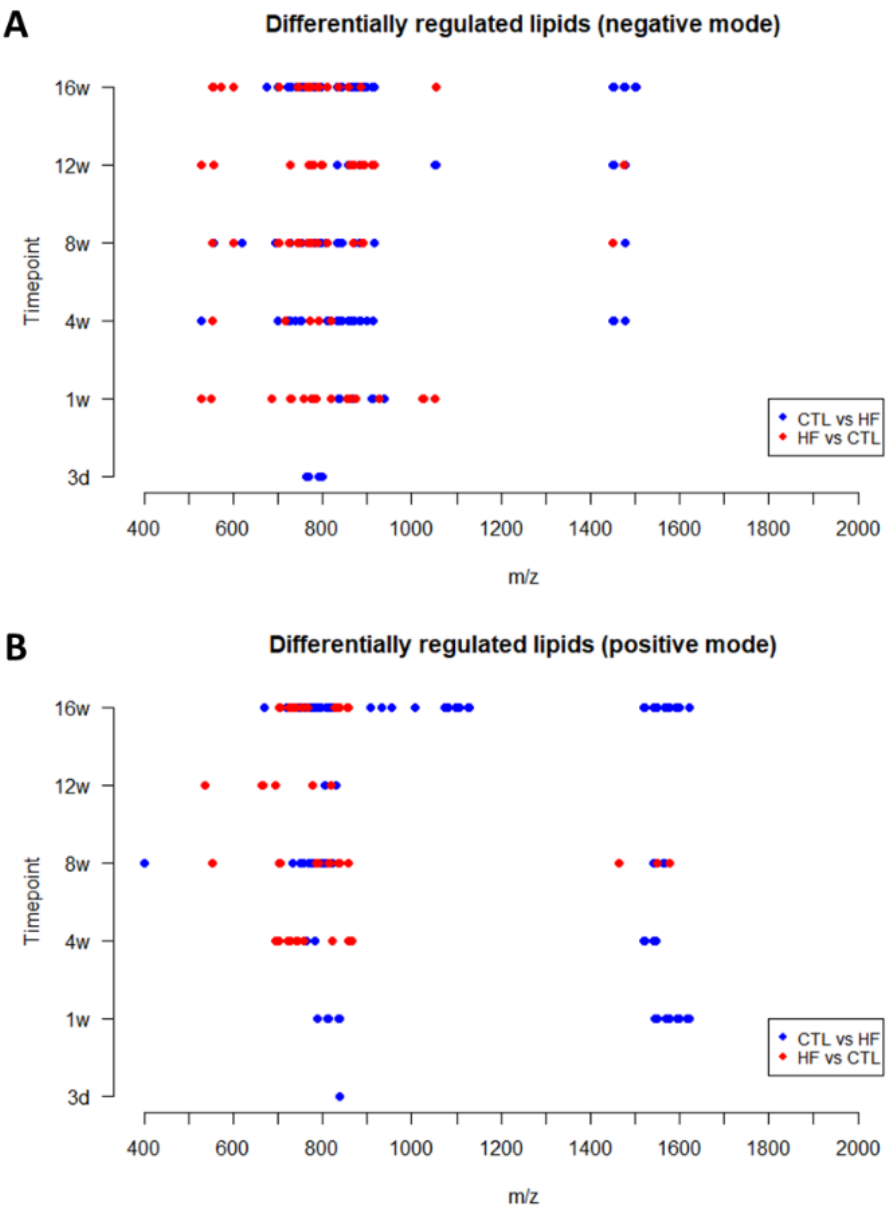


Figure 7. Distribution of molecular weight (*m*/*z*) of the DRLs across all time points detected in negative (**A**) and positive (**B**) reflectron mode. (Legend: HF = HF/HS; CTL = CTRL.)

4.4.2. Assignment of Differentially Regulated Lipids

A total of 206/549 ROC-identified DMs could be putatively annotated in both modes from all time points: 119 DMs in the CTRL vs. HF/HS comparison, and 87 DMs in the HF/HS vs. CTRL comparison. An overview of all ROC-detected DMs and their putative assignments can be found in Tables S3 and S4 as well as a summary of putative annotations in the Supplementary Materials (Table S5). The database with estimated assignments included a few possible annotations for the most detected masses. Therefore, the annotations are described in categories which include all possible assignments. As shown in Figure 8, the majority of the assigned DMs in both CTRL vs. HF/HS and HF/HS vs. CTRL comparisons were found to belong to phospholipids (putatively assigned to either phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), phosphatidylserines (PSs), or to phosphatidylinositols (PIs), and/or sphingomyelins (SMs). Only a few DMs were identified as lysophospholipids (LPs, putatively assigned to either LysoPC (LPC), LysoPE (LPE), LysoPS (LPS), or LysoPI (LPI).

The proportions of PCs, PEs and PSs were altered as early as 3 d in both directions of change, CTRL vs. HF/HS and HF/HS vs. CTRL, and at all time points. DMs specifically assigned to PEs or to the PC/PE category were detected at later time points especially from 8 w onwards in both directions. DMs identified as PIs were relatively more abundant in the CTRL vs. HF/HS comparison than the other direction (in total 34/119 (28.6%) vs. 17/87 (19.5%)). Alterations in lysophospholipids were relatively more abundant in the HF/HS vs. CTRL comparison in all time points starting from 1 w.

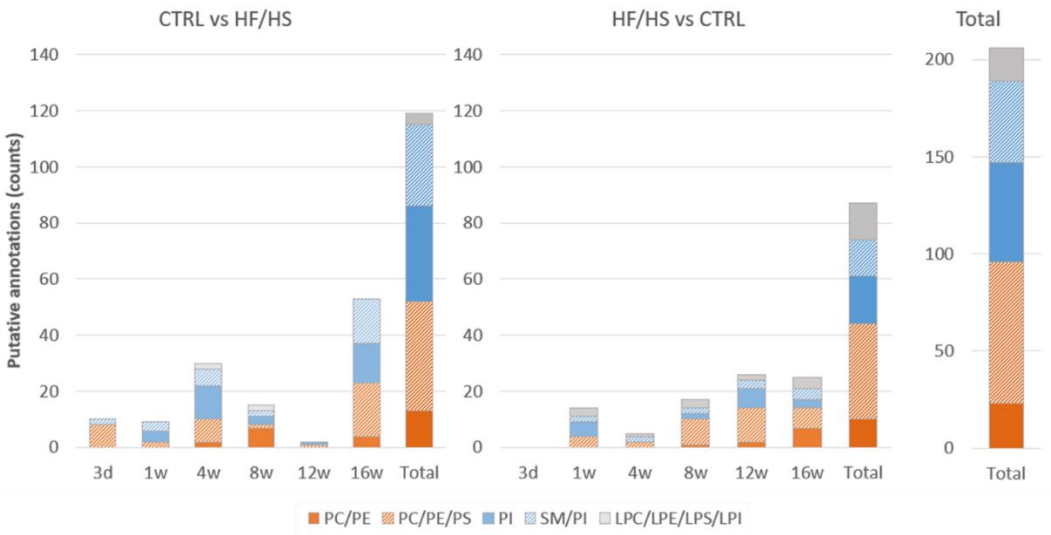


Figure 8. Lipid class categories of the putative annotations for the ROC-identified masses in the CTRL vs. HF/HS and HF/HS vs. CTRL comparisons at each time point and in total. Lipids could be putatively assigned either to phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and phosphatidylserines (PSs); or assigned to phosphatidylinositols (PIs) and/or sphingomyelins (SMs). A few lipid species were identified as LysoPC (LPC), LysoPE (LPE), LysoPS (LPS), and LysoPI (LPI).

5. Discussion

The aim of this study was to investigate whether an HF/HS diet can alter the lipidomic profile of the oviductal epithelium (OE) of mice and to study the patterns of these changes over time as the mice continue to consume the obesogenic diet for short to long-term time periods and develop obesity. This was tested in an outbred Swiss mouse model, to increase the pathophysiological relevance to humans and livestock animals.

The oviductal physiology is very difficult to study, especially in mice, due to the small size and therefore limited access to biological material such as the oviductal cells. For the first time, we were able to apply the use of MALDI mass spectrometry imaging technology on murine oviducts and examine the changes in the lipidomic profile of the OE due to feeding an HF/HS diet. Distinct differences could be detected already at 3 days and 1 week (w) of HF/HS diet feeding. These differences then remained relatively

stable until 12 w before marked lipidomic changes could be detected again at 16 w. The majority of these differences were assigned to phospholipids, sphingomyelins as well as a few lysophospholipids.

We only focused on the ampullar region of the oviduct, where fertilization and the first three days of early embryo development take place (Elsevier, 2014). The ampulla has more secretory cells and is thus more active in de novo lipid synthesis compared to the isthmus (Henault and Killian, 1993) (Kolle et al., 2009). Most of the lipid synthesis occurs in the epithelium (Henault and Killian, 1993). In the present study, the detected masses exhibited very specific spatial distribution patterns which matched the corresponding histological layers of the oviduct, and indeed, a distinct lipid profile of the OE was noticeable in contrast with the surrounding stroma. Such a contrasting lipid profile of the OE could be due to the presence of secretory cells (Behringer, 2012). Distinct lipid profiles of epithelial cell layers compared to stroma have been reported in other tissues such as ovarian tissue (Doria et al., 2016) and prostate tissue (Weisser and Krieg, 1997).

Previous findings from our laboratory showed that mice are metabolically sensitive to high dietary saturated fat and high fructose consumption (Marei et al., 2020). Blood analysis of the mice used in the present study was reported in our previous paper (Moorkens et al., 2022), showing a significant increase in blood total cholesterol concentrations already after feeding the HF/HS diet for 1 w. This acute increase in cholesterol levels was no longer detectable at 4 w of feeding, but the difference became distinct again at 8 w, 12 w and 16 weeks of feeding (Moorkens et al., 2022). Similar duration-specific differences are also observed here in the present study. We saw a distinct lipidomic profile of the OE of the HF/HS-fed mice compared with the controls already at 3 days of feeding. This is shown by the increased average peak intensity of all detected signals in the spectra from the OE, and by the detection of DMs by the ROC analysis of the ion signals. The number of DMs gradually increased over time while the average peak intensity decreased in general, i.e., moving from a non-specific overall

change in the OE lipid profile to a more specific change in certain lipid species. Furthermore, the results showed that the differences were stable from 1 w to 12 w of feeding. At 16 w of feeding, both the detected DMs and the average peak intensity were relatively high. This pattern of change also corresponds to the same distinct phases of systemic metabolic alterations reported in mice fed an HF/HS diet as described by Williams et al. (2014). In that study, an elevated glucose concentration and an acute phase response was reported already after 3 d of feeding. These effects were improved at 1 w of feeding and remained stable until 12 w. Inflammatory markers were undetectable during this period. At 16 w, Williams et al. reported another increase in glucose concentration and inflammation. They concluded that the development of diet-induced obesity and glucose intolerance consists of distinct phases, in which they proposed the time period between 1 w and 12 w as an adaptation phase in the diet-induced metabolic changes. Adaptative mechanisms determining the difference in cellular fatty acid metabolism in short-term vs. long-term HF/HS diet feeding were also described by Ciapaite et al. (2011).

In addition, the pattern of changes in the overall average peak intensity of the OE can also be linked to the gene expression patterns of the OECs collected from the same mice, as previously published (Moorkens et al., 2022). There, we showed that feeding an HF/HS diet significantly increased acute stress responses, as indicated by an increased level of oxidative stress and endoplasmic reticulum (ER) stress marker genes after only 3 d of feeding. This was followed by a phase (between 1 w and 8 w) in which these transcriptomic changes were temporarily normalized (became less distinct). But finally, a significant upregulation of genes associated with OS and ER stress as well as inflammation was detected again at 12 to 16 w in the HF/HS group (Moorkens et al., 2022). The results of these studies show strong evidence of the sensitivity of the oviduct and OE to systemic metabolic changes at both transcriptomic and lipidomic levels.

Further analysis showed that some of the detected discriminative masses (DMs) were common between the different feeding periods, and many of them were detected again at 16 w (see Tables S1 and S2). This can be seen as a validation of the results and strongly suggests that the detected DMs are specifically altered in response to the diet-induced metabolic alteration, potentially for a specific function or signalling response. Persistent alterations in common lipid species over different periods of HFD feeding were also reported in mice and rats in other tissues such as in the liver (Turner et al., 2013) and in the brain (Sighinolfi et al., 2021). Moreover, the lipidomic changes occurring after chronic exposure (16 w) to the HFD were shown to be irreversible, as they persist even after switching to a chow diet (Sighinolfi et al., 2021). Our findings show a relatively low number of common DMs between the early time points up until 12 w of feeding, which may indicate that the changes due to the HF/HS diet during this phase are variable and reversible. The low number of common DMs between the earlier time points may also be due to a high rate of turnover of lipids in the OE. The lipid profile in any tissue is a result of lipid uptake, metabolism and de novo synthesis. In vitro research in our laboratory using bovine oviductal epithelial cells cultured in a polarized set-up confirmed that OE cells are capable of de novo lipid synthesis (Jordaens et al., 2017b).

The main organelle for lipid biosynthesis is the ER as it produces the majority of structural phospholipids (Bell et al., 1981). As shown in our previous study, consuming the HF/HS diet leads to oxidative stress and ER stress in the OECs already after 3 d of feeding (Moorkens et al., 2022). Although the cause–consequence relationship is not certain as the diet leads to a complex network of interacting changes, ER stress is known to be a key component of the pathogenesis of diet-induced lipotoxicity in many tissues and cells and diet-induced alterations in ER functions can indirectly alter the de novo lipid biosynthesis. Also, alterations in the blood lipid concentrations and degree of fatty acid saturation can directly influence the tissue lipid (selective) uptake and impact cellular lipid composition. In the present study, the detected DMs that were

discriminative in the CTRL vs. HF/HS comparison (more abundant in the CTRL OE) were more abundant than those detected in the HF/HS vs. CTRL comparison (as confirmed in the Supplementary Materials (Figure S2). This may suggest that the de novo synthesis of many lipids is hampered in the OE of the HF/HS group, which applies to the detected DMs that were more abundant in the control OE compared to the HF/HS OE from as early as 3 days and up until 16 w. By contrast, DMs that were detected in the HF/HS vs. CTRL comparisons might be, at least in part, a direct effect of the type and concentration of the circulating lipids.

As a final step, we attempt to provide a general overview of the biological relevance and potential functional implications of the diet-induced lipidomic change in the OE. Identifying or annotating lipid *m*/*z* values in lipidomic analysis can be difficult due to structural ambiguities that may be present, such as bond type or the number of hydroxyl groups (Holčapek et al., 2018). Species annotation may be based on assumptions in such cases, and a few putative annotations are often assigned to the same *m*/*z* value (Holčapek et al., 2018). Hence, we focus here on the potential alteration in lipid classes and families, and their ratios, rather than on individual lipid classes.

We found that phospholipids, namely PCs, PEs and PSs constitute almost 50% of the diet-induced lipidomic changes in the OE. This is true both in the CTRL vs. HF/HS and the HF/HS vs. CTRL comparison, i.e., some PCs, PEs, and PSs increase in abundance in the OEs due to diet, while others are reduced. Phospholipids are the major structural lipids in cell membranes (J.E. Vance, 2002) which are important for allowing e.g., fission and fusion, cell division, and intracellular membrane trafficking (van Meer et al., 2008). PCs represent 40%–50% of the total cellular phospholipid content in all cells and play a vital role in cell functions and homeostasis (Yamashita et al., 2014). For example, PC biosynthesis is required for the process of intracellular fatty acid internalization and incorporation into triacylglycerols in lipid droplets. This mechanism helps to reduce the

impact of lipotoxicity (Listenberger et al., 2003). Alteration in PC biosynthesis, as shown here in the OEs, may thus influence cell viability (van der Veen et al., 2017).

Several DMs in both directions of change were also assigned to PEs (see Tables S3–S5) or putatively assigned to the PC/PE or PC/PE/PS categories, especially at 8 w and afterwards (Figure 8). PE is the second most abundant phospholipid in mammalian membranes (Yamashita et al., 2014). Alteration in PE biosynthesis can cause a shift in the PC/PE ratio which can have several crucial consequences. PEs and PCs regulate the expression of sterol regulatory-element-binding proteins (SREBPs) (Lim et al., 2011; Walker et al., 2011), which are binding proteins that regulate the de novo synthesis of lipids by cells (Brown and Goldstein, 1997). PCs and PEs also form the two most abundant phospholipids of mitochondria, with PEs comprising about 40% of total phospholipids in the mitochondrial inner membrane (Yamashita et al., 2014). It is known that mitochondrial dysfunction plays a role in metabolic disorders (Ren et al., 2010). As mitochondrial lipids define the physical properties of mitochondrial membranes, any reduction in mitochondrial PEs, even if subtle, can alter mitochondrial functions (Tasseva et al., 2013) and has been linked with a profound alteration in mitochondrial morphology and embryonic lethality. Increased PC/PE ratio, due to decreased mitochondrial PE synthesis has even been shown to lead to severe impairment of cell survival and growth in mice (Steenbergen et al., 2005). Therefore, the differential abundance of PCs and PEs seen at the later time points in our study may be indicative of a shifted PC/PE ratio and may suggest an altered mitochondrial function and energy metabolism in the oviductal epithelium.

Some DMs were (putatively) annotated as PSs. PS is a plasma membrane component and participates in many signalling pathways (Fadok et al., 1998). PS is highly enriched in the cytosolic leaflet of the membranes of recycling endosomes, which replenish the lipids and proteins of the plasma membrane, and it is essential for their function (Skotland and Sandvig, 2019).

The second main category of lipids that were altered in the OE due to the HF/HS diet is that assigned to PI and SM. PIs were particularly abundant in the CTRL vs. HF/HS comparison starting from 1 w, indicating a reduced biosynthesis in the HF/HS group. PIs identify endocytic membranes and enable them to attract proteins from the cytosol, which are important for vesicle trafficking and cellular homeostasis (Di Paolo and De Camilli, 2006), and thus play a key role in signalling and recognition (van Meer et al., 2008). Alterations in their expression may affect their role in endocytic pathways in the OE, with a potential impact on cellular homeostasis and cell-to-cell communication. The structural lipid SM, part of the sphingolipids class, was differentially regulated at all time points after the start of feeding the HF/HS diet (van Meer and Lisman, 2002). SMs are inflammatory intracellular lipid species and, as for PCs, SMs have been reported to be crucial precursors of many biomolecules, such as prostaglandins and lipid mediators that play a role in many cell signalling pathways (Eyster, 2007). The backbone of SM is formed by CER, which is produced by the ER, through the action of SM synthases (SMSs) (van Meer and Lisman, 2002). SM is involved in the regulation of endocytosis and receptor-mediated ligand uptake, ion channel and G-protein coupled receptor function, and protein sorting (Slotte, 2013). SM also has an important functional role as a precursor of sphingolipid signalling molecules (Slotte and Ramstedt, 2007). Alterations in these lipids in OEs might thus have several functional consequences.

Lipids also play a key role as first and second messengers in signal transduction and molecular recognition processes (van Meer et al., 2008). Lysophospholipids, such as LPC and LPA, are a group of messenger lipids that are formed by the hydrolysis of phospholipids and sphingolipids (Meyer zu Heringdorf and Jakobs, 2007). In our study lysophospholipids LPC, LPE, LPI and LPS appeared to have a higher abundance in the HF/HS OE compared to the CTRL OE over the different time points, which may indicate that cell signalling processes might be affected due to the HF/HS diet.

We did not focus on the putative annotations of the individual lipid species and their molecular weight and degree of saturation (Tables S3–S5) because of the potential ambiguity in these assignments. However, we have noticed that the identified DMs in the HF/HS vs. CTRL comparison are mostly higher in molecular weight than those in the CTRL vs. HF/HS direction of change. Belaz et al. (2016) (Belaz et al., 2016) have reported that the molecular weight (MW) of lipid ions in the bovine uterus and oviduct are linked to embryo receptivity and fertility. High-MW PCs (e.g., PC(38:7), PC(38:5), PC(40:7) and PC(40:6)) were linked with lower receptivity and low fertility, whereas the low-MW PCs and SMs (e.g., PC(32:1), PC(35:2), SM(34:1) and SM(34:2)) were associated with higher receptivity (Belaz et al., 2016). The potential role of these specific lipids and their MW in the oviduct under obesogenic conditions needs further investigation.

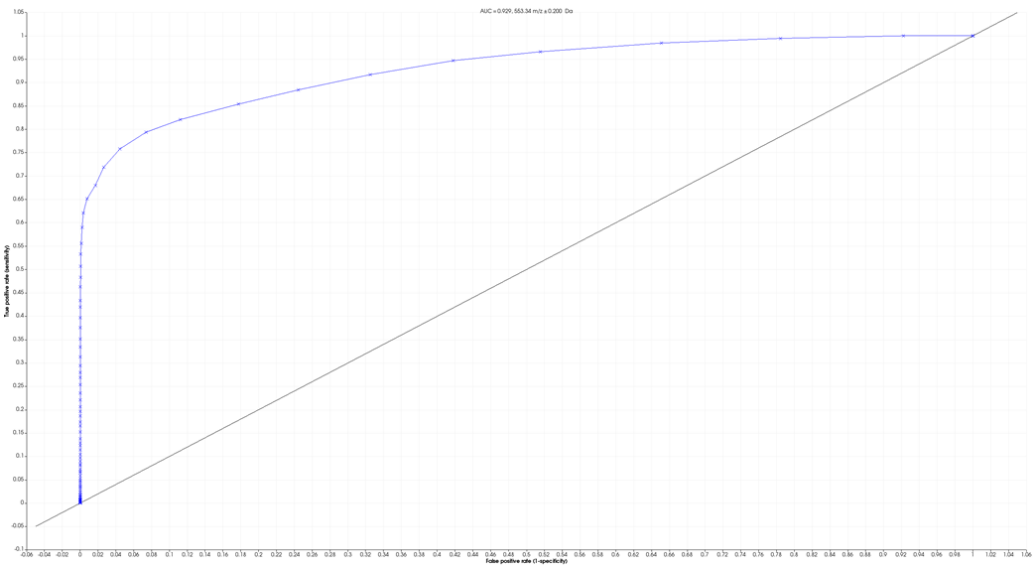
It is important to highlight that lipidomic alterations in the OEs can be reflected in the oviductal microenvironment and alter the interaction between the OE and the gametes and embryos. This may impact all the reproductive events that take place in the oviduct, such as fertilization and early embryo development. The lipid composition of the oviduct was shown to be reflected in the lipidomic profile of the oviductal secretions, including its extracellular vesicles content, which in turn has been shown to alter the lipid composition of the embryos upon direct contact (Banliat et al., 2020). Early embryos internalise and utilise lipids from their microenvironment (Abe et al., 2002), and thus the quantity and type of lipids determine embryo metabolic activity, ROS levels, developmental capacity, as well as embryonic transcriptomic profile and epigenetic programming (Cagnone and Sirard, 2014; Desmet et al., 2016; Aljahdali et al., 2020; Smits et al., 2020). The diet-induced alterations in the lipidomic profile of the OEs, detected as early as after 3 days of feeding, could therefore impact early embryo development and quality. Alterations in signalling molecules that are derived from lipids, such as prostaglandin synthesis in the oviduct, can also impact embryo development and quality (Maillo et al., 2012; Anamthathmakula and Winuthayanon,

2021), ultimately leading to lower pregnancy success or even to offspring health defects (Velazquez, 2015; Robertson et al., 2018). The magnitude of such impact is yet to be determined.

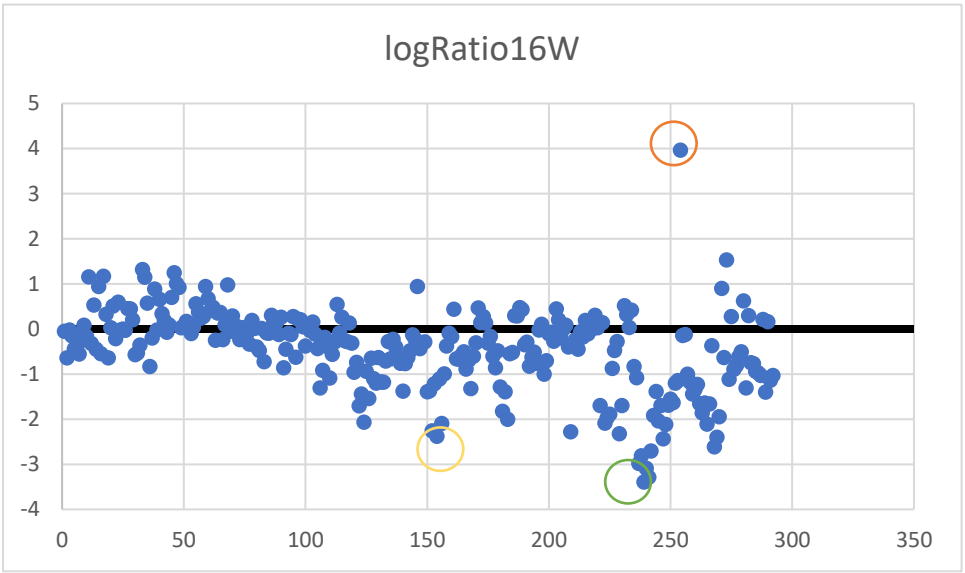
6. Conclusions

We are the first to describe the effect of an HF/HS diet on the lipid profile of the oviductal microenvironment *in vivo*. We can conclude that exposure to an obesogenic diet results in acute changes in the lipid profile in the OE after a short-term feeding period of only 3 days in mice, and thus even before the development of an obese phenotype. At the subsequent early time points, the effects appear to be variable and reversible but the changes in the lipid profile of the OE progressively increase and become more persistent after long-term exposure to the HF/HS diet. Functional annotation revealed a differential abundance of lipids, particularly phospholipids, sphingomyelins and lysophospholipids, which are involved in plasma membrane functions, cell signalling, mitochondrial functions and cellular homeostasis. These alterations appear to be not only due to the direct accumulation of the excess dietary fat in the OEs but also due to a reduction in the de novo synthesis of several lipid classes, most likely due to ER dysfunction.

7. Supplementary material



Supplementary Figure S1. Representative ROC curve of m/z 553,34 detected in negative mode.



Supplementary Figure S2. Scatter plot showing the distribution of the average intensity of the peaks of each detected ion signal (mass) in time point 16 weeks. The ratio of the plot was calculated as HF/HS over CTRL. Therefore, the data points above the baseline are masses with a higher abundance in the HF/HS OE, whereas data points below the baseline are more abundant in the CTRL OE compared to the HF/HS OE. The green and yellow highlighted dots were detected as DMs in the ROC analysis in the CTRLvsHF/HS direction. The orange highlighted dot was identified as DM in the ROC analysis in the HF/HSvsCTRL direction.

Supplementary Table S1. Common DMs detected at different time points in negative mode.

Period		**3d**	**1w**	**4w**	**8w**	**12w**	**16w**
	Total	**10**	**40**	**44**	**43**	**55**	**110**
3d	**10**	10	0	1	2	2	6
1w	**40**		40	11	2	16	22
4w	**44**			44	14	13	35
8w	**43**				43	13	31
12w	**55**					55	36
16w	**110**						110

Supplementary Table S2. Common DMs detected at different time points in positive mode.

Period		**3d**	**1w**	**4w**	**8w**	**12w**	**16w**
	Total	**1**	**33**	**33**	**53**	**9**	**117**
3d	**1**	1	1		1		1
1w	**33**		33	2	8		31
4w	**33**			33	8	1	21
8w	**53**				53	1	25
12w	**9**					9	1
16w	**117**						117

Supplementary Table S3: List of putative annotations of the detected molecular species by MALDI MSI in HF/HS vs CTRL OE in Swiss mice in negative acquisition mode

Identification of differentially regulated lipids detected by MALDI MSI in negative mode (175 m/z)						
m/z	**Direction**	**Time point**	**m/z exact**	**Delta**	**Putative annotation for ion M-H-**	**Putative annotation for ion M+Cl-**
764.557	CTLvsHF	3d	764.5	0.1	PC(35:5)/PC(O-36:5)/PC(P-36:4)/PE(38:5)/PS(35:6)/PS(P-36:5)	PC(32:2)/PC(O-33:2)/PC(P-33:1)/PE(35:2)/PE(O-36:2)/PE(P-36:1) /PS(32:3)/PS(P-33:2)
766.574	CTLvsHF	3d	766.2	0.4	None	PC(32:1)/PC(O-33:1)/PC(P-33:0)/PE-NMe(34:1)/PE(35:1)/PE(36:8) /PE(O-36:1)/PE(P-36:0)/PS(32:2)/PS(O-33:2)/PS(P-33:1)
767.582	CTLvsHF	3d	767.5	0.1	None	SM(d36:0)
768.590	CTLvsHF	3d	768.5	0.1	PC(35:3)/PC(O-36:3)/PC(P-36:2)/PE(38:3)/PS(35:4) /PS(O-36:4)/PS(P-36:3)	PC(32:0)/PC(O-33:0)/PE(35:0)/PE(36:7)/PE(O-36:0)/PS(32:1) /PS(O-33:1)/PS(P-33:0)
790.577	CTLvsHF	3d	790.5	0.1	None	PC(34:3)/PC(P-35:2)/PE(37:3)/PE(O-38:3)/PE(P-38:2)/PS(34:4)
792.594	CTLvsHF	3d	792.5	0.1	PC(37:5)/PC(O-38:5)/PC(P-38:4)/PE(40:5) /PS(37:6)/PS(O-38:6)/PS(P-38:5)	PC(34:2)/PC(O-35:2)/PC(P-35:1)/PE-NMe(36:2)/PE(37:2)/PE(38:9) /PE(O-38:2)/PE(P-38:1)/PS(34:3)/PS(P-35:2)
794.610	CTLvsHF	3d	794.5	0.1	PC(37:4)/PC(O-38:4)/PC(P-38:3)/PE(40:4) /PS(37:5)/PS(O-38:5)/PS(P-38:4)	PC(34:1)/PC(O-35:1)/PC(P-35:0)/PE(37:1)/PE(38:8)/PE(O-38:1) /PE(P-38:0)/PS(34:2)/PS(O-35:2)/PS(P-35:1)
795.618	CTLvsHF	3d	795.5	0.1	PI(31:0)/PI(O-32:0)	SM(d38:0)
796.588	CTLvsHF	3d	796.5	0.1	PC(37:3)/PC(O-38:3)/PC(P-38:2)/PE(40:3) /PS(37:4)/PS(O-38:4)/PS(P-38:3)	PC(34:0)/PC(O-35:0)/PE(37:0)/PE-NMe(36:0)/PE(38:7)/PE(O-38:0) /PS(34:1)/PS(O-35:1)/PS(P-35:0)

798.682	CTLvsHF	3d	798.6	0.1	PC(38:9)/PC(37:2)/PC(O-38:2)/PC(P-38:1)/PE(40:2) /PS(37:3)/PS(O-38:3)/PS(P-38:2)	PC(35:6)/PC(P-36:5)/PE(38:6)/PS(34:0)/PS(O-35:0)
837.538	CTLvsHF	1w	837.5	0	None	PI(32:4)
863.583	CTLvsHF	1w	863.6	0	None	SM(d43:1)/PI(34:5)
864.591	CTLvsHF	1w	864.6	0	None	PC(40:8)/PC(39:1)/PC(O-40:1)/PC(P-40:0)/PE(42:1)/PS(39:2) /PS(40:9)/PS(O-40:2)/PS(P-40:1)
865.559	CTLvsHF	1w	865.5	0.1	None	PI(34:4)
911.874	CTLvsHF	1w	911.6	0.3	None	PI(37:2)/PI(38:9)/PI(O-38:2)/PI(P-38:1)
912.572	CTLvsHF	1w	912.6	0	None	PC(44:12)/PC(O-44:5)/PS(43:6)
937.609	CTLvsHF	1w	937.6	0	PI(42:6)	None
528.324	CTLvsHF	4w	528.1	0.2	-/LPE(22:4)	None
529.332	CTLvsHF	4w	529.1	0.2	LPI(13:0)	None
700.569	CTLvsHF	4w	700.5	0.1	PC(30:2)/PC(P-31:1)/PE(33:2)/PE(O-34:2)/PE(P-34:1)/PS(30:3)	None
722.550	CTLvsHF	4w	722.5	0.1	PC(32:5)/PE(35:5)/PE(O-36:5)/PE(P-36:4)	PC(29:2)/PC(P-30:1)/PE(32:2)/PE(O-33:2)/PE(P-33:1)
723.558	CTLvsHF	4w	723.5	0.1	PI(26:1)	None
724.489	CTLvsHF	4w	724.5	0	PC(32:4)/PE(35:4)/PE(O-36:4)/PE(P-36:3)/PS(32:5)	None
725.458	CTLvsHF	4w	725.4	0.1	SM(d36:3)/PI(26:0)	None
750.579	CTLvsHF	4w	750.5	0.1	None	PC(31:2)/PC(O-32:2)/PC(P-32:1)/PE(34:2)/PE(O-35:2)/PE(P-35:1)/PS(31:3)
751.587	CTLvsHF	4w	751.5	0.1	None	SM(d35:1)
809.507	CTLvsHF	4w	809.5	0	SM(d42:3)/PI(32:0)/PI(O-33:0)	PI(30:4)
833.504	CTLvsHF	4w	833.5	0	PI(34:2)/PI(O-35:2)/PI(P-35:1)	None
837.536	CTLvsHF	4w	837.5	0	None	PI(32:4)

843.506	CTLvsHF	4w	843.5	0	SM(d44:0)/PI(35:4)/PI(O-36:4)/PI(P-36:3)	PI(32:1)/PI(O-33:1)/PI(P-33:0)
857.579	CTLvsHF	4w	857.5	0.1	PI(36:4)	PI(33:1)/PI(O-34:1)/PI(P-34:0)
858.587	CTLvsHF	4w	858.5	0.1	None	PC(39:4)/PC(O-40:4)/PC(P-40:3)/PE(42:4)/PS(39:5)/PS(O-40:5)/PS(P-40:4)
859.556	CTLvsHF	4w	859.5	0.1	None	PI(33:0)/PI(O-34:0)
865.565	CTLvsHF	4w	865.5	0.1	None	PI(34:4)
866.573	CTLvsHF	4w	866.5	0.1	None	PC(40:7)/PC(39:0)/PC(O-40:0)/PE(42:0)/PE-NMe2(40:0)/PS(39:1)/PS(40:8)/PS(O-40:1)/PS(P-40:0)
871.574	CTLvsHF	4w	871.6	0	PI(37:4)/PI(O-38:4)/PI(P-38:3)	PI(34:1)/PI(O-35:1)/PI(P-35:0)
872.582	CTLvsHF	4w	872.6	0	PC(42:0)/PS(42:1)	PC(40:4)/PE(43:4)/PS(40:5)
881.537	CTLvsHF	4w	881.5	0	PI(38:6)	None
882.545	CTLvsHF	4w	882.5	0	None	PC(41:6)/PC(O-42:6)/PE(44:6)/PS(40:0)/PS(41:7)/PS(O-41:0)/PS(P-42:6)
883.553	CTLvsHF	4w	883.5	0.1	PI(38:5)	PI(35:2)/PI(O-36:2)/PI(P-36:1)
884.561	CTLvsHF	4w	884.5	0.1	PC(44:8)/PC(43:1)/PE(46:1)/PS(43:2)	PC(41:5)/PC(P-42:4)/PE(44:5)/PS(41:6)/PS(O-42:6)
885.608	CTLvsHF	4w	885.6	0	PI(38:4)	PI(35:1)/PI(36:8)/PI(O-36:1)/PI(P-36:0)
899.603	CTLvsHF	4w	899.6	0	PI(39:4)/PI(O-40:4)/PI(P-40:3)	PI(36:1)/PI(O-37:1)/PI(P-37:0)
913.598	CTLvsHF	4w	913.6	0	None	PI(37:1)/PI(38:8)/PI(O-38:1)/PI(P-38:0)
556.296	CTLvsHF	8w	556.3	0	LPC(21:4)/PC(20:4)	LPC(18:1)/LPC(18:1)18:1[18:1r18:1a18:1c18:1]/PC(O-18:1)/PC(P-18:0)/LPS(18:2)
618.469	CTLvsHF	8w	618.2	0.3	None	LPS(-OMe-221:0)
725.373	CTLvsHF	8w	725.4	0	SM(d36:3)/PI(26:0)	None
795.569	CTLvsHF	8w	795.5	0.1	PI(31:0)/PI(O-32:0)	SM(d38:0)
809.485	CTLvsHF	8w	809.5	0	SM(d42:3)/PI(32:0)/PI(O-33:0)	PI(30:4)
833.478	CTLvsHF	8w	833.5	0	PI(34:2)/PI(O-35:2)/PI(P-35:1)	None

843.517	CTLvsHF	8w	843.5	0	SM(d44:0)/PI(35:4)/PI(O-36:4)/PI(P-36:3)	PI(32:1)/PI(O-33:1)/PI(P-33:0)
915.264	CTLvsHF	8w	914.6	0.7	None	PS(44:12)/PC(43:4)/PC(O-44:4)
856.497	CTLvsHF	12w	856.5	0	PC(42:8)/PC(41:1)/PC(O-42:1)/PC(P-42:0)/PE(44:1)/PS(41:2)/PS(42:9)/PS(O-42:2)/PS(P-42:1)	PC(39:5)/PC(O-40:5)/PC(P-40:4)/PE(42:5)/PS(39:6)/PS(O-40:6)/PS(P-40:5)
912.331	CTLvsHF	12w	911.6	0.7	None	PI(37:2)/PI(38:9)/PI(O-38:2)/PI(P-38:1)
673.490	CTLvsHF	16w	673.4	0.1	SM(d32:1)	None
700.560	CTLvsHF	16w	700.5	0.1	PC(30:2)/PC(P-31:1)/PE(33:2)/PE(O-34:2)/PE(P-34:1)/PS(30:3)	None
702.538	CTLvsHF	16w	702.5	0	PC(30:1)/PC(O-31:1)/PC(P-31:0)/PE(33:1)/PE(O-34:1)/PE(P-34:0)/PS(30:2)/PS(P-31:1)	None
722.550	CTLvsHF	16w	722.5	0.1	PC(32:5)/PE(35:5)/PE(O-36:5)/PE(P-36:4)	PC(29:2)/PC(P-30:1)/PE(32:2)/PE(O-33:2)/PE(P-33:1)
724.528	CTLvsHF	16w	724.5	0	PC(32:4)/PE(35:4)/PE(O-36:4)/PE(P-36:3)/PS(32:5)	None
725.420	CTLvsHF	16w	725.4	0	SM(d36:3)/PI(26:0)	None
725.537	CTLvsHF	16w	725.4	0.1	SM(d36:3)/PI(26:0)	None
750.552	CTLvsHF	16w	750.5	0.1	None	PC(31:2)/PC(O-32:2)/PC(P-32:1)/PE(34:2)/PE(O-35:2)/PE(P-35:1)/PS(31:3)
751.560	CTLvsHF	16w	751.5	0.1	None	SM(d35:1)
764.552	CTLvsHF	16w	764.5	0.1	PC(35:5)/PC(O-36:5)/PC(P-36:4)/PE(38:5)/PS(35:6)/PS(P-36:5)	PC(32:2)/PC(O-33:2)/PC(P-33:1)/PE(35:2)/PE(O-36:2)/PE(P-36:1)/PS(32:3)/PS(P-33:2)
766.569	CTLvsHF	16w	766.2	0.4	None	PC(32:1)/PC(O-33:1)/PC(P-33:0)/PE-NMe(34:1)/PE(35:1)/PE(36:8)/PE(O-36:1)/PE(P-36:0)/PS(32:2)/PS(O-33:2)/PS(P-33:1)
768.547	CTLvsHF	16w	768.5	0	PC(35:3)/PC(O-36:3)/PC(P-36:2)/PE(38:3)/PS(35:4)/PS(O-36:4)/PS(P-36:3)	PC(32:0)/PC(O-33:0)/PE(35:0)/PE(36:7)/PE(O-36:0)/PS(32:1)/PS(O-33:1)/PS(P-33:0)

796.587	CTLvsHF	16w	796.5	0.1	PC(37:3)/PC(O-38:3)/PC(P-38:2)/PE(40:3) /PS(37:4)/PS(O-38:4)/PS(P-38:3)	PC(34:0)/PC(O-35:0)/PE(37:0)/PE-NMe(36:0)/PE(38:7)/PE(O-38:0) /PS(34:1)/PS(O-35:1)/PS(P-35:0)
797.673	CTLvsHF	16w	797.6	0.1	SM(d41:2)	None
809.541	CTLvsHF	16w	809.5	0	SM(d42:3)/PI(32:0)/PI(O-33:0)	PI(30:4)
833.509	CTLvsHF	16w	833.5	0	PI(34:2)/PI(O-35:2)/PI(P-35:1)	None
837.542	CTLvsHF	16w	837.5	0	None	PI(32:4)
843.515	CTLvsHF	16w	843.5	0	SM(d44:0)/PI(35:4)/PI(O-36:4)/PI(P-36:3)	PI(32:1)/PI(O-33:1)/PI(P-33:0)
844.523	CTLvsHF	16w	844.5	0	PC(41:7)/PC(P-42:6)/PC(40:0)/PC(O-41:0)/PE(43:0) /PE(44:7)/PS(40:1)/PS(P-41:0)	PC(38:4)/PE(41:4)/PE(42:11)/PE(O-42:4)/PS(38:5)
859.571	CTLvsHF	16w	859.5	0.1	None	PI(33:0)/PI(O-34:0)
860.540	CTLvsHF	16w	860.5	0	None	PC(40:10)/PC(39:3)/PC(O-40:3)/PC(P-40:2)/PE(42:3)/PS(39:4) /PS(O-40:4)/PS(P-40:3)
861.549	CTLvsHF	16w	861.5	0	PI(36:2)/PI(O-37:2)/PI(P-37:1)	SM(d43:2)/PI(34:6)
863.566	CTLvsHF	16w	863.6	0	None	SM(d43:1)/PI(34:5)
864.574	CTLvsHF	16w	864.6	0	None	PC(40:8)/PC(39:1)/PC(O-40:1)/PC(P-40:0)/PE(42:1)/PS(39:2) /PS(40:9)/PS(O-40:2)/PS(P-40:1)
865.505	CTLvsHF	16w	865.5	0	None	PI(34:4)
866.513	CTLvsHF	16w	866.5	0	None	PC(40:7)/PC(39:0)/PC(O-40:0)/PE(42:0)/PE-NMe2(40:0)/PS(39:1) /PS(40:8)/PS(O-40:1)/PS(P-40:0)
870.547	CTLvsHF	16w	870.5	0	PC(42:1)/PS(42:2)	PC(40:5)/PE(44:12)/PS(40:6)
871.555	CTLvsHF	16w	871.6	0	PI(37:4)/PI(O-38:4)/PI(P-38:3)	PI(34:1)/PI(O-35:1)/PI(P-35:0)
872.563	CTLvsHF	16w	872.6	0	PC(42:0)/PS(42:1)	PC(40:4)/PE(43:4)/PS(40:5)
873.572	CTLvsHF	16w	873.5	0.1	PI(37:3)/PI(O-38:3)/PI(P-38:2)	PI(34:0)/PI(O-35:0)

881.522	CTLvsHF	16w	881.5	0	PI(38:6)	None
882.530	CTLvsHF	16w	882.5	0	None	PC(41:6)/PC(O-42:6)/PE(44:6)/PS(40:0)/PS(41:7)/PS(O-41:0)/PS(P-42:6)
883.539	CTLvsHF	16w	883.5	0	PI(38:5)	PI(35:2)/PI(O-36:2)/PI(P-36:1)
884.547	CTLvsHF	16w	884.5	0	PC(44:8)/PC(43:1)/PE(46:1)/PS(43:2)	PC(41:5)/PC(P-42:4)/PE(44:5)/PS(41:6)/PS(O-42:6)
887.572	CTLvsHF	16w	887.6	0	None	PI(35:0)/PI(36:7)/PI(O-36:0)
888.581	CTLvsHF	16w	888.6	0	PC(44:6)/PS(43:0)/PS(44:7)	PC(42:10)/PC(41:3)/PC(O-42:3)/PC(P-42:2)/PE(44:3)/PS(41:4)/PS(42:11)/PS(O-42:4)
890.597	CTLvsHF	16w	890.6	0	PC(44:5)/PS(44:6)	None
899.595	CTLvsHF	16w	899.6	0	PI(39:4)/PI(O-40:4)/PI(P-40:3)	PI(36:1)/PI(O-37:1)/PI(P-37:0)
909.562	CTLvsHF	16w	909.6	0	PI(40:6)	None
911.579	CTLvsHF	16w	911.6	0	None	PI(37:2)/PI(38:9)/PI(O-38:2)/PI(P-38:1)
913.557	CTLvsHF	16w	913.6	0	None	PI(37:1)/PI(38:8)/PI(O-38:1)/PI(P-38:0)
914.565	CTLvsHF	16w	914.6	0	None	PS(44:12)/PC(43:4)/PC(O-44:4)
915.574	CTLvsHF	16w	915.6	0	None	PI(37:0)/PI(38:7)/PI(O-38:0)
528.297	HFvsCTL	1w	528.1	0.2	-/LPE(22:4)	None
529.304	HFvsCTL	1w	529.1	0.2	LPI(13:0)	None
550.311	HFvsCTL	1w	550.3	0	LPC(20:0)/PC(19:0)/PC(O-20:0)/LPS(20:1)	LPC(18:4)
685.340	HFvsCTL	1w	685.2	0.1	SM(d33:2)	None
773.551	HFvsCTL	1w	773.5	0.1	None	PI(27:1)/PI(P-28:0)
786.495	HFvsCTL	1w	786.5	0	PC(36:1)/PC(O-37:1)/PC(P-37:0)/PE(39:1)/PE(40:8)/PE(O-40:1)/PE(P-40:0)/PS(36:2)/PS(O-37:2)/PS(P-37:1)	PC(34:5)/PE(37:5)/PE(O-38:5)/PE(P-38:4)/PS(34:6)
817.462	HFvsCTL	1w	817.5	0	PI(33:3)/PI(O-34:3)/PI(P-34:2)	SM(d40:3)/PI(30:0)/PI(O-31:0)
857.498	HFvsCTL	1w	857.5	0	PI(36:4)	PI(33:1)/PI(O-34:1)/PI(P-34:0)

858.506	HFvsCTL	1w	858.5	0	None	PC(39:4)/PC(O-40:4)/PC(P-40:3)/PE(42:4)/PS(39:5) /PS(O-40:5)/PS(P-40:4)
859.513	HFvsCTL	1w	859.5	0	None	PI(33:0)/PI(O-34:0)
860.521	HFvsCTL	1w	860.5	0	None	PC(40:10)/PC(39:3)/PC(O-40:3)/PC(P-40:2)/PE(42:3) /PS(39:4)/PS(O-40:4)/PS(P-40:3)
871.567	HFvsCTL	1w	871.6	0	PI(37:4)/PI(O-38:4)/PI(P-38:3)	PI(34:1)/PI(O-35:1)/PI(P-35:0)
872.574	HFvsCTL	1w	872.6	0	PC(42:0)/PS(42:1)	PC(40:4)/PE(43:4)/PS(40:5)
873.543	HFvsCTL	1w	873.5	0	PI(37:3)/PI(O-38:3)/PI(P-38:2)	PI(34:0)/PI(O-35:0)
552.321	HFvsCTL	4w	552.3	0	LPE(24:6)/LPS(20:0)	None
772.600	HFvsCTL	4w	772.5	0.1	PC(36:8)/PC(35:1)/PC(O-36:1)/PC(P-36:0)/PE(38:1)/PE(39:8) /PE(O-39:1)/PE(P-40:7)/PE(P-39:0)/PS(35:2) /PS(O-36:2)/PS(P-36:1)	PC(33:5)/PC(P-34:4)/PE(36:5)
791.557	HFvsCTL	4w	791.5	0.1	PI(31:2)/PI(P-32:1)	SM(d38:2)
818.539	HFvsCTL	4w	818.5	0	PC(39:6)/PC(O-40:6)/PC(P-40:5)/PE(42:6)/PS(38:0) /PS(39:7)/PS(O-39:0)/PS(P-40:6)	PC(36:3)/PC(P-37:2)/PE(39:3)/PE(40:10)/PE(O-40:3) /PE(P-40:2)/PS(36:4)
552.303	HFvsCTL	8w	552.3	0	LPE(24:6)/LPS(20:0)	None
599.321	HFvsCTL	8w	599.1	0.2	LPI(18:0)	None
599.592	HFvsCTL	8w	599.1	0.5	LPI(18:0)	None
700.759	HFvsCTL	8w	700.5	0.3	PC(30:2)/PC(P-31:1)/PE(33:2)/PE(O-34:2)/PE(P-34:1)/PS(30:3)	None
702.736	HFvsCTL	8w	702.5	0.2	PC(30:1)/PC(O-31:1)/PC(P-31:0)/PE(33:1)/PE(O-34:1) /PE(P-34:0)/PS(30:2)/PS(P-31:1)	None
723.745	HFvsCTL	8w	723.5	0.2	PI(26:1)	None

744.792	HFvsCTL	8w	744.5	0.3	PC(33:1)/PC(O-34:1)/PC(P-34:0)/PE(36:1) /PE-NMe2(34:1)/PE(O-37:1) /PE(P-37:0)/PS(33:2)/PS(O-34:2)/PS(P-34:1)	PE(34:5)
746.536	HFvsCTL	8w	746.5	0	PC(33:0)/PC(O-34:0)/PE(36:0)/PE(37:7)/PE(O-37:0)/PE(P-38:6) /PS(33:1)/PS(O-34:1)/PS(P-34:0)	PC(31:4)/PE(34:4)
748.784	HFvsCTL	8w	748.5	0.3	PC(34:6)/PE(37:6)/PE(O-38:6)/PE(P-38:5)/PS(33:0)/PS(O-34:0)	PC(31:3)/PC(O-32:3)/PE(34:3)/PE(P-35:2)/PS(31:4)
768.785	HFvsCTL	8w	768.5	0.3	PC(35:3)/PC(O-36:3)/PC(P-36:2)/PE(38:3) /PS(35:4)/PS(O-36:4)/PS(P-36:3)	PC(32:0)/PC(O-33:0)/PE(35:0)/PE(36:7)/PE(O-36:0) /PS(32:1)/PS(O-33:1)/PS(P-33:0)
772.545	HFvsCTL	8w	772.5	0	PC(36:8)/PC(35:1)/PC(O-36:1)/PC(P-36:0) /PE(38:1)/PE(39:8)/PE(O-39:1) /PE(P-40:7)/PE(P-39:0)/PS(35:2)/PS(O-36:2)/PS(P-36:1)	PC(33:5)/PC(P-34:4)/PE(36:5)
774.793	HFvsCTL	8w	774.5	0.3	PC(36:7)/PC(35:0)/PC(O-36:0)/PE(38:0)/PE-NMe2(36:0) /PE(39:7)/PE(O-39:0) /PE(P-40:6)/PS(35:1)/PS(36:8)/PS(O-36:1)/PS(P-36:0)	PC(33:4)/PC(O-34:4)/PC(P-34:3)/PE(36:4)/PS(33:5)/PS(P-34:4)
788.825	HFvsCTL	8w	788.5	0.3	PC(P-38:6)/PC(36:0)/PC(O-37:0)/PE(39:0)/PE(O-40:0) /PS(O-37:1)/PS(P-37:0)	None
811.733	HFvsCTL	8w	811.5	0.2	SM(d42:2)	None
887.821	HFvsCTL	8w	887.6	0.2	None	PI(35:0)/PI(36:7)/PI(O-36:0)
890.806	HFvsCTL	8w	890.6	0.2	PC(44:5)/PS(44:6)	None
528.589	HFvsCTL	12w	528.1	0.5	-/LPE(22:4)	None
556.545	HFvsCTL	12w	556.3	0.2	LPC(21:4)/PC(20:4)	LPC(18:1)/LPC(18:1)18:1[18:1r18:1a18:1c18:1] /PC(O-18:1)/PC(P-18:0)/LPS(18:2)

768.869	HFvsCTL	12w	768.5	0.4	PC(35:3)/PC(O-36:3)/PC(P-36:2)/PE(38:3)/PS(35:4)/PS(O-36:4)/PS(P-36:3)	PC(32:0)/PC(O-33:0)/PE(35:0)/PE(36:7)/PE(O-36:0)/PS(32:1)/PS(O-33:1)/PS(P-33:0)
770.575	HFvsCTL	12w	770.5	0.1	PC(35:2)/PC(O-36:2)/PC(P-36:1)/PE(38:2)/PE-NMe2(36:2)/PE(P-39:1)/PS(35:3)/PS(O-36:3)/PS(P-36:2)	PE(36:6)/PS(32:0)/PS(O-33:0)
797.678	HFvsCTL	12w	797.6	0.1	SM(d41:2)	None
798.647	HFvsCTL	12w	798.6	0	PC(38:9)/PC(37:2)/PC(O-38:2)/PC(P-38:1)/PE(40:2)/PS(37:3)/PS(O-38:3)/PS(P-38:2)	PC(35:6)/PC(P-36:5)/PE(38:6)/PS(34:0)/PS(O-35:0)
861.577	HFvsCTL	12w	861.5	0.1	PI(36:2)/PI(O-37:2)/PI(P-37:1)	SM(d43:2)/PI(34:6)
863.593	HFvsCTL	12w	863.6	0	None	SM(d43:1)/PI(34:5)
864.601	HFvsCTL	12w	864.6	0	None	PC(40:8)/PC(39:1)/PC(O-40:1)/PC(P-40:0)/PE(42:1)/PS(39:2)/PS(40:9)/PS(O-40:2)/PS(P-40:1)
865.570	HFvsCTL	12w	865.5	0.1	None	PI(34:4)
866.540	HFvsCTL	12w	866.5	0	None	PC(40:7)/PC(39:0)/PC(O-40:0)/PE(42:0)/PE-NMe2(40:0)/PS(39:1)/PS(40:8)/PS(O-40:1)/PS(P-40:0)
871.852	HFvsCTL	12w	871.6	0.3	PI(37:4)/PI(O-38:4)/PI(P-38:3)	PI(34:1)/PI(O-35:1)/PI(P-35:0)
884.569	HFvsCTL	12w	884.5	0.1	PC(44:8)/PC(43:1)/PE(46:1)/PS(43:2)	PC(41:5)/PC(P-42:4)/PE(44:5)/PS(41:6)/PS(O-42:6)
887.594	HFvsCTL	12w	887.6	0	None	PI(35:0)/PI(36:7)/PI(O-36:0)
888.602	HFvsCTL	12w	888.6	0	PC(44:6)/PS(43:0)/PS(44:7)	PC(42:10)/PC(41:3)/PC(O-42:3)/PC(P-42:2)/PE(44:3)/PS(41:4)/PS(42:11)/PS(O-42:4)
890.618	HFvsCTL	12w	890.6	0	PC(44:5)/PS(44:6)	None
890.851	HFvsCTL	12w	890.6	0.3	PC(44:5)/PS(44:6)	None
911.594	HFvsCTL	12w	911.6	0	None	PI(37:2)/PI(38:9)/PI(O-38:2)/PI(P-38:1)
911.866	HFvsCTL	12w	911.6	0.3	None	PI(37:2)/PI(38:9)/PI(O-38:2)/PI(P-38:1)

912.603	HFvsCTL	12w	912.6	0	None	PC(44:12)/PC(O-44:5)/PS(43:6)
913.611	HFvsCTL	12w	913.6	0	None	PI(37:1)/PI(38:8)/PI(O-38:1)/PI(P-38:0)
914.619	HFvsCTL	12w	914.6	0	None	PS(44:12)/PC(43:4)/PC(O-44:4)
914.852	HFvsCTL	12w	914.6	0.3	None	PS(44:12)/PC(43:4)/PC(O-44:4)
915.588	HFvsCTL	12w	915.6	0	None	PI(37:0)/PI(38:7)/PI(O-38:0)
552.37	HFvsCTL	16w	552.3	0.1	LPE(24:6)/LPS(20:0)	None
554.348	HFvsCTL	16w	554.3	0	LPS(-OMe-219:0)	LPC(18:2)/LPC(P-19:1)/LPS(18:3)
571.374	HFvsCTL	16w	571.1	0.3	LPI(16:0)	None
600.383	HFvsCTL	16w	600.3	0.1	None	LPC(21:0)/PC(20:0)/PC(O-21:0)/PE-NMe(22:0)/LPE(24:0)
744.579	HFvsCTL	16w	744.5	0.1	PC(33:1)/PC(O-34:1)/PC(P-34:0)/PE(36:1) /PE-NMe2(34:1)/PE(O-37:1) /PE(P-37:0)/PS(33:2)/PS(O-34:2)/PS(P-34:1)	PE(34:5)
746.596	HFvsCTL	16w	746.5	0.1	PC(33:0)/PC(O-34:0)/PE(36:0)/PE(37:7)/PE(O-37:0)/PE(P-38:6) /PS(33:1)/PS(O-34:1)/PS(P-34:0)	PC(31:4)/PE(34:4)
762.536	HFvsCTL	16w	762.5	0	None	PC(32:3)/PC(P-33:2)/PE(35:3)/PE(O-36:3) /PE(P-36:2)/PS(32:4)
772.580	HFvsCTL	16w	772.5	0.1	PC(36:8)/PC(35:1)/PC(O-36:1)/PC(P-36:0) /PE(38:1)/PE(39:8)/PE(O-39:1) /PE(P-40:7)/PE(P-39:0)/PS(35:2)/PS(O-36:2)/PS(P-36:1)	PC(33:5)/PC(P-34:4)/PE(36:5)
773.589	HFvsCTL	16w	773.5	0.1	None	PI(27:1)/PI(P-28:0)
774.597	HFvsCTL	16w	774.5	0.1	PC(36:7)/PC(35:0)/PC(O-36:0)/PE(38:0)/PE-NMe2(36:0) /PE(39:7)/PE(O-39:0) /PE(P-40:6)/PS(35:1)/PS(36:8)/PS(O-36:1)/PS(P-36:0)	PC(33:4)/PC(O-34:4)/PC(P-34:3)/PE(36:4)/PS(33:5)/PS(P-34:4)
775.567	HFvsCTL	16w	775.5	0.1	PI(30:3)	PI(27:0)/PI(O-28:0)

788.598	HFvsCTL	16w	788.5	0.1	PC(P-38:6)/PC(36:0)/PC(O-37:0)/PE(39:0)/PE(O-40:0) /PS(O-37:1)/PS(P-37:0)	None
789.567	HFvsCTL	16w	789.5	0.1	PI(31:3)	SM(d38:3)/PI(28:0)/PI(O-29:0)
790.576	HFvsCTL	16w	790.5	0.1	None	PC(34:3)/PC(P-35:2)/PE(37:3)/PE(O-38:3)/PE(P-38:2)/PS(34:4)
791.584	HFvsCTL	16w	791.5	0.1	PI(31:2)/PI(P-32:1)	SM(d38:2)
811.557	HFvsCTL	16w	811.5	0.1	SM(d42:2)	None
856.546	HFvsCTL	16w	856.5	0	PC(42:8)/PC(41:1)/PC(O-42:1)/PC(P-42:0) /PE(44:1)/PS(41:2) /PS(42:9)/PS(O-42:2)/PS(P-42:1)	PC(39:5)/PC(O-40:5)/PC(P-40:4)/PE(42:5)/PS(39:6) /PS(O-40:6)/PS(P-40:5)
858.563	HFvsCTL	16w	858.5	0.1	None	PC(39:4)/PC(O-40:4)/PC(P-40:3)/PE(42:4)/PS(39:5) /PS(O-40:5)/PS(P-40:4)
885.556	HFvsCTL	16w	885.6	0	PI(38:4)	PI(35:1)/PI(36:8)/PI(O-36:1)/PI(P-36:0)
886.603	HFvsCTL	16w	886.6	0	None	PC(42:11)/PC(41:4)/PC(O-42:4)/PE(44:4) /PS(41:5)/PS(P-42:4)

Supplementary Table S4: List of putative annotation of the detected molecular species by MALDI MSI in HF/HS vs CTRL OE in Swiss mice in positive acquisition mode;

Identification of differentially regulated lipids detected by MALDI MSI (31 m/z)							
m/z	**Direction**	**Time point**	**m/z exact**	**Delta**	**Putative annotation for ion M.H+**	**Putative annotation for ion M.Na+**	**Putative annotation for ion M.K+**
810.334	CTLvsHF	1w	809.6	0.7	None	SM(d40:1)	None
812.315	CTLvsHF	1w	811.6	0.7	None	SM(d40:0)	None
758.315	CTLvsHF	4w	757.6	0.7	SM(d38:2)	None	None
759.323	CTLvsHF	4w	758.6	0.7	PC(34:2)/PC(O-35:2)/PC(P-35:1) /PE-NMe(36:2)/PE(37:2) /PE(38:9)/PE(O-38:2)/PE(P-38:1)	None	PC(31:0)/PC(O-32:0)/PE-NMe2(32:0) /PE(O-35:0)
760.330	CTLvsHF	4w	759.6	0.7	SM(d38:1)	None	None
749.329	CTLvsHF	8w	748.6	0.7	PC(33:0)/PC(O-34:0)/PE(36:0) /PE(37:7)/PE(O-37:0)/PE(P-38:6)	PC(32:4)/PE(35:4)/PE(O-36:4) /PE(P-36:3)	PE(34:5)
769.027	CTLvsHF	8w	768.6	0.4	PC(35:4)/PC(O-36:4)/PC(P-36:3)/PE(38:4)	PC(33:1)/PC(O-34:1)/PC(P-34:0) /PE(36:1)/PE-NMe2(34:1) /PE(O-37:1)/PE(P-37:0)	None
775.011	CTLvsHF	8w	774.6	0.4	PC(36:8)/-/PC(35:1)/PC(O-36:1) /PC(P-36:0)/PE(38:1) /PE(39:8)/PE(O-39:1)/PE(P-40:7)/PE(P-39:0)	PC(34:5)/PE(37:5) /PE(O-38:5)/PE(P-38:4)	PE(36:6)
781.033	CTLvsHF	8w	780.6	0.4	None	None	PC(33:3)/PC(O-34:3)/PC(P-34:2) /PE(36:3)/PE(P-37:2)
781.266	CTLvsHF	8w	780.6	0.7	None	None	PC(33:3)/PC(O-34:3)/PC(P-34:2) /PE(36:3)/PE(P-37:2)

799.022	CTLvsHF	8w	798.6	0.4	PC(37:3)/PC(O-38:3)/PC(P-38:2)/PE(40:3)	PC(36:7)/PC(35:0)/PC(O-36:0)/PE(38:0)/PE-NMe2(36:0)/PE(39:7)/PE(O-39:0)/PE(P-40:6)	PC(34:1)/PC(O-35:1)/PC(P-35:0)/PE(37:1)/PE(38:8)/PE(O-38:1)/PE(P-38:0)
805.316	CTLvsHF	8w	804.6	0.7	PC(38:7)/PC(37:0)/PC(O-38:0)/PE(40:0)/PE(41:7)/PE(O-41:0)/PE(P-42:6)	PC(36:4)/PC(O-37:4)/PE(39:4)/PE(O-40:4)/PE(P-40:3)	PC(35:5)/PC(O-36:5)/PC(P-36:4)/PE(38:5)
746.283	CTLvsHF	16w	745.6	0.7	SM(d37:1)	None	None
758.305	CTLvsHF	16w	757.6	0.7	SM(d38:2)	None	None
759.313	CTLvsHF	16w	758.6	0.7	PC(34:2)/PC(O-35:2)/PC(P-35:1)/PE-NMe(36:2)/PE(37:2)/PE(38:9)/PE(O-38:2)/PE(P-38:1)	None	PC(31:0)/PC(O-32:0)/PE-NMe2(32:0)/PE(O-35:0)
760.321	CTLvsHF	16w	759.6	0.7	SM(d38:1)	None	None
768.309	CTLvsHF	16w	767.6	0.7	None	None	SM(d36:2)
769.318	CTLvsHF	16w	768.6	0.7	PC(35:4)/PC(O-36:4)/PC(P-36:3)/PE(38:4)	PC(33:1)/PC(O-34:1)/PC(P-34:0)/PE(36:1)/PE-NMe2(34:1)/PE(O-37:1)/PE(P-37:0)	None
774.320	CTLvsHF	16w	773.6	0.7	SM(d39:1)	None	None
781.300	CTLvsHF	16w	780.6	0.7	None	None	PC(33:3)/PC(O-34:3)/PC(P-34:2)/PE(36:3)/PE(P-37:2)
784.325	CTLvsHF	16w	783.6	0.7	SM(d40:3)	None	None
794.329	CTLvsHF	16w	793.6	0.7	None	SM(d39:2)	None
757.966	HFvsCTL	4w	757.6	0.4	SM(d38:2)	None	None
812.348	HFvsCTL	8w	811.6	0.7	None	SM(d40:0)	None
695.147	HFvsCTL	12w	694.5	0.6	None	PE(31:3)	None

777.297	HFvsCTL	12w	776.6	0.7	None	PC(34:4)/PC(O-35:4)/PE(37:4) /PE(O-38:4)/PE(P-38:3)	None
705.256	HFvsCTL	16w	704.6	0.7	PC(30:1)/PC(O-31:1)/PC(P-31:0) /PE(33:1)/PE(O-34:1)/PE(P-34:0)	PE(32:5)	None
767.301	HFvsCTL	16w	766.6	0.7	None	PC(33:2)/PC(O-34:2)/PC(P-34:1) /PE(36:2) /PE(O-37:2)/PE(P-37:1)	PC(32:3)/PC(P-33:2)/PE(35:3) /PE(O-36:3)/PE(P-36:2)
829.346	HFvsCTL	16w	828.6	0.7	PC(40:9)/PC(39:2)/PC(O-40:2) /PC(P-40:1)/PE(42:2)	PC(38:6)/PE(41:6)/PE(O-42:6)	PC(37:7)/PC(P-38:6)/PC(36:0) /PC(O-37:0) /PE(39:0)/PE(40:7)/PE(O-40:0)
837.101	HFvsCTL	16w	836.6	0.5	None	PC(38:2)/PC(P-39:1)/PE(41:2) /PE(42:9) /PE(O-42:2)/PE(P-42:1)	None
838.109	HFvsCTL	16w	837.6	0.5	None	SM(d42:1)	None

Supplementary Table S5: Overview of putative annotations

CTRL vs HF/HS								
Putative Annotations (CTRL vs HF/HS)	3d	1w	4w	8w	12w	16w	Total	Total (%)
PC/PE			2	7		4	13	10.9
PC/PE/PS	8	2	8	1	1	19	39	32.8
PI		4	12	3	1	14	34	28.6
SM/PI	2	3	6	2	0	16	29	24.4
LPC/LPE/LPS/LPI			2	2			4	3.4
								119

HF/HS vs CTRL								
Putative Annotations (HF/HS vs CTRL)	3d	1w	4w	8w	12w	16w	Total	Total (%)
PC/PE	0			1	2	7	10	11.5
PC/PE/PS	0	4	2	9	12	7	34	39.1
PI		5		2	7	3	17	19.5
SM/PI	0	2	2	2	3	4	13	14.9
LPC/LPE/LPS/LPI	0	3	1	3	2	4	13	14.9
								87

Putative annotations both comparisons	Total	Total (%)
PC/PE	23	11.16505
PC/PE/PS	73	35.43689
PI	51	24.75728
SM/PI	42	20.38835
LPC/LPE/LPS/LPI	17	8.252427
	206	

8. References

ABE, H., YAMASHITA, S., SATOH, T. & HOSHI, H. 2002. Accumulation of cytoplasmic lipid droplets in bovine embryos and cryotolerance of embryos developed in different culture systems using serum-free or serum-containing media. *Mol Reprod Dev,* 61**,** 57-66.

ALEXANDROV, T. & KOBARG, J. H. 2011. Efficient spatial segmentation of large imaging mass spectrometry datasets with spatially aware clustering. *Bioinformatics,* 27**,** i230-8.

ALJAHDALI, A., AIRINA, R., VELAZQUEZ, M. A., SHETH, B., WALLEN, K., OSMOND, C., WATKINS, A. J., ECKERT, J. J., SMYTH, N. R. & FLEMING, T. P. 2020. The duration of embryo culture after mouse IVF differentially affects cardiovascular and metabolic health in male offspring. *Hum Reprod,* 35**,** 2497-2514.

ANAMTHATHMAKULA, P. & WINUTHAYANON, W. 2021. Prostaglandin-Endoperoxide Synthase 2 (PTGS2) in the Oviduct: Roles in Fertilization and Early Embryo Development. *Endocrinology,* 162.

AVILES, M., GUTIERREZ-ADAN, A. & COY, P. 2010. Oviductal secretions: will they be key factors for the future ARTs? *Mol Hum Reprod,* 16**,** 896-906.

BANLIAT, C., LE BOURHIS, D., BERNARDI, O., TOMAS, D., LABAS, V., SALVETTI, P., GUYONNET, B., MERMILLOD, P. & SAINT-DIZIER, M. 2020. Oviduct Fluid Extracellular Vesicles Change the Phospholipid Composition of Bovine Embryos Developed In Vitro. *Int J Mol Sci,* 21.

BANLIAT, C., TOMAS, D., TEIXEIRA-GOMES, A. P., UZBEKOVA, S., GUYONNET, B., LABAS, V. & SAINT-DIZIER, M. 2019. Stage-dependent changes in oviductal phospholipid profiles throughout the estrous cycle in cattle. *Theriogenology,* 135**,** 65-72.

BEHRINGER, C. A. S. A. R. R. 2012. Mouse Oviduct Development. *Mouse Development.* Springer-Verlag Berlin Heidelberg.

BELAZ, K. R., TATA, A., FRANCA, M. R., SANTOS DA SILVA, M. I., VENDRAMINI, P. H., FERNANDES, A. M., D'ALEXANDRI, F. L., EBERLIN, M. N. & BINELLI, M. 2016. Phospholipid Profile and Distribution in the Receptive Oviduct and Uterus During Early Diestrus in Cattle. *Biol Reprod,* 95**,** 127.

BELL, R. M., BALLAS, L. M. & COLEMAN, R. A. 1981. Lipid topogenesis. *J Lipid Res,* 22**,** 391-403.

BERTEVELLO, P. S., TEIXEIRA-GOMES, A. P., SEYER, A., VITORINO CARVALHO, A., LABAS, V., BLACHE, M. C., BANLIAT, C., CORDEIRO, L. A. V., DURANTHON, V., PAPILLIER, P., MAILLARD, V., ELIS, S. & UZBEKOVA, S. 2018. Lipid Identification and Transcriptional Analysis of Controlling Enzymes in Bovine Ovarian Follicle. *Int J Mol Sci,* 19.

BESENFELDER, U., HAVLICEK, V. & BREM, G. 2012. Role of the oviduct in early embryo development. *Reprod Domest Anim,* 47 Suppl 4**,** 156-63.

BROWN, M. S. & GOLDSTEIN, J. L. 1997. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell,* 89**,** 331-40.

CAGNONE, G. & SIRARD, M. A. 2014. The impact of exposure to serum lipids during in vitro culture on the transcriptome of bovine blastocysts. *Theriogenology,* 81**,** 712-22 e1-3.

CARDIFF, R. D., MILLER, C. H. & MUNN, R. J. 2014. Manual hematoxylin and eosin staining of mouse tissue sections. *Cold Spring Harb Protoc,* 2014**,** 655-8.

CHILDS, S., HENNESSY, A. A., SREENAN, J. M., WATHES, D. C., CHENG, Z., STANTON, C., DISKIN, M. G. & KENNY, D. A. 2008. Effect of level of dietary n-3 polyunsaturated fatty acid supplementation on systemic and tissue fatty acid concentrations and on selected reproductive variables in cattle. *Theriogenology,* 70**,** 595-611.

CIAPAITE, J., VAN DEN BROEK, N. M., TE BRINKE, H., NICOLAY, K., JENESON, J. A., HOUTEN, S. M. & PROMPERS, J. J. 2011. Differential effects of short- and long-term high-fat diet feeding on hepatic fatty acid metabolism in rats. *Biochim Biophys Acta,* 1811**,** 441-51.

COCKCROFT, S. 2021. Mammalian lipids: structure, synthesis and function. *Essays Biochem,* 65**,** 813-845.

COOK, N. R. 2008. Statistical evaluation of prognostic versus diagnostic models: beyond the ROC curve. *Clin Chem,* 54**,** 17-23.

DAG, Z. O. & DILBAZ, B. 2015. Impact of obesity on infertility in women. *J Turk Ger Gynecol Assoc,* 16**,** 111-7.

DE MOURA, E. D. M., DOS REIS, S. A., DA CONCEICAO, L. L., SEDIYAMA, C., PEREIRA, S. S., DE OLIVEIRA, L. L., GOUVEIA PELUZIO, M. D. C., MARTINEZ, J. A. & MILAGRO, F. I. 2021. Diet-induced obesity in animal models: points to consider and influence on metabolic markers. *Diabetol Metab Syndr,* 13**,** 32.

DEININGER, S. O., CORNETT, D. S., PAAPE, R., BECKER, M., PINEAU, C., RAUSER, S., WALCH, A. & WOLSKI, E. 2011. Normalization in MALDI-TOF imaging datasets of proteins: practical considerations. *Anal Bioanal Chem,* 401**,** 167-81.

DESMET, K. L., VAN HOECK, V., GAGNE, D., FOURNIER, E., THAKUR, A., O'DOHERTY, A. M., WALSH, C. P., SIRARD, M. A., BOLS, P. E. & LEROY, J. L. 2016. Exposure of bovine oocytes and embryos to elevated non-esterified fatty acid concentrations: integration of epigenetic and transcriptomic signatures in resultant blastocysts. *BMC Genomics,* 17**,** 1004.

DI PAOLO, G. & DE CAMILLI, P. 2006. Phosphoinositides in cell regulation and membrane dynamics. *Nature,* 443**,** 651-7.

DORIA, M. L., MCKENZIE, J. S., MROZ, A., PHELPS, D. L., SPELLER, A., ROSINI, F., STRITTMATTER, N., GOLF, O., VESELKOV, K., BROWN, R., GHAEM-MAGHAMI, S. & TAKATS, Z. 2016. Epithelial ovarian carcinoma diagnosis by desorption electrospray ionization mass spectrometry imaging. *Sci Rep,* 6**,** 39219.

ELSEVIER 2014. Gamete Transport. *Reference Module in Biomedical Sciences.*

ENGIN, A. 2017a. The Definition and Prevalence of Obesity and Metabolic Syndrome. *Adv Exp Med Biol,* 960**,** 1-17.

ENGIN, A. B. E., A. (EDS.) 2017b. *Obesity and Lipotoxicity,* New York, NY, USA, Springer.

EYSTER, K. M. 2007. The membrane and lipids as integral participants in signal transduction: lipid signal transduction for the non-lipid biochemist. *Adv Physiol Educ,* 31**,** 5-16.

FADOK, V. A., BRATTON, D. L., FRASCH, S. C., WARNER, M. L. & HENSON, P. M. 1998. The role of phosphatidylserine in recognition of apoptotic cells by phagocytes. *Cell Death & Differentiation,* 5**,** 551-562.

FEDORCSAK, P., DALE, P. O., STORENG, R., ERTZEID, G., BJERCKE, S., OLDEREID, N., OMLAND, A. K., ABYHOLM, T. & TANBO, T. 2004. Impact of overweight and underweight on assisted reproduction treatment. *Hum Reprod,* 19**,** 2523-8.

FERNANDEZ-SANCHEZ, A., MADRIGAL-SANTILLAN, E., BAUTISTA, M., ESQUIVEL-SOTO, J., MORALES-GONZALEZ, A., ESQUIVEL-CHIRINO, C., DURANTE-MONTIEL, I., SANCHEZ-RIVERA, G., VALADEZ-VEGA, C. & MORALES-GONZALEZ, J. A. 2011. Inflammation, oxidative stress, and obesity. *Int J Mol Sci,* 12**,** 3117-32.

FRERET, S., OSEIKRIA, M., BOURHIS, D. L., DESMARCHAIS, A., BRIANT, E., DESNOES, O., DUPONT, M., LE BERRE, L., GHAZOUANI, O., BERTEVELLO, P. S., TEIXEIRA-GOMES, A. P., LABAS, V., UZBEKOVA, S., SALVETTI, P., MAILLARD, V. & ELIS, S. 2019. Effects of a n-3 polyunsaturated fatty acid-enriched diet on embryo production in dairy cows. *Reproduction,* 158**,** 71-83.

GONZALEZ, M. B., LANE, M., KNIGHT, E. J. & ROBKER, R. L. 2018. Inflammatory markers in human follicular fluid correlate with lipid levels and Body Mass Index. *J Reprod Immunol,* 130**,** 25-29.

GONZALEZ, M. B., ROBKER, R. L. & ROSE, R. D. 2022. Obesity and oocyte quality: significant implications for ART and emerging mechanistic insights. *Biol Reprod,* 106**,** 338-350.

HALEY, M. J., KRISHNAN, S., BURROWS, D., DE HOOG, L., THAKRAR, J., SCHIESSL, I., ALLAN, S. M. & LAWRENCE, C. B. 2019. Acute high-fat feeding leads to disruptions in glucose homeostasis and worsens stroke outcome. *J Cereb Blood Flow Metab,* 39**,** 1026-1037.

HENAULT, M. A. & KILLIAN, G. J. 1993. Synthesis and secretion of lipids by bovine oviduct mucosal explants. *J Reprod Fertil,* 98**,** 431-8.

HERDT, T. H. 2013. Metabolic diseases of dairy cattle. *Vet Clin North Am Food Anim Pract,* 29**,** xi-xii.

HOLČAPEK, M., LIEBISCH, G. & EKROOS, K. 2018. Lipidomic Analysis. *Analytical Chemistry,* 90**,** 4249-4257.

IGOSHEVA, N., ABRAMOV, A. Y., POSTON, L., ECKERT, J. J., FLEMING, T. P., DUCHEN, M. R. & MCCONNELL, J. 2010. Maternal Diet-Induced Obesity Alters Mitochondrial Activity and Redox Status in Mouse Oocytes and Zygotes. *Plos One,* 5.

J.E. VANCE, D. E. V. 2002. *Biochemistry of Lipids, Lipoproteins and Membranes,* Amsterdam, Elsevier.

JORDAENS, L., ARIAS-ALVAREZ, M., PINTELON, I., THYS, S., VALCKX, S., DEZHKAM, Y., BOLS, P. E. & LEROY, J. L. 2015. Elevated non-esterified fatty acid concentrations hamper bovine oviductal epithelial cell physiology in three different in vitro culture systems. *Theriogenology,* 84**,** 899-910.

JORDAENS, L., VAN HOECK, V., DE BIE, J., BERTH, M., MAREI, W. F. A., DESMET, K. L. J., BOLS, P. E. J. & LEROY, J. 2017a. Non-esterified fatty acids in early luteal bovine oviduct fluid mirror plasma concentrations: An ex vivo approach. *Reprod Biol,* 17**,** 281-284.

JORDAENS, L., VAN HOECK, V., MAILLO, V., GUTIERREZ-ADAN, A., MAREI, W. F. A., VLAEMINCK, B., THYS, S., STURMEY, R. G., BOLS, P. E. J. & LEROY, J. 2017b. Maternal metabolic stress may affect oviduct gatekeeper function. *Reproduction,* 153**,** 759-773.

JUNGHEIM, E. S., SCHOELLER, E. L., MARQUARD, K. L., LOUDEN, E. D., SCHAFFER, J. E. & MOLEY, K. H. 2010. Diet-induced obesity model: abnormal oocytes and persistent growth abnormalities in the offspring. *Endocrinology,* 151**,** 4039-46.

JUNGHEIM, E. S., TRAVIESO, J. L., CARSON, K. R. & MOLEY, K. H. 2012. Obesity and reproductive function. *Obstet Gynecol Clin North Am,* 39**,** 479-93.

KOLLE, S., DUBIELZIG, S., REESE, S., WEHREND, A., KONIG, P. & KUMMER, W. 2009. Ciliary transport, gamete interaction, and effects of the early embryo in the oviduct: ex vivo analyses using a new digital videomicroscopic system in the cow. *Biol Reprod,* 81**,** 267-74.

LEROY, J., MEULDERS, B., MOORKENS, K., XHONNEUX, I., SLOOTMANS, J., DE KEERSMAEKER, L., SMITS, A., BOGADO PASCOTTINI, O. & MAREI, W. F. A. 2022. Maternal metabolic health and fertility: we should not only care about but also for the oocyte! *Reprod Fertil Dev,* 35**,** 1-18.

LI, S. & WINUTHAYANON, W. 2017. Oviduct: roles in fertilization and early embryo development. *J Endocrinol,* 232**,** R1-R26.

LIISBERG, U., FAUSKE, K. R., KUDA, O., FJAERE, E., MYRMEL, L. S., NORBERG, N., FROYLAND, L., GRAFF, I. E., LIASET, B., KRISTIANSEN, K., KOPECKY, J. & MADSEN, L. 2016. Intake of a Western diet containing cod instead of pork alters fatty acid composition in tissue phospholipids and attenuates obesity and hepatic lipid accumulation in mice. *J Nutr Biochem,* 33**,** 119-27.

LIM, H. Y., WANG, W., WESSELLS, R. J., OCORR, K. & BODMER, R. 2011. Phospholipid homeostasis regulates lipid metabolism and cardiac function through SREBP signaling in Drosophila. *Genes Dev,* 25**,** 189-200.

LIN, J., YANG, R., TARR, P. T., WU, P. H., HANDSCHIN, C., LI, S., YANG, W., PEI, L., ULDRY, M., TONTONOZ, P., NEWGARD, C. B. & SPIEGELMAN, B. M. 2005. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. *Cell,* 120**,** 261-73.

LISTENBERGER, L. L., HAN, X., LEWIS, S. E., CASES, S., FARESE, R. V., JR., ORY, D. S. & SCHAFFER, J. E. 2003. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci U S A,* 100**,** 3077-82.

MAILLO, V., RIZOS, D., BESENFELDER, U., HAVLICEK, V., KELLY, A. K., GARRETT, M. & LONERGAN, P. 2012. Influence of lactation on metabolic characteristics and embryo development in postpartum Holstein dairy cows. *J Dairy Sci,* 95**,** 3865-76.

MAILLO, V., SANCHEZ-CALABUIG, M. J., LOPERA-VASQUEZ, R., HAMDI, M., GUTIERREZ-ADAN, A., LONERGAN, P. & RIZOS, D. 2016. Oviductal response to gametes and early embryos in mammals. *Reproduction,* 152**,** R127-41.

MAREI, W. F. A., ALVAREZ, M. A., VAN HOECK, V., GUTIERREZ-ADAN, A., BOLS, P. E. J. & LEROY, J. 2017. Effect of nutritionally induced hyperlipidaemia on in vitro bovine embryo quality depends on the type of major fatty acid in the diet. *Reprod Fertil Dev,* 29**,** 1856-1867.

MAREI, W. F. A., SMITS, A., MOHEY-ELSAEED, O., PINTELON, I., GINNEBERGE, D., BOLS, P. E. J., MOERLOOSE, K. & LEROY, J. 2020. Differential effects of high fat diet-induced obesity on oocyte mitochondrial functions in inbred and outbred mice. *Sci Rep,* 10**,** 9806.

MEDING, S. & WALCH, A. 2013. MALDI imaging mass spectrometry for direct tissue analysis. *Methods Mol Biol,* 931**,** 537-46.

MEYER ZU HERINGDORF, D. & JAKOBS, K. H. 2007. Lysophospholipid receptors: signalling, pharmacology and regulation by lysophospholipid metabolism. *Biochim Biophys Acta,* 1768**,** 923-40.

MOORKENS, K., LEROY, J., VERHEYEN, S. & MAREI, W. F. A. 2022. Effects of an obesogenic diet on the oviduct depend on the duration of feeding. *PLoS One,* 17**,** e0275379.

NIEMANN, H. & WRENZYCKI, C. 2000. Alterations of expression of developmentally important genes in preimplantation bovine embryos by in vitro culture conditions: implications for subsequent development. *Theriogenology,* 53**,** 21-34.

PAKIET, A., JAKUBIAK, A., MIERZEJEWSKA, P., ZWARA, A., LIAKH, I., SLEDZINSKI, T. & MIKA, A. 2020. The Effect of a High-Fat Diet on the Fatty Acid Composition in the Hearts of Mice. *Nutrients,* 12.

PANCHAL, S. K. & BROWN, L. 2011. Rodent models for metabolic syndrome research. *J Biomed Biotechnol,* 2011**,** 351982.

PANDEY, S., PANDEY, S., MAHESHWARI, A. & BHATTACHARYA, S. 2010. The impact of female obesity on the outcome of fertility treatment. *J Hum Reprod Sci,* 3**,** 62-7.

PERCIE DU SERT, N., AHLUWALIA, A., ALAM, S., AVEY, M. T., BAKER, M., BROWNE, W. J., CLARK, A., CUTHILL, I. C., DIRNAGL, U., EMERSON, M., GARNER, P., HOLGATE, S. T., HOWELLS, D. W., HURST, V., KARP, N. A., LAZIC, S. E., LIDSTER, K., MACCALLUM, C. J., MACLEOD, M., PEARL, E. J., PETERSEN, O. H., RAWLE, F., REYNOLDS, P., ROONEY, K., SENA, E. S., SILBERBERG, S. D., STECKLER, T. & WURBEL, H. 2020. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol,* 18**,** e3000411.

PEREIRA, R. M., BOTEZELLI, J. D., RODRIGUES, K. C. D., MEKARY, R. A., CINTRA, D. E., PAULI, J. R., DA SILVA, A. S. R., ROPELLE, E. R. & DE MOURA, L. P. 2017. Fructose Consumption in the Development of Obesity and the Effects of Different Protocols of Physical Exercise on the Hepatic Metabolism. *Nutrients,* 9.

REN, J., PULAKAT, L., WHALEY-CONNELL, A. & SOWERS, J. R. 2010. Mitochondrial biogenesis in the metabolic syndrome and cardiovascular disease. *J Mol Med (Berl),* 88**,** 993-1001.

RIZOS, D., CARTER, F., BESENFELDER, U., HAVLICEK, V. & LONERGAN, P. 2010. Contribution of the female reproductive tract to low fertility in postpartum lactating dairy cows. *J Dairy Sci,* 93**,** 1022-9.

ROBERTSON, S. A., CHIN, P. Y., FEMIA, J. G. & BROWN, H. M. 2018. Embryotoxic cytokines-Potential roles in embryo loss and fetal programming. *J Reprod Immunol,* 125**,** 80-88.

ROBKER, R. L. 2008. Evidence that obesity alters the quality of oocytes and embryos. *Pathophysiology,* 15**,** 115-21.

SAINT-DIZIER, M., SCHOEN, J., CHEN, S., BANLIAT, C. & MERMILLOD, P. 2019. Composing the Early Embryonic Microenvironment: Physiology and Regulation of Oviductal Secretions. *Int J Mol Sci,* 21.

SCOTT, A. J., FLINDERS, B., CAPPELL, J., LIANG, T., PELC, R. S., TRAN, B., KILGOUR, D. P., HEEREN, R. M., GOODLETT, D. R. & ERNST, R. K. 2016. Norharmane Matrix Enhances Detection of Endotoxin by MALDI-MS for Simultaneous Profiling of Pathogen, Host, and Vector Systems. *Pathog Dis,* 74.

SIGHINOLFI, G., CLARK, S., BLANC, L., COTA, D. & RHOURRI-FRIH, B. 2021. Mass spectrometry imaging of mice brain lipid profile changes over time under high fat diet. *Sci Rep,* 11**,** 19664.

SILVESTRIS, E., DE PERGOLA, G., ROSANIA, R. & LOVERRO, G. 2018. Obesity as disruptor of the female fertility. *Reprod Biol Endocrinol,* 16**,** 22.

SKAZNIK-WIKIEL, M. E., SWINDLE, D. C., ALLSHOUSE, A. A., POLOTSKY, A. J. & MCMANAMAN, J. L. 2016. High-Fat Diet Causes Subfertility and Compromised Ovarian Function Independent of Obesity in Mice. *Biol Reprod,* 94**,** 108.

SKOTLAND, T., HESSVIK, N. P., SANDVIG, K. & LLORENTE, A. 2019. Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology. *J Lipid Res,* 60**,** 9-18.

SKOTLAND, T. & SANDVIG, K. 2019. The role of PS 18:0/18:1 in membrane function. *Nature Communications,* 10**,** 2752.

SLOTTE, J. P. 2013. Biological functions of sphingomyelins. *Prog Lipid Res,* 52**,** 424-37.

SLOTTE, J. P. & RAMSTEDT, B. 2007. The functional role of sphingomyelin in cell membranes. *European Journal of Lipid Science and Technology,* 109**,** 977-981.

SMITS, A., LEROY, J., BOLS, P. E. J., DE BIE, J. & MAREI, W. F. A. 2020. Rescue Potential of Supportive Embryo Culture Conditions on Bovine Embryos Derived from Metabolically Compromised Oocytes. *Int J Mol Sci,* 21.

SMITS, A., MAREI, W. F. A., MOORKENS, K., BOLS, P. E. J., DE NEUBOURG, D. & LEROY, J. 2022. Obese outbred mice only partially benefit from diet normalization or calorie restriction as preconception care interventions to improve metabolic health and oocyte quality. *Hum Reprod,* 37**,** 2867-2884.

SPOONER, H. C., DERRICK, S. A., MAJ, M., MANJARIN, R., HERNANDEZ, G. V., TAILOR, D. S., BASTANI, P. S., FANTER, R. K., FIOROTTO, M. L., BURRIN, D. G., LA FRANO, M. R., SIKALIDIS, A. K. & BLANK, J. M. 2021. High-Fructose, High-Fat Diet Alters Muscle Composition and Fuel Utilization in a Juvenile Iberian Pig Model of Non-Alcoholic Fatty Liver Disease. *Nutrients,* 13.

STEENBERGEN, R., NANOWSKI, T. S., BEIGNEUX, A., KULINSKI, A., YOUNG, S. G. & VANCE, J. E. 2005. Disruption of the phosphatidylserine decarboxylase gene in mice causes embryonic lethality and mitochondrial defects. *J Biol Chem,* 280**,** 40032-40.

TAPPY, L. & LE, K. A. 2010. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev,* 90**,** 23-46.

TASSEVA, G., BAI, H. D., DAVIDESCU, M., HAROMY, A., MICHELAKIS, E. & VANCE, J. E. 2013. Phosphatidylethanolamine deficiency in Mammalian mitochondria impairs oxidative phosphorylation and alters mitochondrial morphology. *J Biol Chem,* 288**,** 4158-73.

TREDE, D., SCHIFFLER, S., BECKER, M., WIRTZ, S., STEINHORST, K., STREHLOW, J., AICHLER, M., KOBARG, J. H., OETJEN, J., DYATLOV, A., HELDMANN, S., WALCH, A., THIELE, H., MAASS, P. & ALEXANDROV, T. 2012. Exploring three-dimensional matrix-assisted laser desorption/ionization imaging mass spectrometry data: three-dimensional spatial segmentation of mouse kidney. *Anal Chem,* 84**,** 6079-87.

TREMELLEN, K., PEARCE, K. & ZANDER-FOX, D. 2017. Increased miscarriage of euploid pregnancies in obese women undergoing cryopreserved embryo transfer. *Reprod Biomed Online,* 34**,** 90-97.

TURNER, N., KOWALSKI, G. M., LESLIE, S. J., RISIS, S., YANG, C., LEE-YOUNG, R. S., BABB, J. R., MEIKLE, P. J., LANCASTER, G. I., HENSTRIDGE, D. C., WHITE, P. J., KRAEGEN, E. W., MARETTE, A., COONEY, G. J., FEBBRAIO, M. A. & BRUCE, C. R. 2013. Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia,* 56**,** 1638-48.

UZBEKOVA, S., ELIS, S., TEIXEIRA-GOMES, A. P., DESMARCHAIS, A., MAILLARD, V. & LABAS, V. 2015. MALDI Mass Spectrometry Imaging of Lipids and Gene Expression Reveals Differences in Fatty Acid Metabolism between Follicular Compartments in Porcine Ovaries. *Biology (Basel),* 4**,** 216-36.

VALCKX, S. D., DE PAUW, I., DE NEUBOURG, D., INION, I., BERTH, M., FRANSEN, E., BOLS, P. E. & LEROY, J. L. 2012. BMI-related metabolic composition of the follicular fluid of women undergoing assisted reproductive treatment and the consequences for oocyte and embryo quality. *Hum Reprod,* 27**,** 3531-9.

VALCKX, S. D., VAN HOECK, V., ARIAS-ALVAREZ, M., MAILLO, V., LOPEZ-CARDONA, A. P., GUTIERREZ-ADAN, A., BERTH, M., CORTVRINDT, R., BOLS, P. E. & LEROY, J. L. 2014. Elevated non-esterified fatty acid concentrations during in vitro murine follicle growth alter follicular physiology and reduce oocyte developmental competence. *Fertil Steril,* 102**,** 1769-76 e1.

VALSESIA, A., SARIS, W. H., ASTRUP, A., HAGER, J. & MASOODI, M. 2016. Distinct lipid profiles predict improved glycemic control in obese, nondiabetic patients after a low-caloric diet intervention: the Diet, Obesity and Genes randomized trial. *Am J Clin Nutr,* 104**,** 566-75.

VAN DER VEEN, J. N., KENNELLY, J. P., WAN, S., VANCE, J. E., VANCE, D. E. & JACOBS, R. L. 2017. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. *Biochim Biophys Acta Biomembr,* 1859**,** 1558-1572.

VAN MEER, G. & LISMAN, Q. 2002. Sphingolipid transport: rafts and translocators. *J Biol Chem,* 277**,** 25855-8.

VAN MEER, G., VOELKER, D. R. & FEIGENSON, G. W. 2008. Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Biol,* 9**,** 112-24.

VELAZQUEZ, M. A. 2015. Impact of maternal malnutrition during the periconceptional period on mammalian preimplantation embryo development. *Domest Anim Endocrinol,* 51**,** 27-45.

WALKER, A. K., JACOBS, R. L., WATTS, J. L., ROTTIERS, V., JIANG, K., FINNEGAN, D. M., SHIODA, T., HANSEN, M., YANG, F., NIEBERGALL, L. J., VANCE, D. E., TZONEVA, M., HART, A. C. & NAAR, A. M. 2011. A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. *Cell,* 147**,** 840-52.

WEISSER, H. & KRIEG, M. 1997. Lipid composition in epithelium and stroma of human benign prostatic hyperplasia. *Prostate,* 30**,** 41-6.

WHITTEN, W. K. 1956. Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *J Endocrinol,* 13**,** 399-404.

WILLIAMS, L. M., CAMPBELL, F. M., DREW, J. E., KOCH, C., HOGGARD, N., REES, W. D., KAMOLRAT, T., THI NGO, H., STEFFENSEN, I. L., GRAY, S. R. & TUPS, A. 2014. The development of diet-induced obesity and glucose intolerance in C57BL/6 mice on a high-fat diet consists of distinct phases. *PLoS One,* 9**,** e106159.

WITTEMER, C., OHL, J., BAILLY, M., BETTAHAR-LEBUGLE, K. & NISAND, I. 2000. Does body mass index of infertile women have an impact on IVF procedure and outcome? *J Assist Reprod Genet,* 17**,** 547-52.

WONNACOTT, K. E., KWONG, W. Y., HUGHES, J., SALTER, A. M., LEA, R. G., GARNSWORTHY, P. C. & SINCLAIR, K. D. 2010. Dietary omega-3 and -6 polyunsaturated fatty acids affect the composition and development of sheep granulosa cells, oocytes and embryos. *Reproduction,* 139**,** 57-69.

WU, L. L., NORMAN, R. J. & ROBKER, R. L. 2011. The impact of obesity on oocytes: evidence for lipotoxicity mechanisms. *Reprod Fertil Dev,* 24**,** 29-34.

YAMASHITA, A., HAYASHI, Y., NEMOTO-SASAKI, Y., ITO, M., OKA, S., TANIKAWA, T., WAKU, K. & SUGIURA, T. 2014. Acyltransferases and transacylases that determine the fatty acid composition of glycerolipids and the metabolism of bioactive lipid mediators in mammalian cells and model organisms. *Prog Lipid Res,* 53**,** 18-81.

ZACHUT, M., ARIELI, A. & MOALLEM, U. 2011. Incorporation of dietary n-3 fatty acids into ovarian compartments in dairy cows and the effects on hormonal and behavioral patterns around estrus. *Reproduction,* 141**,** 833-40.

ZOU, K. H., O'MALLEY, A. J. & MAURI, L. 2007. Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. *Circulation,* 115**,** 654-7.

CHAPTER 5:

EFFECTS OF A WESTERN TYPE DIET ON THE OVARY AND OVARIAN CELLS OVER TIME: FOCUS ON DYNAMIC CHANGES IN GRANULOSA CELL TRANSCRIPTOMICS AND OOCYTE QUALITY

Effects of an obesogenic diet on the ovary and ovarian cells after different periods of feeding in an outbred mouse model

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1. Abstract

Objectives: Continuous consumption of western-type diets (high in fat and sugar, HF/HS diets) results in systemic metabolic changes including low-grade inflammation, lipotoxicity and oxidative stress. This impairs different tissue functions, and eventually leads to obesity and increased risk of metabolic disorders. Consumption of a HF/HS diet and the development of obesity have also been linked with reduced fertility, partially by impacting ovarian follicle development and oocyte quality. Previous studies have shown that diet-induced alterations in metabolism and tissue functions are dependent on the exposure time and occur in a multiphasic pattern. Similar patterns were reported in the alterations in oviductal cell functions, but the dynamics of diet-induced alterations in the ovarian follicles have not been previously described. The aim of this study was to determine the onset and progression of changes in the ovary, oocyte quality and the granulosa cell transcriptomic profile after starting HF/HS diet feeding.

Design: Five week (wk) old female outbred Swiss mice were fed a control (CTRL, 10% fat) or HF/HS (60% fat in diet, 20% fructose in drinking water) diet for up to 16wk. Mice (n= 5 per treatment per timepoint) were sacrificed at 24 hours (h), 3 days, 1wk, 4wk, 8wk, 12wk and 16wk. Total cholesterol concentrations in serum were measured and triglyceride content in whole ovaries was quantified. To study oocyte quality, ooplasmic total lipid droplet volume was quantified using Bodipy staining and mitochondrial ultrastructure was examined using transmission electron microscopy. Data were analysed using 2-way ANOVA, and T-tests within each timepoint. Granulosa cells were collected and used for RNA sequencing. Differential gene expression analysis was performed as a series of pairwise comparisons at all time points using edgeR. Gene Set Enrichment Analysis was then done with GAGE and significant GO terms ($q<0.1$) were summarised using the “Reduce and Visualize GO” tool (REVIGO).

Results: The HF/HS diet significantly increased blood cholesterol levels at 1wk. This difference could not be detected at 4wk but was more distinct again at 8wk, 12wk and 16wk. Triglyceride content of the ovary was not significantly affected by the HF/HS diet.

Oocytes from the HF/HS group already showed a significant increase in the volume of lipid droplets at 24h compared to the CTRLs. This increase was persistent until 8wk but was not significant at 12wk and 16wk compared to their respective CTRLs. On the other hand, from 8wk onwards HF/HS-oocytes exhibited a progressive increase in the proportion of ultrastructural mitochondrial abnormalities. A few significant GO term annotations were detected already at 3 days, the majority of which were also detected at 1wk, together with the dysregulation of another 228 GO terms. These acute changes were mainly related to endoplasmic reticulum and mitochondrial functions, translation, cell differentiation and cell signalling. No significant GO terms could be detected at 4wk, which may indicate transient metabolic adaptations. Subsequently, the highest number of significant GO term annotations was detected at 8wk (495, including 103 of those detected at 1wk). The majority of these annotations were also detected at 12wk and 16wk. These chronic changes are related to cell metabolism, steroid biosynthesis, immune responses, autophagy, biogenesis, and mRNA processing. **Conclusions:** We conclude that exposure to HF/HS diet results in an acute increase in oocyte lipid droplet volume just after 24h, potentially due to an acute postprandial hyperlipidemia being reflected in the follicular fluid. Whereas the impact of the obesogenic diet on mitochondrial ultrastructure was only evident after 8w. We demonstrate that the ovarian follicle microenvironment is sensitive to very short-term changes in the diet composition, and that the alterations in ovarian cell biological functions follow a time-dependent multiphasic cascade. Defining and understanding these progressive changes should increase the awareness needed to protect reproductive health and improve efficiency of preconception care interventions.

2. Introduction

One of the biggest global health challenges of this century is the rapidly increasing prevalence of obesity, with one billion adults currently being overweight and 650 million adults classified as obese (WHO, 2016). Obese women of childbearing age have shown to be more likely to have higher miscarriage rates, lower rates of fertilization and poor oocyte and embryo quality, and thus reduced fertility, compared to normal-weight women (Pandey et al., 2010; Klenov and Jungheim, 2014; Dag and Dilbaz, 2015; Silvestris et al., 2018). Over the past decades, various studies have demonstrated that pregnancy losses mostly occur already in the first 2 weeks after fertilization, highlighting the importance of early preimplantation embryo mortality and failure of implantation (Fedorcsak et al., 2004; Tremellen et al., 2017), in which reduced oocyte quality may play a pivotal role (Leroy et al., 2008; Pandey et al., 2010; Wu et al., 2011; Gonzalez et al., 2022; Leroy et al., 2023). The disturbed metabolic state that is linked with obesity (Samuelsson et al., 2008; Jungheim and Moley, 2010) is reflected in the follicular fluid (FF), the microenvironment of the oocyte, and may directly impact oocyte quality (Leroy et al., 2015). Obesity is mainly caused by the lack of physical activity and an increased consumption of the obesogenic diet (Saklayen, 2018; Blüher, 2019). The substantial amounts of saturated fats in an obesogenic diet (Panchal and Brown, 2011) lead to hyperlipidemia, increased adiposity with elevated lipolytic rates and inflammation, causing cellular dysfunction due to lipotoxicity, reduced insulin sensitivity and oxidative stress (Lin et al., 2005; Fernandez-Sanchez et al., 2011; Herdt, 2013; Engin). This disturbed metabolic state is even further aggravated (Stanhope, 2016; Taskinen et al., 2019) by the high sugar content in the obesogenic diets (Panchal and Brown, 2011).

The pathogenesis of obesity and the effects of an obesogenic diet on fertility is thus often studied by using high-fat/high-sugar(HF/HS)-diet-induced obese mouse models (Dias et al., 2021). The effects induced by feeding an obesogenic diet to mice have shown to be acute, as early as three days of feeding (Williams et al., 2014; Haley et al.,

2019; Moorkens et al., 2022; Moorkens et al., 2023), as well as appearing after long-term feeding (Lin et al., 2005; Fernandez-Sanchez et al., 2011; Herdt, 2013; Engin, 2017a; Engin, 2017b). Long-term feeding of an obesogenic diet results in insulin resistance and increased hyperlipidemia, which leads to increased adiposity and accumulation of lipids in non-adipose tissues, resulting in lipotoxicity, oxidative stress and ultimately cellular dysfunctions (Cuevas et al., 2004; Lin et al., 2005; Fernandez-Sanchez et al., 2011; Herdt, 2013; Engin, 2017a; Engin, 2017b). Previously, various tissues such as muscles, heart and liver, demonstrated a changed lipid content due to an obesogenic diet. This was linked with changes in cellular functions and metabolism (Liisberg et al., 2016; Valsesia et al., 2016; Pakiet et al., 2020; Spooner et al., 2021). Whether such changes also appear in reproductive tissue has to be further investigated.

Furthermore, effects of an obesogenic diet are reflected in the ovarian follicular environment (Reynolds et al., 2015). Several studies reported increased lipid content in the oocytes and FF microenvironment of diet-induced obese mice (Robker, 2008; Igosheva et al., 2010; Wu et al., 2011; Luzzo et al., 2012; Valckx et al., 2014b; Marei et al., 2020) and women (Wittemer et al., 2000; Valckx et al., 2012; Gonzalez et al., 2018). Feeding an obesogenic diet to mice leads to an abnormal lipid accumulation and distribution within their oocytes (Wu et al., 2010) and a dramatically increased lipid content in both mature and immature oocytes (Reynolds et al., 2015). HFD-fed mice also exhibit a significantly different number and type of lipids in the ovarian granulosa cells compared to control mice (Gao et al., 2023). Many of the studies demonstrated a direct lipotoxic impact on oocyte development. The duration of exposure to the HFD diet in these studies is long, ranging between 4 weeks and 13 weeks. This makes it difficult to dissect the effects of obesity and its associated metabolic alterations (such as reduced insulin sensitivity and hyperlipidaemia) from the direct effects of the diet. Our research group, as well as others, reported that oocytes matured under lipotoxic conditions exhibited oxidative stress and altered mitochondrial functions, as well as a

devious proteomic and metabolic profile, leading to reduced oocyte developmental capacity and lower embryo quality (Robker, 2008; Igosheva et al., 2010; Van Hoeck et al., 2013; Marei et al., 2017; Smits et al., 2021). Thus, lipotoxicity in the ovarian microenvironment results in the development of oocyte defects in animal models of maternal obesity, eventually even impacting offspring health (Gonzalez et al., 2022). In addition, high-fat diet-induced obese mouse models confirmed have demonstrated a higher proportion of mitochondrial ultrastructural abnormalities, altered mitochondrial membrane potential as well as higher lipid droplet volume in oocytes (Wu et al., 2010; Marei et al., 2020; Smits et al., 2021). Moreover, the obesogenic diet appears to be the main driver in impacting ovarian functions, regardless of the development or the presence of the obese phenotype (Skaznik-Wikiel et al., 2016). Although many studies have investigated the effects of obesity and the obesogenic diet on the ovarian microenvironment, the mechanisms underlying the effects on oocytes and granulosa cell function as well as the development of the effects over time while consuming the obesogenic diet, are not yet fully understood.

The lipid composition of the ovary was reported to be cell specific and undergoes dynamic changes during follicular development (Campbell et al., 2012; Uzbekova et al., 2015). Based on these insights, we assume that an obesogenic diet or an obese metabolic state may impact these changes. Feeding a HF/HS diet for only as short as 3days results in acute oxidative and mitochondrial stress in oviductal epithelial cells. While these effects appeared before developing an obese phenotype, they initiated a cascade of changes during the prolonged feeding of mice, leading to a persistent upregulation of oxidative stress and ER-stress after 8w and 12w, eventually resulting in local and systemic inflammation after 16w HF/HS-diet feeding (Moorkens et al., 2022). Whether the effects of an obesogenic diet on the ovary and the ovarian cells progress in such timely manner, has yet to be investigated.

Taken together, the direct impact of an obesogenic diet and obesity on the ovarian follicular fluid microenvironment and oocyte quality is relatively well documented in

animal models and women. It is known that the impact of lipotoxicity, caused by an obesogenic diet, on oocyte quality is mediated by oxidative stress, endoplasmic reticulum stress and mitochondrial dysfunction. However, the nature and magnitude of these changes may be dependent on the duration of feeding (and thus the development of the obesogenic phenotype), which is still to be investigated.

Therefore, we hypothesized that feeding a HF/HS diet can lead to acute and/or long-term changes in the ovarian cells and oocytes of mice and that the development of these changes depends on the duration of feeding the obesogenic diet. To test this hypothesis, we aimed to examine and compare serum, oocytes, granulosa cells and whole ovarian tissues collected from Swiss mice that were fed either a control or a HF/HS diet for different periods of time, namely 24 hours (h), 3 days (d), 1 week (w), 4w, 8w, 12w and 16 weeks. We focused on changes in lipid droplet volume and mitochondrial abnormalities in oocytes, neutral lipid content in the ovaries, and transcriptomic changes in the granulosa cells.

3. Materials and Method

3.1 Ethical approval

All animal procedures in this study were approved by and performed according to the ethical committee of the University of Antwerp (ECD approval number nr. 2014-57). All methods were performed in accordance with the relevant ethical guidelines and regulations as well as the ARRIVE guidelines (Percie du Sert et al., 2020).

3.2 Experimental animals, diet and experimental design

Non-pregnant, sexually mature female outbred Rj:Orl Swiss mice (hereafter referred to as “Swiss” mice, n=42, Janvier labs) were used. At the age of five weeks the mice were randomly divided into two groups with ad libitum access to either a control diet (CTRL) or a high-fat/high-sugar diet (HF/HS). When compared to a high-fat diet (HFD), a HF/HS diet is more representative for the typical Western style diet (Panchal and Brown, 2011). The HF/HS group was fed with a 60 kJ% fat (coming from beef tallow which contains more palmitic and stearic acids compared to lard) and 9.4% sucrose diet

(E15741-34, Sniff diets, Soest, Germany) combined with drinking water containing 20% w/v fructose (Merck, 102109450). A high consumption of fructose is known to cause insulin resistance and obesity in rodents (Tappy and Le, 2010) and thus a high-fat/high-fructose diet can lead to metabolic syndrome and type 2 diabetes in mice (Panchal and Brown, 2011). The control group mice had ad libitum access to fructose-free water and were fed with a matched, purified (not a grain-based chow diet) CTRL diet (E157453-04, Sniff diets, Soest, Germany), containing 10 kJ% fat (coming from lard) and 7% sucrose, which was also lower in saturated fatty acids. Mice of both groups were fed with their respective diets up to 16 weeks.

At seven different time points of experimental feeding the mice were euthanized (n=3 mice per treatment per time point, mice were housed as 6 mice per cage) by decapitation, namely at 24 hours(h) 3 days (d), 1 week (1w), 4w, 8w, 12w and 16w after the start of feeding the CTRL or the HF/HS diet. Blood serum, whole ovaries or granulosa cells and oocytes were collected as explained hereafter to study different outcome parameters. Before euthanasia the estrous cycles of the female mice were synchronised by exposing the mice to bedding from male cages for 24 hours (Whitten effect) (Whitten, 1956) to collect the samples during the follicular phase (before ovulation). The six time points of sample collection are based on previous studies in which multiphasic acute and long-term effects of a HFD on general metabolic features in mice were reported (Williams et al., 2014).

3.3 Assessment of live body weight

During the whole experiment, the weight of the mice was recorded weekly. At the first time point the weight gain data were derived from 21 mice per dietary group after which the number of mice were reduced at each time point due to culling of a subset of animals (n=3) for sample collection.

3.4 Serum collection and analysis

At decapitation of the mice, the blood was immediately collected and centrifuged after 30 min at 6000xg for 2 min at 4˚C after which serum was stored at -80˚C. Serum samples of all time points were analysed for total cholesterol concentrations in a commercial certified laboratory (Algemeen Medisch Labo, AML, Antwerp, Belgium) using automated facilities. Total cholesterol concentrations were measured on an Abbott Architect c16000 (Abbott, Illinois, U.S.A).

3.5 Collection and processing of ovaries, oocytes and granulosa cells

At each time point, mice were dissected and the ovaries were collected in L15 medium (Thermo Fisher Scientific, Belgium) supplemented with 50 IU/mL penicillin G sodium salt (Merck, Belgium). Fat and surrounding tissue were removed under a stereomicroscope. One ovary per mouse was washed two times in phosphate buffered saline (PBS; Life Technologies, Belgium) after which it was transferred to a vial, snap frozen in liquid nitrogen (LN2) and stored at -80°C. Meanwhile, the other ovary of each mouse was punctured with two 27 G needles for the collection of granulosa cells (GCs), oocytes and cumulus-oocyte-complexes (COCs) in L15 medium supplemented with 50 IU/mL penicillin G sodium salt (Merck, Belgium), which was done in a DNAse and RNAse-free environment. Contamination with theca or somatic cells was checked and ruled out using cell specific qPCR markers. The collected GCs were transferred to a vial and centrifuged at 600xg for 10 minutes at 25˚C after which the supernatant was removed. After adding RNAse-free PBS (Life Technologies, Belgium) the centrifugation step was repeated for pellet washing. After removing the supernatant the pellets were snap frozen in LN2 and stored at -80˚C. The oocytes and COCs were washed in fresh L15 medium (Thermo Fisher Scientific, Belgium) supplemented with 50 IU/mL penicillin G sodium salt (Merck, Belgium) and 3mg/mL PolyVinylPyrrolidone (PVP) (Sigma, Belgium). At every time point five to six denuded oocytes per mouse were transferred to paraformaldehyde 4% for 1 hour and then stored in PBS-PVP (1mg/mL) at 4°C until

they were used for staining to determine intracellular lipid droplet content. One COC per mouse with compact cumulus cell mass, regular zona pellucida, spherical oocyte and a translucent, homogenously coloured ooplasm without inclusions was selected and fixed in glutaraldehyde solution for transmission electron microscopy (TEM).

3.6 Lipid extraction and Quantification of triglyceride content in whole ovaries

The triglyceride content in whole ovaries of 5 mice per diet group was quantified after lipid extraction. Lipids were isolated by first manually homogenising the whole ovary in sterile water (10mg tissue per 100µL sterile water) in microcentrifuge tubes using a pestula. This was followed by vortexing for 10 seconds (s) and sonicating the tubes for 10 minutes (m), after which the tube was vortexed again for 10s. To 10µL of the homogenised tissue (1mg/400µL) 190µL sterile water and 200µL 2X homogenising buffer (DiPhosphate Buffered Saline (DPBS) (Thermofisher Sci, 70011-036) + EDTA (Merck, ED4SS)) were added to start the lipid extraction process. A standard curve, on which following steps were performed as well, was prepared using 0, 3.125, 6.25, 12.5, 25 and 50 µg triolein (Merck, D6584) in 400µL homogenizing buffer 1X. Subsequently, 2ml isopropanol (Merck, 1.09634.1000):hexane (Merck, 34859) (4:1) was added to the samples followed by vortexing for 5s and incubation in the dark for 30m at room temperature. Next, 500µL of hexane:diethylether (VWR, 23807.297) (1:1) was added and the samples were vortexed for 5s and incubated in the dark for 10m at room temperature. After that, 1mL of water was added and the samples were mixed and left on the bench for 30min to separate the phases. Then 800µL of the top phase (supernatant) was transferred to a new tube and evaporated completely in an evaporator for approximately 30m. The triglyceride content was measured using the Triglyceride GPO Method kit (Biolabo, LP80619), following the manufacturers' protocol (Biolabo).

3.7 Assessment of oocyte total lipid droplet volume

BODIPY staining was used to quantify the intracellular lipid droplets in the oocytes according to the protocol described by (Van Hoeck et al., 2015). In short, the fixed oocytes were permeabilized using 0.1% (w/v) saponin (Fiers, Kuurne, Belgium) and 0.1 M glycine in PBS after which they were incubated for 1 h in 20 µg/ml BODIPY 493/503 (Thermo Fisher Scientific, Belgium) in PBS. After each step of the staining procedure the oocytes were washed two times in PBS containing 3 mg/ml PVP. The oocytes were then mounted on glass-bottom dishes in droplets of PBS-PVP and directly examined. A Nikon Eclipse Ti-E inverted microscope attached to a microlens-enhanced dual spinning disk confocal system (UltraVIEW VoX; PerkinElmer, Zaventem, Belgium) equipped with 488 nm diode lasers for excitation of green fluorophores was used to obtain high-resolution images. In the lower half of each oocyte, which is closest to the objective lens and thus where the image is the most sharp, a z-stack of 40 µm (with steps of 1 µm) was acquired. The obtained images were analysed using Volocity 6.0.1 software (PerkinElmer) to quantify the total volume of lipid droplet content in each oocyte. Only particles with the size of 0.5 μm^3 or higher were considered as relevant lipid droplets and included in the analysis to overcome false positive counting of background pixels.

3.8 Assessment of mitochondrial morphology in oocytes using Transmission Electron Microscopy (TEM)

The mitochondrial ultrastructure of COCs was assessed according to the protocol described by Marei et al. (2020). Since the examination is time-consuming and expensive, this was done for the HF/HS COCs of time point 1w, 4w, 8w, 12w and 16w as well as the control oocytes of 24h, 8w and 16w. Briefly, freshly collected whole COCs were fixed in 0.1 M sodium cacodylate-buffered (pH 7.4) 2.5% glutaraldehyde solution and kept at 4°C for a maximum of 1 month. Individual COCs were then embedded in 2% agar blocks to enable handling. The blocks were washed three times in 0.1 M sodium cacodylate-buffered (pH 7.4) 7.5% saccharose solution and then postfixated in

1% OsO4 solution for 2 h. After dehydration in an ethanol gradient, samples were embedded in EM-bed812. Ultrathin sections were stained with lead citrate and examined in a Tecnai G2 Spirit Bio TWIN microscope (Fei, Europe BV, Zaventem, Belgium) at 120 kV. For each COC, images of at least 10 random fields in the oocyte (covering most of the oocyte area) were acquired at 16500–25000x. The morphology of the mitochondria in the acquired images was evaluated by an expert, blinded to the corresponding treatment group, and classified as described by Marei et al. (2020). Mitochondrial morphology was classified to be normal if the mitochondria were spherical and electron dense, with or without regular vacuoles. Their morphology was considered to be abnormal when they were either vacuolated with loose membranous structures, were elongated, dumbbell and rose petal in shape, contained highly electron dense foci or when they were degenerated.

3.9 Assessment of differential gene expression in granulosa cells using RNA sequencing

RNA sequencing was performed on granulosa cells (GCs) collected from 5 mice per treatment group per time point, for time points 3d, 1w, 4w, 8w, 12w and 16weeks.

3.9.1 Total RNA isolation from granulosa cells

Total RNA isolation was done using the Qiagen PicoPure RNA Isolation kit (Qiagen, #KIT0204) by performing the manufacturers' protocol. DNA was removed by DNAse treatment (RNase-Free DNase Set Qiagen, #79254) using the manufacturers' protocol.

3.9.2 RNA sequencing

RNA sequencing was performed on CTRL en HFHS samples from time point 3d, 1w, 4w, 8w, 12w and 16weeks, for each diet group three to four biological replicates. A total RNA of 47 samples was transferred to NxtGnt where RNA sequencing was performed. For each sample, a sequencing library was constructed using the QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen) and the UMI Second Strand Synthesis Module for QuantSeq FWD (Illumina, Read 1). This incorporates a unique molecular

identifier (UMI) in the first 6 nucleotides (nt) of each read, allowing to identify PCR duplicates and eliminate amplification bias. The libraries were sequenced as single-end 76 on a NextSeq device (Illumina). (Used methods for read preparation, adapter and quality trimming, read mapping and feature counts, library saturation can be found in the Supplementary material.)

3.9.3 Analysis of RNAseq data

RNA sequencing data was filtered to discard lowly expressed features which were unreliable, using edgeR's filterByExpr() function. A PCA and scree plot of the rlog-transformed filtered data were explored to investigate if the variation between the samples reflects the expected biological differences. Feature counts were TMM-normalized and dispersions were estimated. The preferred and more robust quasi likelihood model and F-test were used for a more reliable error rate. Correction of the p-values for repeated testing (PAdj) was done with the Benjamini-Hochberg method. Data from at least three replicates per time point were assessed statistical and biological significant when PAdj < 0.05 and |log2FC|>=1. The fold-changes of all genes were used to investigate affected pathways. Up- and downregulated GO terms were reported using fold-changes of all genes with GAGE and murine GO data in R (using q < 0.05 for statistical significance). The lists of upregulated and downregulated GO terms were summarized using REVIGO (REduce and VIsualize GO) (Supek et al., 2011). This tool removes redundant GO terms and generates a representative subset of the terms using a simple clustering algorithm that relies on semantic similarity measures. This is a better approach compared to only reporting e.g. the top 10 or 20 GO terms.

3.10 Statistical analysis

Each mouse is considered as an experimental unit. Power analysis was performed to achieve a power (1-β) of 90% to detect statistical differences at P-value (P) <0.05 based on the expected mean differences from similar experiments that were previously

performed in our laboratory. The power analysis was performed using the PS program for Power and Sample Size calculations (version 3.1.2, 2014 (from Vanderbilt University)). IBM Statistics SPSS (IBM SPSS statistics version 26) was used for all statistical analyses.

All numerical data, e.g. live body weight, serum cholesterol, ovary triglyceride content, were checked for normality of distribution and homogeneity of variance. To study the main effects and interaction of treatment and time in the live body weight of the mice, repeated measures ANOVA was performed. Additionally, the effect of treatment on body weight, serum cholesterol, Bodipy, TEM and lipid extraction data within each time point was studied with two-tailed independent sample T-tests. To check for potential aging effects in the serum cholesterol, Bodipy and TEM data a univariate ANOVA test on the control groups of each time point was performed. For the analysis of the lipid extraction data a 2-way ANOVA was performed. All data are expressed as mean +/- S.E.M (standard error of mean). Data were considered significantly different at $P \leq 0.05$. The numbers of replicates and oocytes used to generate the data are described in the results section for each parameter.

4. Results

4.1 The impact of the HF/HS diet on body weight and serum total cholesterol concentrations

The live body weight and serum total cholesterol concentrations analysis are already previously described in Moorkens et al. (2022), where the same mice were used to examine oviductal cell functions. In short, feeding HF/HS diet increased the body weight progressively compared to the controls, which was already significant ($P<0.001$) from 1w onwards. The body weight reached a plateau at 12w after the onset of the dietary treatment (Moorkens et al., 2022). Feeding the HF/HS diet led to a significant increase in blood total cholesterol concentrations already after 1w ($P=0.004$). This significant difference was not observed at 4w ($P=0.136$) of feeding the HF/HS diet but

became more distinct again at 8w (P=0.006), 12w (P=0.068) and 16weeks (P=0.085) on the diet. Furthermore, Pearson correlation showed a positive correlation between body weight and serum cholesterol concentration.

4.2 The impact of the HF/HS diet on triglyceride content of whole ovaries

No significant difference was observed in the triglyceride content of the control and HF/HS ovaries within each time point (Fig. 1).

4.3 The impact of the HF/HS diet on the oocyte quality

4.3.1 Total lipid droplet volume in oocytes

Oocytes coming from the HF/HS group already showed a significant increase (63%;*P*<0.05) in the total volume of lipid droplets at 24h compared to the control mice. This increase was persistent at the following time points up until 8w (76%;*P*<0.05), reaching its maximum at 1w, but was not significant different anymore at 12w and 16w (*P*>0.1) compared to their respective controls at each timepoint (Fig 2.).

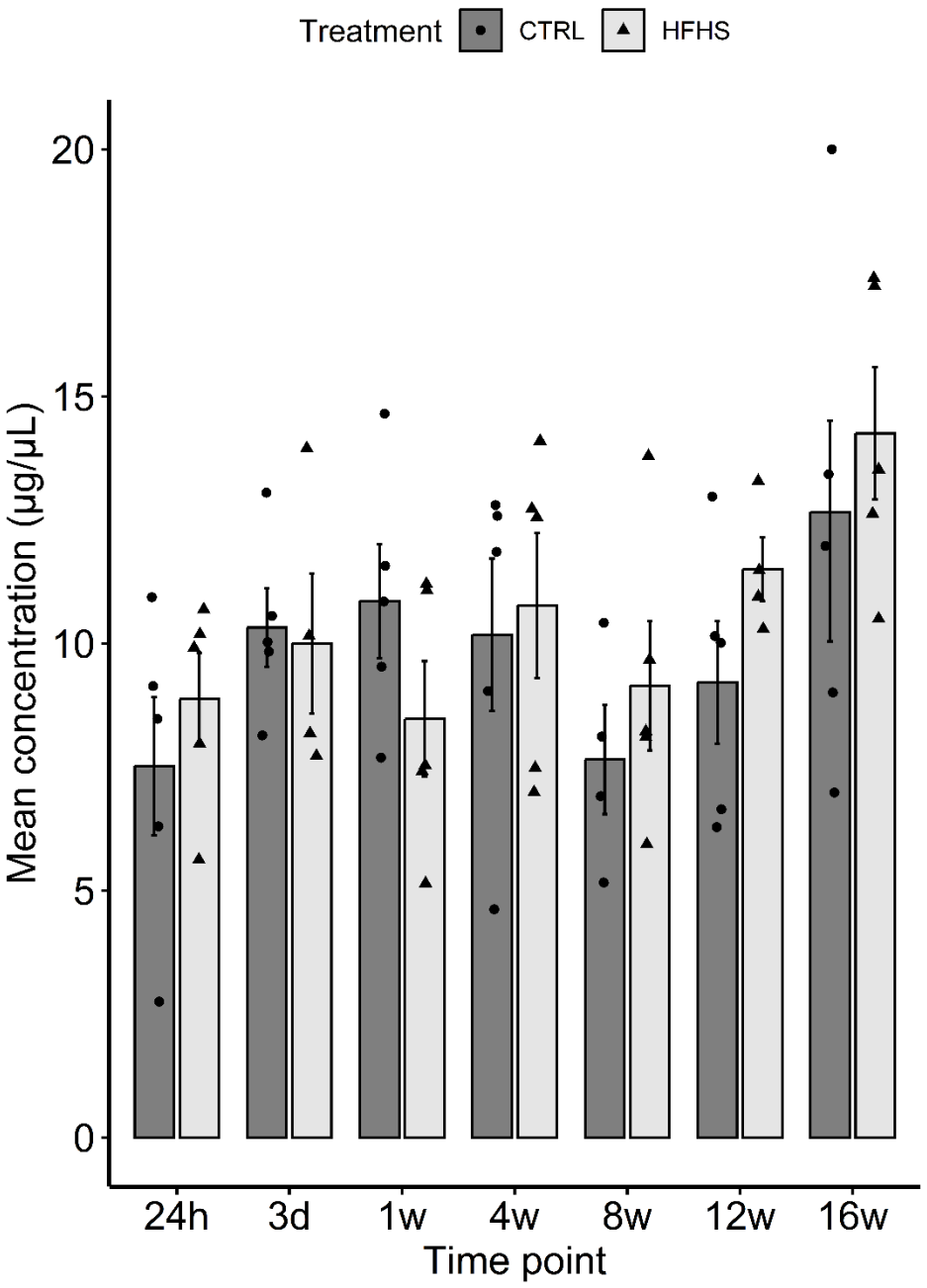


Figure 1. Mean triglyceride content in whole ovaries per diet group per treatment. Data is shown in a scatter plot as mean ± SEM from 5 ovaries coming from 5 mice for each treatment group at each time point.

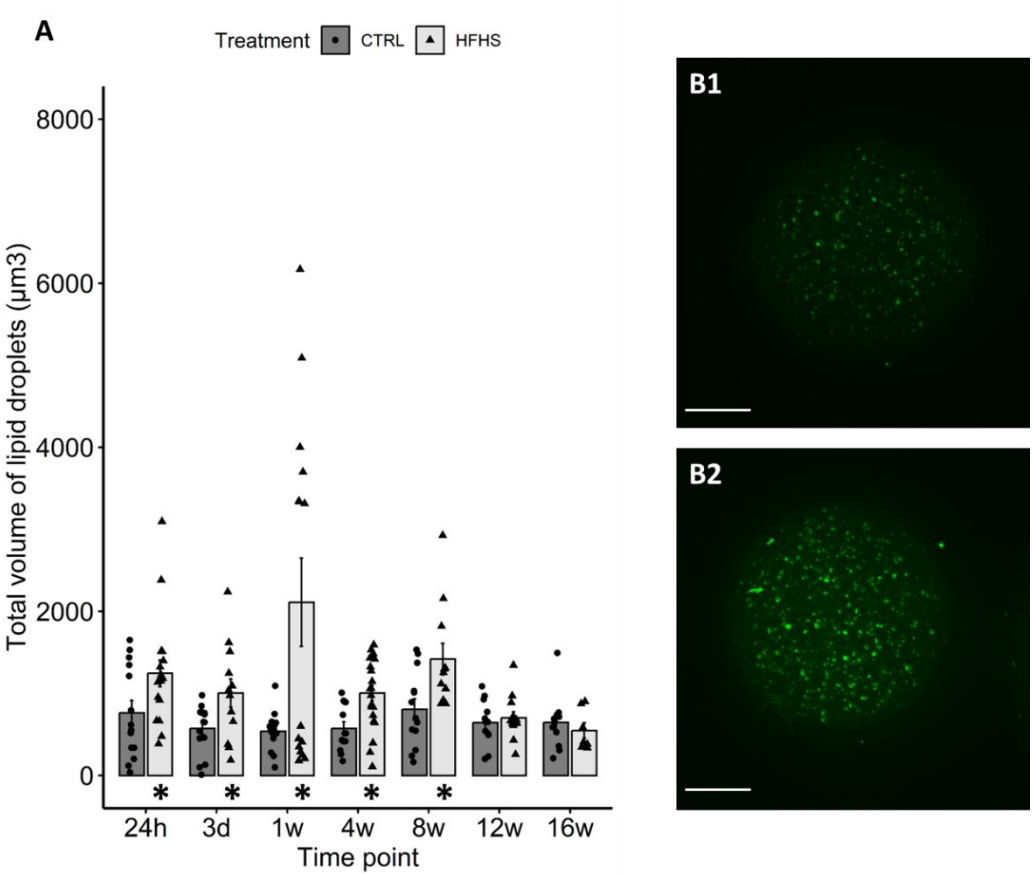


Figure 2: Assessment of lipid droplet volume in oocytes collected from mice fed either a control or a HF/HS diet for different durations (from 24h up to 16weeks). **A**. Scatter plot showing means ± SEM from 12 to 21 oocytes per dietary treatment, collected from 4 to 5 mice per treatment per time point. A significant difference between HF/HS and control within a time point is indicated by an asterisk (*) below the bars. **B1** and **B2**. Representative confocal images of a control oocyte (B1) and a HF/HS oocyte (B2) at 8w on the diet. Each confocal image is a z-stack projection of 40 x 1 µm steps.

4.3.2 Mitochondrial ultrastructure in oocytes

From 8w onwards HF/HS oocytes exhibited a progressive increase in the proportion of ultrastructural mitochondrial abnormalities (vacuolization, low electron density and rose petal appearance (Fig.3)) shown by a significant increase in abnormalities of 16.40% at 8w, 20.70% at 12w and 29.20% at 16w compared to 24h (Fig. 4A). Ultrastructural mitochondrial abnormalities at 8w and 16w HF/HS oocytes were

significantly higher compared to their controls of the same timepoints (15.8% and 20.40% respectively; *P*<0.05) (Fig. 4B).

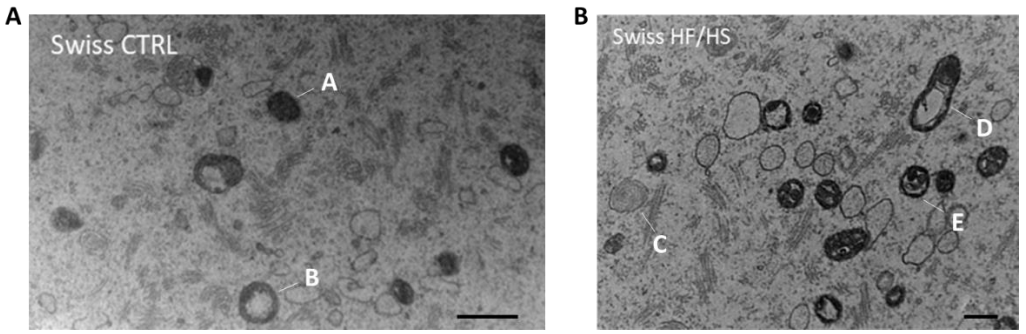


Figure 3. Representative TEM micrographs showing the ultrastructure of mitochondria in oocytes from mice fed a control (Fig.3A) or HF/HS (Fig3.B) diet for 16w. Different forms of normal (A: spherical; B: regular vacuoles) and abnormal (C: degenerated; D: elongated; E: irregular vacuoles) mitochondria can be observed classified according to Supplementary Fig. 1.

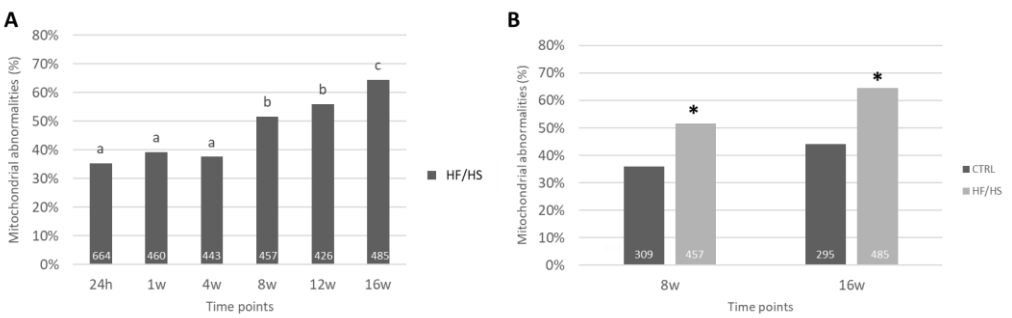


Figure 4: Proportions of mitochondria with abnormal ultrastructure in oocytes per time point (using TEM). The total number of assessed mitochondria is displayed at the bottom of each bar. **A.** A significant difference between the HF/HS groups of different time points is indicated with letters a, b, c (*P*<0.05). **B.** A significant difference between HF/HS oocytes and control oocytes within a time point is indicated by an asterisk (*).

4.4 Effect of HF/HS feeding on differential gene expression in granulosa cells

RNA sequencing showed that differential gene expression in the GCs is dependent on the duration of feeding the HF/HS diet at different timepoints. Additional PCA analysis of the data (including all 47 samples from both treatment groups and all time points together) showed that the samples clustered more by time points and less by diet. However, for the later time point a separation by diet may be observed (Supplementary figure 2).

RNA sequencing analysis revealed no differentially expressed genes (DEG) in the GCs at any of the time points after the start of HF/HS diet feeding, except at 8w (11 upregulated and 69 downregulated). These 80 DEGs resulted in a distinct separation between the HF/HS and CTRL groups as shown in the supplementary heatmap and volcano plot (supplementary figure 3 and 4). A list of these 80 DEGs can be found in supplementary table 1. A functional annotation analysis of these genes was performed using DAVID (Huang et al., 2009). A list of the enriched GO Biological Process (BP) and Molecular Function (MF) terms is shown in supplementary table 2.

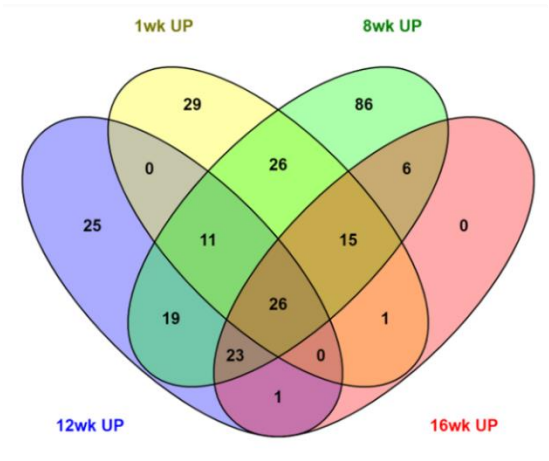
Moreover, Gene Set Enrichment Analysis (GSEA) revealed that several GO terms are significantly enriched GO terms at all timepoints except at 4w (Fig. 5).

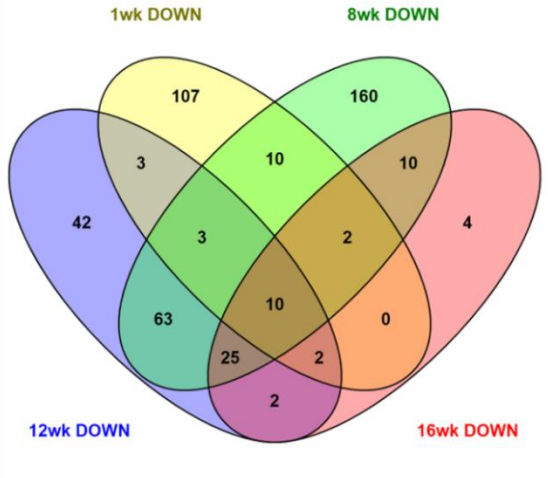
A few significant up- and downregulated GO term annotations (following the GSEA) were already detected at 3d (4 and 17 respectively). This number increased at 1w, and reached its maximum at 8w (212 up- and 283 downregulated), after which it remained moderate at 12w and slightly decreased at 16w on the HF/HS diet. None of the upregulated annotations detected at 3d of HF/HS feeding were detected at later time points. All downregulated 3d annotations were also detected at 1w of HF/HS diet feeding, but none of them were detected at later time points. Some, but not all, of the annotations detected at 8w were also detected at 1w, and the majority of them were persistently detected at 12w and/or 16wk. These patterns clearly show distinct acute and late responses to the HFHS diet. Because of the high number of detected significant

up- and down-regulated GO term annotations, Revigo was used to merge GO-terms based on their semantic similarity (Supek et al., 2011) after which the reduced list of GO-terms was classified into common classes focusing on biological processes (BP) (Fig. 6). The pattern by which these annotations start, increase and persist overtime illustrate the cascade of transcriptomic changes in biological functions in the affected GCs (Fig 6).

The up-regulated GO-terms detected in the GCs at 3d after the start of HF/HS diet feeding were related to ER functions, whereas the 1w annotations were related to cell differentiation and cell signaling. All up-regulated annotations in the GCs from 1w after the start of HF/HS diet feeding as well as at the later time points were related to biogenesis and chromosome organization. The annotations detected in the GCs at 8w and the later time points were related to RNA and DNA regulatory processes and mechanisms.

All downregulated 3d and 1w annotations appeared to be related to translation. At 1w of HF/HS diet feeding the downregulated GO-terms were also related to mitochondrial functions and metabolic alterations. The annotations that were detected in the GCs at the later time points were also related to metabolic alterations, as well as immune responses, inflammation, angiogenesis, and autophagy (Fig. 6).





UP	n GO-terms	3d	1wk	8wk	12wk
3d	4				
1wk	108	0			
8wk	212	0	78		
12wk	105	0	37	79	
16wk	72	0	42	64	24

DOWN	n GO-terms	3d	1wk	8wk	12wk
3d	17				
1wk	137	17			
8wk	283	0	25		
12wk	150	0	15	101	
16wk	55	0	0	12	39

Figure 5. Number of common up- and down-regulated GO-terms detected in the GCs at different timepoints after the start of HF/HS diet feeding.

Figure 6. A reduced visualization of the up- and down-regulated GO-terms detected in the GCs at different time points after the start of HF/HS diet feeding. Go-term annotations in each time point are represented by a color code based on the Log10 of the average PAdj value of GO-terms in each class. As there were no significant GO-terms at 4w after the start of HF/HS diet feeding (even at Padj <0.1), this time point is not included in the table.

5. Discussion

The aim of this study was to investigate the effects of feeding a HF/HS diet for different periods (from 24h till 16w) on the ovary and ovarian cells of mice and describe the development and patterns of the changes over time as the mice continue to consume the obesogenic diet and develop an obese phenotype. To increase the pathophysiological relevance to humans and livestock animals, an outbred Swiss mouse model was used.

While most diet-induced obese mouse models are based on inbred mice such as C57Bl/6, we confirmed in our present study that Swiss mice are also sensitive to HFHS diets and develop adiposity and hypercholesterolemia. The body weight and total serum cholesterol results demonstrate that our Swiss outbred model is metabolically sensitive to the high fructose and saturated fat content of the HF/HS diet, as was shown in our previous reports as well (Marei et al., 2020; Moorkens et al., 2022; Smits et al., 2022). The analysis of live body weight was reported in our previous study (Moorkens et al., 2022) and was able to confirm the findings of Marei et al. (2020) as the weight gain of the HF/HS diet fed mice was already significantly higher after 1w of feeding compared to the controls. Furthermore, serum cholesterol concentrations were also significantly increased after 1w on the HF/HS diet compared to the control diet and remained high during the later time points. It has been reported that an increase in weight gain is correlated with higher cholesterol synthesis (Nestel et al., 1973). Our study could confirm this by showing a positive correlation between weight and total serum concentrations. The weight of the mice reached a plateau at 12w of feeding, which might be linked to a metabolic adaption that was reported in older Swiss mice with increased adiposity (Krishna et al., 2016). This may be linked to the cholesterol concentrations in the serum collected from HF/HS-fed at 12w and 16w as they remained high but only showed a tendency of a significant difference compared to the control mice at these latest time points.

Many studies reported the impact of obesogenic diets on the lipid droplet content in the ovarian cells (Akamine et al., 2010; Wu et al., 2010; Marei et al., 2020). It has also been shown that increased triglycerides due to obesity are reflected in the follicular fluid (Wu et al., 2010). The impact of obesity or feeding an obesogenic diet on the triglyceride content in ovarian tissue was not been described up until now. Our data could not indicate a significant difference in triglyceride content of the ovarian tissue after any of the time periods of feeding the HF/HS diet. The absence of a detectable overall change in the lipid content at the organ level in the ovary, does however not rule out cell-specific effects on the follicular cells and oocytes.

These duration specific changes were also observed in the the Bodipy staining of the oocytes. Namely, while the lipid droplet (LD) volume in the oocytes already showed to be significantly increased as early as 24h of HF/HS diet feeding and remained significantly different up to 8w of feeding compared to the controls, there was no significant difference detected anymore at 12w and 16w of feeding. Our data shows a very acute increase in LD volume already 24h after the start of HF/HS diet feeding. As far as we know, such short-term acute effects of feeding an obesogenic diet on the LD volume in oocytes have not been reported before. An increase in LD content in oocytes after relatively short-term feeding a HFD for 21 days was shown by Aizawa et al (Aizawa et al., 2021). Previously, our research group reported that hyperlipidemia was reflected in the lipid composition of the FF which was linked with reduced oocyte quality (Valckx et al., 2014a). The detected increase in intracellular lipid in the oocytes may be due to diffusion from the serum or follicular fluid, as it has previously been shown *in vitro* that oocytes are capable of directly incorporating free fatty acids (FFA) from their microenvironment when exposed to FFA-rich media (Yang et al., 2012). Although an adequate amount of lipid droplets is essential for preimplantation embryonic development (Tatsumi et al., 2018), the excessive accumulation of lipid droplets in cells other than adipocytes may result in lipotoxicity, due to the induction of altered metabolic rates and oxidative stress (Szendroedi and Roden, 2009). To protect itself

from lipotoxicity the cell stores intracellular lipids in a neutral status (LD) in lipid droplets (Jarc and Petan, 2019). Various other studies have reported increased lipid content in the oocytes and FF microenvironment of diet-induced obese mice (Robker, 2008; Igosheva et al., 2010; Wu et al., 2011; Luzzo et al., 2012; Valckx et al., 2014b; Marei et al., 2020). Wu et al (2010) also reported a dramatically increase in the lipid droplet content of oocytes of mice after 4w of HFD-feeding (Wu et al., 2010). This could however not be confirmed by Li et al (Li et al., 2018) as they did not detect an increase in the accumulation of lipid droplets in mice oocytes after the same period on the HFD. (Jarc and Petan, 2019). They suggested this may be due to the protective function of estradiol (E_2), which is converted by P450 aromatase in the cumulus cells from testosterone, which in its turn is synthesized in follicular membrane cells (Kato et al., 2013). Serum cholesterol has been found to promote P450 aromatase expression. Therefore the E_2 level in the serum may be impacted by a HFD due to an increased level of cholesterol and increased expression of P450 aromatase (Li et al., 2018). The protective function of E_2 was previously demonstrated by Chaube et al. (Chaube et al 2005). In our study, the significant increase of serum cholesterol may have led to an increase in E_2 in the later time points, causing the lipid droplet volume to decrease as a protection mechanism for the oocyte. Li et al reported that E2 was probably able to maintain oocyte quality due to its protective function, however, the HFD was still able to induce mitochondrial dysfunction in the cumulus cells, demonstrated by the reduced expression of genes involved in mitochondrial function after 4w of HFD-feeding (Li et al., 2018). As it is known that cumulus cells are in direct communication with oocytes via gap junctions (Turathum et al., 2021), this may be an explanation for the significant higher proportion of mitochondrial abnormalities that was detected from 8w onwards on the HF/HS diet in our present study. Our data confirms our previous study by Marei et al. (2020) showing a significantly higher proportion of mitochondrial abnormalities in Swiss mice fed a HFD for 13 weeks, whereas our data shows a significant difference in mitochondrial abnormalities from 8w onwards. The study of Luzzo et al (2012)

reported an increase already at 4w after the start of a HFD (Luzzo et al., 2012). Previously, we have demonstrated that administration of a HF/HS diet to mice in a similar experimental setup resulted in an acute increase of (mitochondrial) oxidative stress (OS) and (mild) ER stress in their oviductal epithelial cells (OECs) already after 3d of feeding, even before developing the obesogenic phenotype. This was followed by the initiation of a sequence of transcriptomic changes to control ER-stress and mitochondrial ROS-production, however, long-term HF/HS diet feeding resulted in a persistent upregulation of mitochondrial OS and ER-stress, ultimately even leading to local and systemic inflammation 16w after the start of the HF/HS diet (Moorkens et al., 2022). These duration specific changes in which first a cascade of transcriptomic changes is initiated to control mitochondrial ROS-production which wasn't able to prevent persistent upregulation of mitochondrial OS, may explain the observation of a significant increase in mitochondrial abnormalities only starting from the mid/later time points after the start of HF/HS-diet feeding. The proportion of ultrastructural mitochondrial abnormalities increased over time while mice continued to consume the HF/HS diet, as is shown by the significant difference of mitochondrial abnormalities of time period of 8w to 12w on the diet and long-term feeding of 16w compared to the proportion of abnormalities detected at 24h of feeding. This indicates that the impact of an obesogenic diet on the mitochondrial ultrastructure progressively increases over time after continued exposure to the diet. This was also confirmed when comparing 8w and 16w HF/HS oocytes to their controls, which demonstrated a significant difference in mitochondrial abnormalities between the HF/HS and CTRL oocytes, with the highest proportion of mitochondrial abnormalities detected at 16w on the diet.

The main focus of our study is to investigate the development of effects in the ovary and ovarian cells after different durations of feeding an obesogenic diet. The detected GO-term annotations by RNA sequencing of the granulosa cells (GCs), show a very interesting evolution cascade of changes (Fig. 6) over time which matches with our previous findings of gene expression changes reported in the OECs of these mice as

well (Moorkens et al., 2022). Namely, their GCs showed a very acute upregulation of GO-terms related to ER stress as early as 3days after the start of the HF/HS diet, whereas a downregulation of GO-terms related to mitochondrial functions was already observed after 1w of feeding. Granulosa cells are very important with regards to nutritional support and hormone regulations in the ovary (Gao et al., 2023). Together with FF and cumulus cells (CCs), they form the closest follicular environment of the oocyte, which is known to be of crucial importance on achieving oocyte developmental competence (Leroy et al., 2005; Revelli et al., 2009; Assidi et al., 2010; Dumesic et al., 2015) and has found to have molecular factors predicting oocyte developmental potential (Bunel et al., 2014; Matoba et al., 2014; Warzych et al., 2014). Gene enrichment analysis has shown that feeding the obesogenic diet leads to downregulation of Go-term annotations related to cell-signaling already from 1w onwards. After 8w on the HF/HS diet a downregulation of cell-to-cell interaction related GO-terms was detected. These results suggest that feeding of the HF/HS diet may hamper the direct communication between the GCs and the oocytes, thereby having a detrimental impact on oocyte quality and oocyte developmental competence. The OECs of these mice showed an increased expression level of genes related to mitochondrial OS and ER stress after long-term feeding. It has been reported that mitochondrial and ER stress in somatic cells controls protein misfolding and maintains cell survival and function by initiating the unfolded protein response (UPR) (Hetz, 2012). This may be impacted in the GCs of mice on the HF/HS diet, as our data shows a downregulation of Go-terms related to UPRs from 8w onwards, up until long-term feeding of 16w. DNA repair related GO-term annotations however, were upregulated after 8w on the diet up until 16w on the diet. Reduced DNA repair in GCs has found to be contributing to ovarian aging, which is closely related to a decrease in oocyte quality (Zhang et al., 2015). Wu et al (2010) previously demonstrated that mice fed a HFD for 4w showed increased apoptosis in their GCs (Wu et al., 2010). The upregulation of DNA repair detected from 8w onwards in the GCs in our study may indicate the capability of

the cells to prevent apoptosis (Winship et al., 2018). While long-term HF/HS diet feeding of 16w resulted in upregulation of inflammation in OECs, the GCs demonstrated a downregulation of inflammation at 8w and 12w of feeding, even showing no signs of inflammation anymore at 16w of feeding. Few of the acute effects, both up- and down-regulated, appearing as early as 3d and 1w after the start of the obesogenic diet are also observed after long-term feeding. However, the majority of up- and downregulated annotations at 8w were also detected at the latest time points (Fig. 5), indicating that the effects of the obesogenic diet are persistent and may aggravate over time, while the mice develop the obese phenotype. These results further confirm the distinct phases in which responses to metabolic changes are developed in the reproductive tissues and cells, as was previously reported (Moorkens et al., 2022).

Feeding a HF/HS diet for only 24h already results in an acute increase in lipid droplet volume in oocytes from 24h of feeding onwards. ER and mitochondrial related pathways appeared to be impacted as early as 3d and 1w of feeding. These acute responses were followed by an increase in mitochondrial abnormalities in the oocyte from 8w onwards and the initiation of changes in up- and downregulated pathways due to the obesogenic diet and the development of obesity.

6. Conclusions

We can confirm that the ovarian cells can sense and react to systemic diet-induced hyperlipideamia. Exposure to an obesogenic diet results in an acute increase in the lipid content of oocytes and in acute upregulated ER and downregulated mitochondrial functions in granulosa cells after short-term feeding of only 24h till 1w, thus even before the development of an obese phenotype. The effects of the obesogenic diet progressively increase over time, showing increased mitochondrial abnormalities in oocytes and different affected pathways, linked to DNA repair, cell signalling, UPRs and many others in the granulosa cells. The responses in the oocytes and granulosa cells

have shown to be cell specific and may directly impact oocyte quality and early embryo development, ultimately leading to reduced fertility.

Taken together, the ovarian follicle microenvironment is sensitive to very short-term changes in the diet composition, and the alterations in ovarian cell biological functions follow a time-dependent multiphasic cascade. The impact of the detected progressive changes after long-term obesogenic feeding on pregnancy success, further oocyte and embryo development and postnatal health needs to be further investigated. Defining and understanding these progressive changes should increase the awareness of the impact of the consumption of an obesogenic diet and the development of obesity, which is needed to protect reproductive health.

7. Supplementary material

Supplementary file 1: RNA sequencing: methods for read preparation, adapter and quality trimming, read mapping and feature counts, library saturation

Read preparation

Raw read quality control

UMI were removed using UMI-tools (v1.1.1), resulting in raw reads with a remaining length of 66 nt (removed 6 nt for the UMI + 4 nt spacer). The raw sequencing reads were inspected using FastQC (v0.11.9) for their quality and length. Putative contaminations were checked using FastQ Screen (v0.15.1) and a set of genomes from common lab organisms. The read quality based on phred scores was good. Contamination screening with FastQScreen showed that up to 95% of the reads map on the mouse genome. A small amount (around 1%) of reads mapped uniquely to the human genome for all samples. Limited cross-mapping (i.e. reads mapping to multiple genomes) to other organisms is normal and caused by homology between organisms.

Adapter and quality trimming

Adaptor trimming was done using cutadapt (v3.4) with added filtering of reads containing ambiguities or not passing the phred score threshold of 20. The quality of the remaining read pairs was checked using FastQC as before.

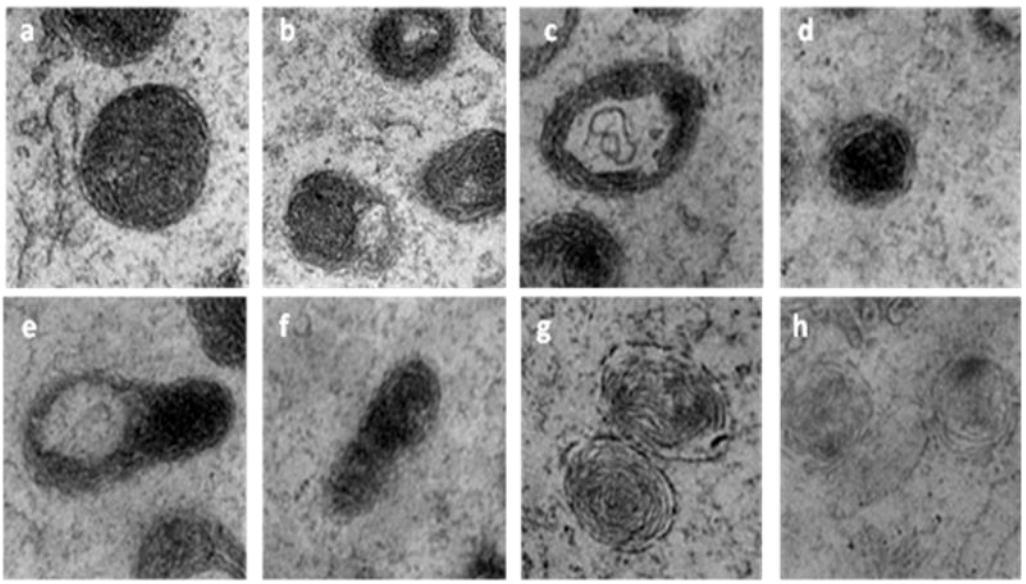
Gene expression quantification

Read mapping and feature counts

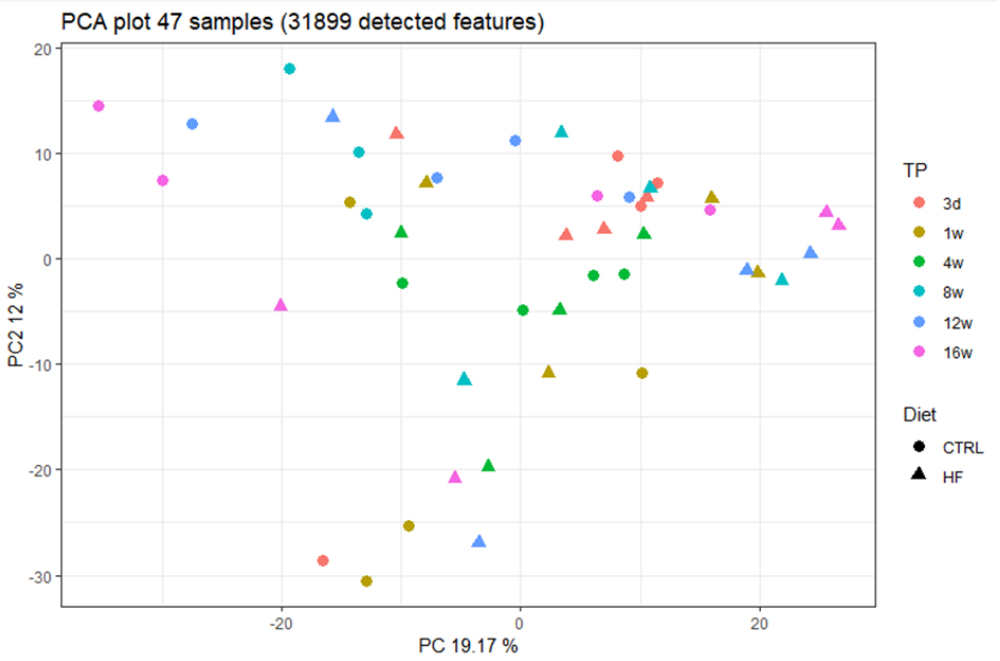
For each sample, trimmed reads were mapped on the latest mouse genome (GRCm39, ENSEMBL release 107) using the splice-aware STAR (v2.7.9a) mapper. UMI-based removal of PCR duplicates from the mapped reads was done with UMI-tools (v1.1.1). Feature counting at the gene and transcript isoform level was done using rsem-calculate-expression (RSEM v1.3.0). The gene-level counts were used for all subsequent analyses.

Library saturation

Whether the sequencing depth reflects the sample's active gene diversity was checked by looking at a rarefaction (a.k.a. saturation or complexity) plot. The data for such plot was generated using preseq (v2.0.1) and the result was plotted using python (v3.6). Inspection of the plot reveals that saturation is satisfactory for all samples, meaning most of the diversity is captured and additional sequencing would not add significantly more information.

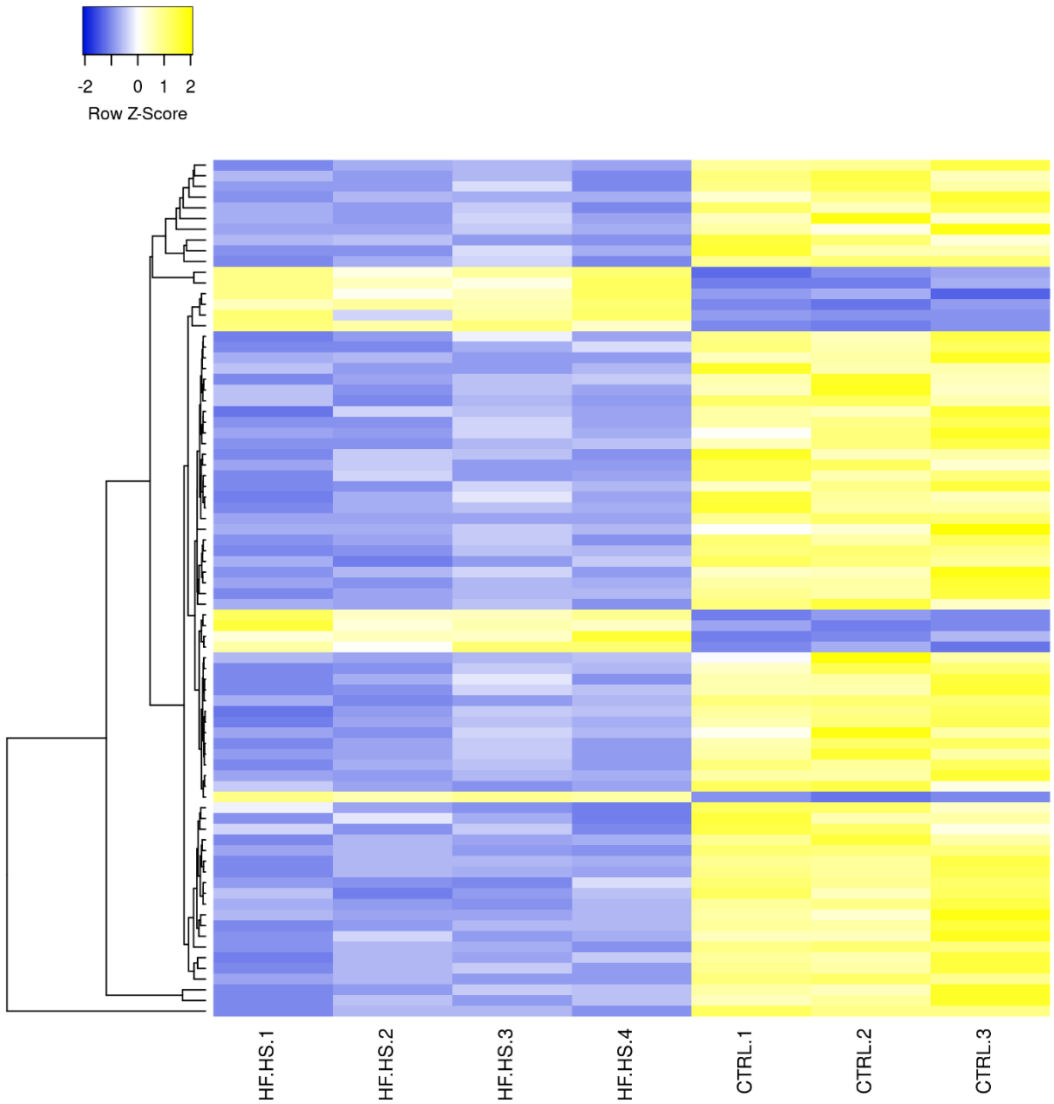


Supplementary Figure 1. Classification of mitochondria by Marei et al. (2020).
a. Spherical, b. Regular vacuoles, c. Membranous vacuoles, d. Electron dense foci, e. Dumbbell shape, f. Elognated, g. Rose petal shaped, h. Degenerated mitochondrion.

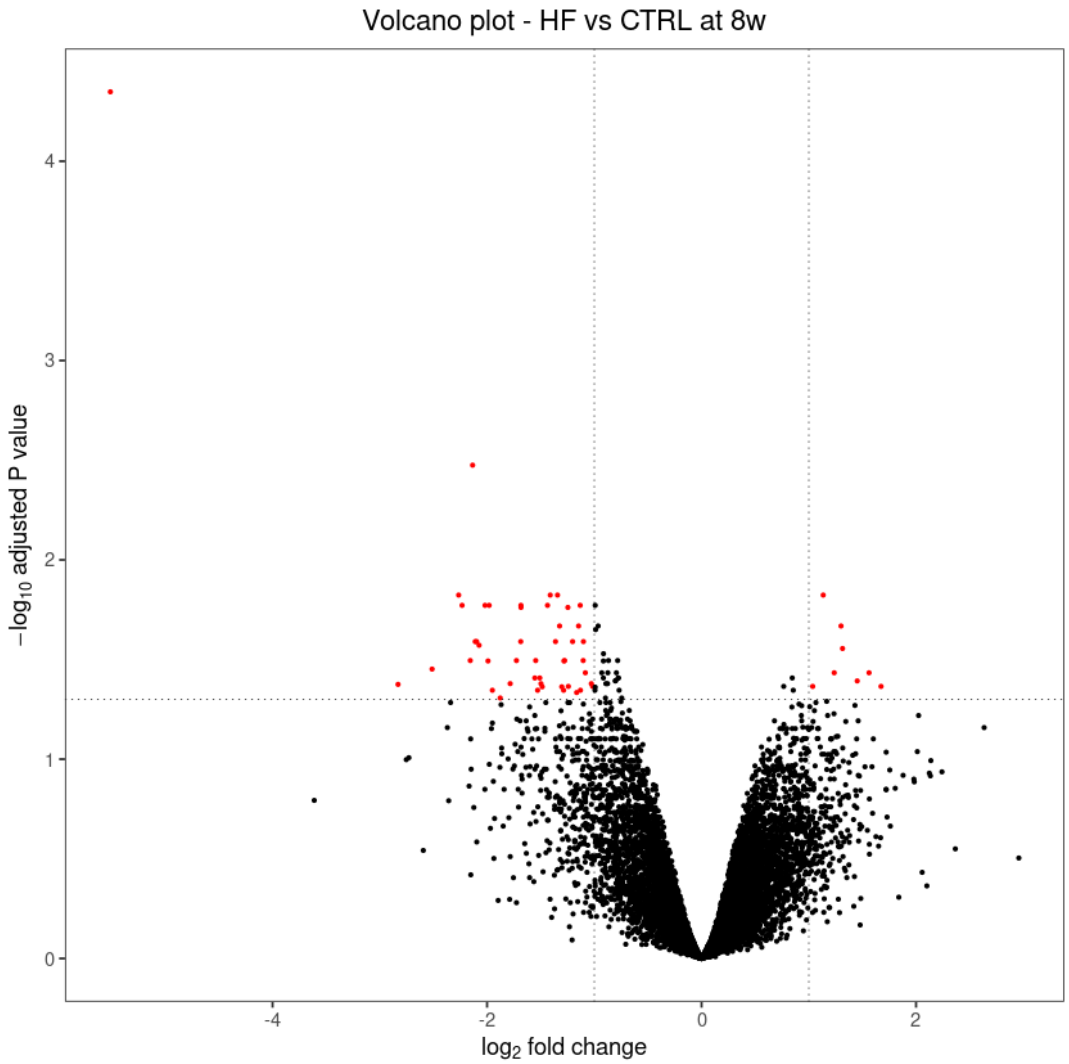


Supplementary Figure 2. A PCA analysis of the GC transcriptomic profiles of the control and HF/HS samples collected at different timepoints after the start of HF/HS diet feeding.

Supplementary Figure 3: Heatmap of the 80 DEGs of the HF/HS vs CTRL comparison at 8 weeks of feeding



Supplementary Figure 4: Volcano plot of the 80 DEGs of the HF/HS vs CTRL comparison at 8 weeks of feeding



Supplementary Table 1: List of 80 DEGs detected at 8w of feeding the HF/HS diet

gene_id	entrezgene_id	gene_symbol	gene_description	FC	logFC	PValue	PAdj
ENSMUSG00000109658		Gm45660	predicted gene 45660 [Source:MGI Symbol; Acc:MGI:5791496]	0.02	-5.51	3.76E-09	4.49E-05
ENSMUSG00000001131	21857	Timp1	tissue inhibitor of metalloproteinase 1 [Source:MGI Symbol;Acc:MGI:98752]	0.23	-2.13	5.62E-07	3.35E-03
ENSMUSG00000014782	102075	Plekhg4	pleckstrin homology domain containing, family G (with RhoGef domain) member 4 [Source:MGI Symbol; Acc:MGI:2142544]	0.21	-2.26	5.40E-06	1.50E-02
ENSMUSG00000096768	170942	Gm47283	predicted gene, 47283 [Source:MGI Symbol; Acc:MGI:6096131]	2.20	1.13	6.64E-06	1.50E-02
ENSMUSG00000031751	23802	Amfr	autocrine motility factor receptor [Source:MGI Symbol; Acc:MGI:1345634]	0.38	-1.41	6.81E-06	1.50E-02
ENSMUSG00000022969	16155	Il10rb	interleukin 10 receptor, beta [Source:MGI Symbol; Acc:MGI:109380]	0.39	-1.34	7.56E-06	1.50E-02
ENSMUSG00000073802	12579	Cdkn2b	cyclin dependent kinase inhibitor 2B [Source:MGI Symbol; Acc:MGI:104737]	0.25	-2.02	1.35E-05	1.69E-02
ENSMUSG00000090877	15511	Hspa1b	heat shock protein 1B [Source:MGI Symbol;Acc:MGI:99517]	0.31	-1.68	1.47E-05	1.69E-02
ENSMUSG00000058492		Scp2-ps2	sterol carrier protein 2, pseudogene 2 [Source:MGI Symbol;Acc:MGI:107679]	0.50	-0.99	1.56E-05	1.69E-02
ENSMUSG00000024424	72747	Ttc39c	tetratricopeptide repeat domain 39C [Source:MGI Symbol;Acc:MGI:1919997]	0.25	-1.98	1.64E-05	1.69E-02
ENSMUSG00000025647	66940	Shisa5	shisa family member 5 [Source:MGI Symbol;Acc:MGI:1915044]	0.37	-1.44	1.68E-05	1.69E-02
ENSMUSG00000026535	26388	Ifi202b	interferon activated gene 202B [Source:MGI Symbol;Acc:MGI:1347083]	0.21	-2.23	1.79E-05	1.69E-02
ENSMUSG00000036390	13197	Gadd45a	growth arrest and DNA-damage-inducible 45 alpha [Source:MGI Symbol;Acc:MGI:107799]	0.46	-1.13	1.84E-05	1.69E-02
ENSMUSG00000042460	94192	C1galt1	core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase, 1 [Source:MGI Symbol;Acc:MGI:2151071]	0.42	-1.25	2.15E-05	1.73E-02

ENSMUSG00000041828	217258	Abca8a	ATP-binding cassette, sub-family A (ABC1), member 8a [Source:MGI Symbol;Acc:MGI:2386846]	0.31	-1.68	2.18E-05	1.73E-02
ENSMUSG00000039202	54608	Abhd2	abhydrolase domain containing 2 [Source:MGI Symbol;Acc:MGI:1914344]	0.40	-1.32	2.93E-05	2.15E-02
ENSMUSG00000069601	11735	Ank3	ankyrin 3, epithelial [Source:MGI Symbol;Acc:MGI:88026]	2.46	1.30	3.16E-05	2.15E-02
ENSMUSG00000052656	22644	Rnf103	ring finger protein 103 [Source:MGI Symbol;Acc:MGI:109483]	0.45	-1.15	3.33E-05	2.15E-02
ENSMUSG00000022533	224088	Atp13a3	ATPase type 13A3 [Source:MGI Symbol;Acc:MGI:2685387]	0.51	-0.96	3.42E-05	2.15E-02
ENSMUSG00000049047	71703	Armcx3	armadillo repeat containing, X-linked 3 [Source:MGI Symbol;Acc:MGI:1918953]	0.50	-0.99	3.76E-05	2.24E-02
ENSMUSG00000008540	56615	Mgst1	microsomal glutathione S-transferase 1 [Source:MGI Symbol;Acc:MGI:1913850]	0.44	-1.20	4.87E-05	2.57E-02
ENSMUSG00000030351	68498	Tspan11	tetraspanin 11 [Source:MGI Symbol;Acc:MGI:1915748]	0.23	-2.10	5.03E-05	2.57E-02
ENSMUSG00000024659	16952	Anxa1	annexin A1 [Source:MGI Symbol;Acc:MGI:96819]	0.39	-1.36	5.07E-05	2.57E-02
ENSMUSG00000026817	11636	Ak1	adenylate kinase 1 [Source:MGI Symbol;Acc:MGI:87977]	0.23	-2.11	5.45E-05	2.57E-02
ENSMUSG00000030605	17304	Mfge8	milk fat globule EGF and factor V/VIII domain containing [Source:MGI Symbol;Acc:MGI:102768]	0.47	-1.10	5.55E-05	2.57E-02
ENSMUSG00000021190	19141	Lgmn	legumain [Source:MGI Symbol;Acc:MGI:1330838]	0.31	-1.69	5.61E-05	2.57E-02
ENSMUSG00000024292	64385	Cyp4f14	cytochrome P450, family 4, subfamily f, polypeptide 14 [Source:MGI Symbol;Acc:MGI:1927669]	0.24	-2.07	6.08E-05	2.68E-02
ENSMUSG00000110928		Gm48114	predicted gene, 48114 [Source:MGI Symbol; Acc:MGI:6097467]	2.49	1.31	6.54E-05	2.78E-02
ENSMUSG00000022365	67819	Derl1	Der1-like domain family, member 1 [Source:MGI Symbol;Acc:MGI:1915069]	0.53	-0.91	7.19E-05	2.96E-02
ENSMUSG00000054619	70152	Mettl7a1	methyltransferase like 7A1 [Source:MGI Symbol; Acc:MGI:1916523]	0.47	-1.10	8.65E-05	3.20E-02
ENSMUSG00000028249	53378	Sdcbp	syndecan binding protein [Source:MGI Symbol; Acc:MGI:1337026]	0.58	-0.78	8.73E-05	3.20E-02
ENSMUSG00000030218	17313	Mgp	matrix Gla protein [Source:MGI Symbol;Acc:MGI:96976]	0.41	-1.28	9.46E-05	3.20E-02
ENSMUSG00000045414	68861	Dipk2a	divergent protein kinase domain 2A [Source:MGI Symbol;Acc:MGI:1916111]	0.22	-2.16	9.51E-05	3.20E-02

ENSMUSG00000043419		Rnf227	ring finger protein 227 [Source:MGI Symbol; Acc:MGI:1915359]	0.34	-1.55	9.60E-05	3.20E-02
ENSMUSG00000014867	20932	Surf4	surfeit gene 4 [Source:MGI Symbol;Acc:MGI:98445]	0.55	-0.87	9.62E-05	3.20E-02
ENSMUSG00000032802	76650	Srxn1	sulfiredoxin 1 homolog (S. cerevisiae) [Source:MGI Symbol;Acc:MGI:104971]	0.30	-1.73	9.67E-05	3.20E-02
ENSMUSG00000027447	13010	Cst3	cystatin C [Source:MGI Symbol;Acc:MGI:102519]	0.53	-0.92	1.03E-04	3.22E-02
ENSMUSG00000001020	20198	S100a4	S100 calcium binding protein A4 [Source:MGI Symbol;Acc:MGI:1330282]	0.25	-1.99	1.04E-04	3.22E-02
ENSMUSG00000048332	108927	Lhfp	lipoma HMGIC fusion partner [Source:MGI Symbol;Acc:MGI:1920048]	0.41	-1.28	1.05E-04	3.22E-02
ENSMUSG00000096942		Rps19-ps6	ribosomal protein S19, pseudogene 6 [Source:MGI Symbol;Acc:MGI:5011818]	0.18	-2.51	1.19E-04	3.53E-02
ENSMUSG00000085194		Platr32	pluripotency associated transcript 32 [Source:MGI Symbol;Acc:MGI:3801726]	2.95	1.56	1.28E-04	3.69E-02
ENSMUSG00000036275	213673	9530068E07Rik	RIKEN cDNA 9530068E07 gene [Source:MGI Symbol;Acc:MGI:2654705]	0.58	-0.79	1.33E-04	3.69E-02
ENSMUSG00000002733	83435	Plekha3	pleckstrin homology domain-containing, family A (phosphoinositide binding specific) member 3 [Source:MGI Symbol;Acc:MGI:1932515]	0.53	-0.93	1.37E-04	3.69E-02
ENSMUSG00000016427	54405	Ndufa1	NADH:ubiquinone oxidoreductase subunit A1 [Source:MGI Symbol;Acc:MGI:1929511]	0.55	-0.87	1.37E-04	3.69E-02
ENSMUSG00000021054	81535	Sgpp1	sphingosine-1-phosphate phosphatase 1 [Source:MGI Symbol;Acc:MGI:2135760]	0.47	-1.08	1.41E-04	3.69E-02
ENSMUSG00000021055	13983	Esr2	estrogen receptor 2 (beta) [Source:MGI Symbol; Acc:MGI:109392]	2.36	1.24	1.42E-04	3.69E-02
ENSMUSG00000000058	12390	Cav2	caveolin 2 [Source:MGI Symbol;Acc:MGI:107571]	0.35	-1.51	1.59E-04	3.91E-02
ENSMUSG00000025351	12512	Cd63	CD63 antigen [Source:MGI Symbol;Acc:MGI:99529]	0.53	-0.92	1.64E-04	3.91E-02
ENSMUSG00000038463	320078	Olfml2b	olfactomedin-like 2B [Source:MGI Symbol; Acc:MGI:2443310]	0.34	-1.55	1.64E-04	3.91E-02
ENSMUSG00000037706	12520	Cd81	CD81 antigen [Source:MGI Symbol;Acc:MGI:1096398]	0.58	-0.78	1.66E-04	3.91E-02

ENSMUSG00000005802	22785	Slc30a4	solute carrier family 30 (zinc transporter), member 4 [Source:MGI Symbol;Acc:MGI:1345282]	0.58	-0.78	1.69E-04	3.91E-02
ENSMUSG00000035954	238130	Dock4	dedicator of cytokinesis 4 [Source:MGI Symbol; Acc:MGI:1918006]	1.80	0.85	1.71E-04	3.91E-02
ENSMUSG00000037316	67384	Bag4	BCL2-associated athanogene 4 [Source:MGI Symbol;Acc:MGI:1914634]	0.57	-0.80	1.80E-04	4.04E-02
ENSMUSG00000017707	26943	Serinc3	serine incorporator 3 [Source:MGI Symbol;Acc:MGI:1349457]	0.57	-0.80	1.84E-04	4.05E-02
ENSMUSG00000034209	75668	Rasl10a	RAS-like, family 10, member A [Source:MGI Symbol;Acc:MGI:1922918]	2.74	1.45	1.87E-04	4.05E-02
ENSMUSG00000022816	14314	Fstl1	follistatin-like 1 [Source:MGI Symbol;Acc:MGI:102793]	0.54	-0.89	1.96E-04	4.18E-02
ENSMUSG00000028603	20280	Scp2	sterol carrier protein 2, liver [Source:MGI Symbol;Acc:MGI:98254]	0.49	-1.03	2.01E-04	4.18E-02
ENSMUSG00000029998	66881	Pcyox1	prenylcysteine oxidase 1 [Source:MGI Symbol;Acc:MGI:1914131]	0.55	-0.88	2.06E-04	4.18E-02
ENSMUSG00000070287	245020	Slc35g2	solute carrier family 35, member G2 [Source:MGI Symbol;Acc:MGI:2685365]	0.29	-1.78	2.10E-04	4.18E-02
ENSMUSG00000056870	70676	Gulp1	GULP, engulfment adaptor PTB domain containing 1 [Source:MGI Symbol;Acc:MGI:1920407]	0.35	-1.50	2.10E-04	4.18E-02
ENSMUSG00000032373	76459	Car12	carbonic anhydrase 12 [Source:MGI Symbol;Acc:MGI:1923709]	0.14	-2.83	2.16E-04	4.22E-02
ENSMUSG00000029098	80911	Acox3	acyl-Coenzyme A oxidase 3, pristanoyl [Source:MGI Symbol;Acc:MGI:1933156]	0.50	-1.02	2.26E-04	4.31E-02
ENSMUSG00000046997	211949	Spsb4	splA/ryanodine receptor domain and SOCS box containing 4 [Source:MGI Symbol;Acc:MGI:2183445]	3.19	1.67	2.34E-04	4.31E-02
ENSMUSG00000049553	20019	Polr1a	polymerase (RNA) I polypeptide A [Source:MGI Symbol;Acc:MGI:1096397]	1.70	0.77	2.35E-04	4.31E-02
ENSMUSG00000039217	16173	Il18	interleukin 18 [Source:MGI Symbol;Acc:MGI:107936]	0.42	-1.24	2.37E-04	4.31E-02
ENSMUSG00000000861	14025	Bcl11a	B cell CLL/lymphoma 11A (zinc finger protein) [Source:MGI Symbol;Acc:MGI:106190]	2.05	1.04	2.39E-04	4.31E-02
ENSMUSG00000056313	69068	Tcim	transcriptional and immune response regulator [Source:MGI Symbol;Acc:MGI:1916318]	0.36	-1.49	2.44E-04	4.33E-02

ENSMUSG00000027800	17112	Tm4sf1	transmembrane 4 superfamily member 1 [Source:MGI Symbol;Acc:MGI:104678]	0.41	-1.30	2.47E-04	4.33E-02
ENSMUSG00000020225	68212	Tmbim4	transmembrane BAX inhibitor motif containing 4 [Source:MGI Symbol;Acc:MGI:1915462]	0.50	-0.99	2.52E-04	4.35E-02
ENSMUSG00000037720	67878	Tmem33	transmembrane protein 33 [Source:MGI Symbol;Acc:MGI:1915128]	0.50	-0.99	2.68E-04	4.51E-02
ENSMUSG00000034903	319876	Cobll1	Cobl-like 1 [Source:MGI Symbol;Acc:MGI:2442894]	0.35	-1.53	2.75E-04	4.51E-02
ENSMUSG00000023186	67776	Vwa5a	von Willebrand factor A domain containing 5A [Source:MGI Symbol;Acc:MGI:1915026]	0.46	-1.13	2.85E-04	4.51E-02
ENSMUSG00000060675	225845	Plaat3	phospholipase A and acyltransferase 3 [Source:MGI Symbol; Acc:MGI:2179715]	0.41	-1.29	2.85E-04	4.51E-02
ENSMUSG00000054321	72504	Taf4b	TATA-box binding protein associated factor 4b [Source:MGI Symbol;Acc:MGI:2152345]	1.81	0.86	2.86E-04	4.51E-02
ENSMUSG00000029650	71706	Slc46a3	solute carrier family 46, member 3 [Source:MGI Symbol;Acc:MGI:1918956]	0.26	-1.95	2.87E-04	4.51E-02
ENSMUSG00000016534	16784	Lamp2	lysosomal-associated membrane protein 2 [Source:MGI Symbol;Acc:MGI:96748]	0.59	-0.77	2.88E-04	4.51E-02
ENSMUSG00000031438	66889	Rnf128	ring finger protein 128 [Source:MGI Symbol;Acc:MGI:1914139]	0.45	-1.16	2.99E-04	4.63E-02
ENSMUSG00000024150	193813	Mcfd2	multiple coagulation factor deficiency 2 [Source:MGI Symbol; Acc:MGI:2183439]	0.60	-0.74	3.29E-04	4.93E-02
ENSMUSG00000012889	244550	Podnl1	podocan-like 1 [Source:MGI Symbol;Acc:MGI:2685352]	0.27	-1.88	3.31E-04	4.93E-02
ENSMUSG00000029338	71914	Antxr2	anthrax toxin receptor 2 [Source:MGI Symbol;Acc:MGI:1919164]	0.54	-0.90	3.31E-04	4.93E-02

Supplementary Table 2: List of the enriched GO Biological Process (BP) and Molecular Function (MF) terms following functional annotation analysis of the 80 DEGs at week 8 performed using DAVID (Huang et al., 2009).

Category	Term	P-Value	Benjamini
GOTERM_BP_DIRECT	estrous cycle	4.2E-03	1.0E+00
GOTERM_BP_DIRECT	localization	1.3E-02	1.0E+00
GOTERM_BP_DIRECT	neutrophil activation	3.2E-02	1.0E+00
GOTERM_BP_DIRECT	positive regulation of NIK/NF-kappaB signaling	3.3E-02	1.0E+00
GOTERM_BP_DIRECT	positive regulation of protein kinase C activity	4.5E-02	1.0E+00
GOTERM_BP_DIRECT	protein localization to plasma membrane	5.4E-02	1.0E+00
GOTERM_BP_DIRECT	ERAD pathway	6.5E-02	1.0E+00
GOTERM_BP_DIRECT	ubiquitin-dependent ERAD pathway	6.5E-02	1.0E+00
GOTERM_BP_DIRECT	regulation of protein stability	9.8E-02	1.0E+00
GOTERM_BP_DIRECT	positive regulation of apoptotic process	9.9E-02	1.0E+00
GOTERM_MF_DIRECT	lipid binding	2.4E-03	5.50E-01
GOTERM_MF_DIRECT	protease binding	1.9E-02	1.0E+00
GOTERM_MF_DIRECT	calcium-dependent protein binding	3.9E-02	1.0E+00
GOTERM_MF_DIRECT	hormone binding	5.0E-02	1.0E+00
GOTERM_MF_DIRECT	steroid hormone receptor activity	5.6E-02	1.0E+00
GOTERM_MF_DIRECT	protein heterodimerization activity	5.7E-02	1.0E+00

Supplementary Table 3: Revigo reduced list of downregulated GO-terms.

GO term annotation
Translation
Mitochonrial functions
Metabolism
Unfolded protein responses
Detoxification
Immune response
Inflammation
Ion regulation
Steroid Biosynthesis
Angiogenesis
Signalling
Autophagy
Cell-to-cell interaction

Name	3d	1wk	8wk	12wk	16wk
ribonucleoprotein complex biogenesis	-4.53	-5.53			
ribosome biogenesis	-4.50	-7.58			
rRNA metabolic process	-3.22	-4.56			
ncRNA metabolic process	-2.44	-3.11			
cytoplasmic translation	-1.68	-5.13			
mitochondrion organization		-6.75			
mitochondrial respiratory chain complex assembly		-4.93			
mitochondrial transport		-3.94			
cellular respiration		-3.40			
generation of precursor metabolites and energy		-3.35			
monocarboxylic acid metabolic process		-3.27			
establishment of protein localization to mitochondrion		-3.17			
NADH dehydrogenase complex assembly		-3.01			
sulfur compound metabolic process		-2.28			
mitochondrial transmembrane transport		-2.28			
protein folding		-1.87			
purine-containing compound metabolic process		-1.80			
energy coupled proton transport, down electrochemical gradient		-1.37			
cellular modified amino acid metabolic process		-1.37			
thioester metabolic process		-1.34			
polyamine biosynthetic process		-1.14			
cytochrome complex assembly		-1.05			
angiogenesis			-4.84	-5.01	-2.17
intracellular monoatomic cation homeostasis			-2.49		
lymphocyte mediated immunity			-2.39		

cell-cell adhesion			-2.37		
immune effector process			-2.33		
regulation of immune response			-2.33		
leukocyte migration			-2.33		
monoatomic ion transmembrane transport			-2.30	-1.98	
cellular glucuronidation			-2.27	-1.18	-1.34
positive regulation of cell adhesion			-2.17	-1.62	
organic hydroxy compound metabolic process			-2.08	-3.97	-1.62
regulation of cell adhesion			-2.08	-2.66	
lipid biosynthetic process			-2.01		
cytokine production			-1.78		
ubiquitin-dependent ERAD pathway			-1.73		
G protein-coupled receptor signaling pathway			-1.73	-1.12	
extracellular matrix organization			-1.68	-1.99	
external encapsulating structure organization			-1.68	-1.99	
regulation of cytokine production			-1.68		
positive regulation of MAPK cascade			-1.68		
hemostasis			-1.64		
extracellular structure organization			-1.61	-1.94	
antigen processing and presentation of peptide antigen			-1.59		
cell killing			-1.59		
regulation of angiogenesis			-1.59	-2.05	-1.89
cellular response to vascular endothelial growth factor stimulus			-1.50		-1.20
response to endoplasmic reticulum stress			-1.50		-1.07
leukocyte differentiation			-1.50		
response to unfolded protein			-1.50		

positive regulation of cytokine production			-1.45		
regulation of lipid metabolic process			-1.37	-1.32	
MAPK cascade			-1.36		
adenylate cyclase-activating G protein-coupled receptor signaling pathway			-1.35		
interleukin-1 production			-1.34		
type 2 immune response			-1.25		-1.08
inorganic anion transport			-1.22		
carbohydrate derivative catabolic process			-1.16		
inflammatory response			-1.08		
autophagy			-1.08		-1.06
process utilizing autophagic mechanism			-1.08		-1.06
response to growth factor			-1.08		
transmembrane receptor protein tyrosine kinase signaling pathway			-1.06		
toll-like receptor 9 signaling pathway			-1.05		
regulation of steroid biosynthetic process			-1.03		
regulation of monoatomic ion transport			-1.02	-1.19	
xenobiotic metabolic process			-1.01		
carbohydrate derivative transport			-1.01		
regulation of type 2 immune response			-1.00		
steroid metabolic process				-3.50	-1.20
cellular response to growth factor stimulus				-2.71	
cell-substrate adhesion				-2.17	
cell junction assembly				-2.17	
inorganic ion homeostasis				-1.99	
positive regulation of phosphatidylinositol 3-kinase signaling				-1.90	
response to xenobiotic stimulus				-1.76	

regulation of vascular permeability				-1.28	
circulatory system process				-1.23	
regulation of phosphatidylinositol 3-kinase signaling				-1.20	
regulation of cellular response to growth factor stimulus				-1.20	
regulation of hormone levels				-1.20	
chemotaxis				-1.15	
cell junction organization				-1.13	
phosphatidylinositol-mediated signaling				-1.12	
inositol lipid-mediated signaling				-1.10	
response to metal ion				-1.03	
vascular endothelial growth factor receptor signaling pathway				-1.02	
antigen processing and presentation of peptide antigen via MHC class I					-1.20
positive regulation of autophagy					-1.08
endothelial cell migration					-1.07
regulation of mononuclear cell migration					-1.07

Supplementary Table 4: Revigo reduced list of upregulated GO-terms.

Upregulated GO terms
ER functions
Chromosome organization and DNA replication
DNA repair
Post-translational modification
mRNA processing
RNA and DNA metabolic processes
Cell differentiaiton (-)
Cell signalling
Biogenesis

Name	3d	1wk	8wk	12wk	16wk
endoplasmic reticulum membrane	-1.78				
endoplasmic reticulum subcompartment	-1.78				
nuclear outer membrane-endoplasmic reticulum membrane network	-1.78				
cell surface receptor signaling pathway involved in cell-cell signaling		-1.02			
positive regulation of protein phosphorylation		-1.05			
negative regulation of cell differentiation		-1.42			
small GTPase mediated signal transduction		-1.42			
kinetochore organization		-1.48			
regulation of chromosome organization		-1.66	-4.15	-2.85	-2.98
regulation of small GTPase mediated signal transduction		-1.87			
DNA replication		-2.61			

organelle fission		-2.94	-4.15	-1.71	-1.31
negative regulation of cell cycle		-3.05			
microtubule cytoskeleton organization		-3.14	-8.24	-3.54	-1.24
cell division		-3.38	-5.44	-3.10	-2.17
nuclear division		-3.38			
chromosome segregation		-4.34	-8.24	-6.03	-5.37
regulation of chromatin organization			-1.01		
protein acetylation			-1.03		
RNA catabolic process			-1.04		
chromosome localization			-1.10		
regulation of RNA splicing			-1.14	-2.78	
post-transcriptional regulation of gene expression			-1.29		
regulation of mRNA processing			-1.30		
protein alkylation			-1.48		
regulation of DNA repair			-1.62		
chromosome organization			-1.91		
regulation of DNA metabolic process			-1.97		
methylation			-1.98	-1.21	
ribosome biogenesis			-2.10	-6.28	-1.94
protein localization to chromosome			-2.18		-1.15
regulation of mRNA metabolic process			-2.32	-1.43	
macromolecule methylation			-2.33	-1.52	
mitotic sister chromatid separation			-2.46		-1.29
peptidyl-lysine modification			-2.48		

ncRNA metabolic process			-2.85	-6.28	-1.54
non-membrane-bounded organelle assembly			-3.03	-2.98	
regulation of cell cycle process			-3.09	-2.49	
histone modification			-3.34	-1.43	
ribonucleoprotein complex biogenesis			-3.55	-7.06	-3.00
mRNA processing			-4.18	-6.28	
chromatin organization			-4.37		-2.55
DNA repair			-9.14	-2.54	-4.98
nucleocytoplasmic transport				-1.11	
regulation of mRNA splicing, via spliceosome				-2.19	
protein-RNA complex organization				-2.72	
regulation of chromosome organization				-2.85	-2.98
RNA splicing					-3.33

8. References

AIZAWA, R., IBAYASHI, M., HATAKEYAMA, T., TATSUMI, T. & TSUKAMOTO, S. 2021. Impact of short-term high-fat feeding on lipid droplet content in mouse oocytes. *J Reprod Dev,* 67**,** 73-77.

AKAMINE, E. H., MARCAL, A. C., CAMPOREZ, J. P., HOSHIDA, M. S., CAPERUTO, L. C., BEVILACQUA, E. & CARVALHO, C. R. 2010. Obesity induced by high-fat diet promotes insulin resistance in the ovary. *J Endocrinol,* 206**,** 65-74.

ASSIDI, M., DIELEMAN, S. J. & SIRARD, M. A. 2010. Cumulus cell gene expression following the LH surge in bovine preovulatory follicles: potential early markers of oocyte competence. *Reproduction,* 140**,** 835-52.

BLÜHER, M. 2019. Obesity: global epidemiology and pathogenesis. *Nature Reviews Endocrinology,* 15**,** 288-298.

BUNEL, A., NIVET, A. L., BLONDIN, P., VIGNEAULT, C., RICHARD, F. J. & SIRARD, M. A. 2014. Cumulus cell gene expression associated with pre-ovulatory acquisition of developmental competence in bovine oocytes. *Reprod Fertil Dev,* 26**,** 855-65.

CAMPBELL, D. I., FERREIRA, C. R., EBERLIN, L. S. & COOKS, R. G. 2012. Improved spatial resolution in the imaging of biological tissue using desorption electrospray ionization. *Anal Bioanal Chem,* 404**,** 389-98.

CUEVAS, A., MIQUEL, J. F., REYES, M. S., ZANLUNGO, S. & NERVI, F. 2004. Diet as a risk factor for cholesterol gallstone disease. *J Am Coll Nutr,* 23**,** 187-96.

DAG, Z. O. & DILBAZ, B. 2015. Impact of obesity on infertility in women. *J Turk Ger Gynecol Assoc,* 16**,** 111-7.

DIAS, M. D. E., DOS REIS, S. A., DA CONCEIÇAO, L. L., SEDIYAMA, C. M. N. D., PEREIRA, S. S., DE OLIVEIRA, L. L., PELUZIO, M. D. G., MARTINEZ, J. A. & MILAGRO, F. I. 2021. Diet-induced obesity in animal models: points to consider and influence on metabolic markers. *Diabetology & Metabolic Syndrome,* 13.

DUMESIC, D. A., MELDRUM, D. R., KATZ-JAFFE, M. G., KRISHER, R. L. & SCHOOLCRAFT, W. B. 2015. Oocyte environment: follicular fluid and cumulus cells are critical for oocyte health. *Fertility and Sterility,* 103**,** 303-316.

ENGIN, A. 2017a. The Definition and Prevalence of Obesity and Metabolic Syndrome. *Adv Exp Med Biol,* 960**,** 1-17.

ENGIN, A. B. 2017b. Obesity and Lipotoxocity. *In:* ENGIN, A. (ed.). New York, NY, USA: Springer.

FEDORCSAK, P., DALE, P. O., STORENG, R., ERTZEID, G., BJERCKE, S., OLDEREID, N., OMLAND, A. K., ABYHOLM, T. & TANBO, T. 2004. Impact of overweight and underweight on assisted reproduction treatment. *Hum Reprod,* 19**,** 2523-8.

FERNANDEZ-SANCHEZ, A., MADRIGAL-SANTILLAN, E., BAUTISTA, M., ESQUIVEL-SOTO, J., MORALES-GONZALEZ, A., ESQUIVEL-CHIRINO, C., DURANTE-MONTIEL, I., SANCHEZ-RIVERA, G., VALADEZ-VEGA, C. & MORALES-GONZALEZ, J. A. 2011. Inflammation, oxidative stress, and obesity. *Int J Mol Sci,* 12**,** 3117-32.

GAO, J., LIU, M., LIU, J., SHI, P., CUI, H., ZHAO, S., ZHANG, X. & TAO, C. 2023. Effect of high-fat diet on the lipid profile of ovarian granulosa cells and female reproduction in mice. *PLoS One,* 18**,** e0287534.

GONZALEZ, M. B., LANE, M., KNIGHT, E. J. & ROBKER, R. L. 2018. Inflammatory markers in human follicular fluid correlate with lipid levels and Body Mass Index. *J Reprod Immunol,* 130**,** 25-29.

GONZALEZ, M. B., ROBKER, R. L. & ROSE, R. D. 2022. Obesity and oocyte quality: significant implications for ART and emerging mechanistic insights. *Biol Reprod,* 106**,** 338-350.

HALEY, M. J., KRISHNAN, S., BURROWS, D., DE HOOG, L., THAKRAR, J., SCHIESSL, I., ALLAN, S. M. & LAWRENCE, C. B. 2019. Acute high-fat feeding leads to disruptions in glucose homeostasis and worsens stroke outcome. *Journal of Cerebral Blood Flow and Metabolism,* 39**,** 1026-1037.

HERDT, T. H. 2013. Metabolic diseases of dairy cattle. *Vet Clin North Am Food Anim Pract,* 29**,** xi-xii.

HETZ, C. 2012. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol,* 13**,** 89-102.

Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. Nature Protoc. 2009;4(1):44-57.

IGOSHEVA, N., ABRAMOV, A. Y., POSTON, L., ECKERT, J. J., FLEMING, T. P., DUCHEN, M. R. & MCCONNELL, J. 2010. Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. *PLoS One,* 5**,** e10074.

JARC, E. & PETAN, T. 2019. Lipid Droplets and the Management of Cellular Stress. *Yale Journal of Biology and Medicine,* 92**,** 435-452.

JUNGHEIM, E. S. & MOLEY, K. H. 2010. Current knowledge of obesity's effects in the pre- and periconceptional periods and avenues for future research. *Am J Obstet Gynecol,* 203**,** 525-30.

KATO, N., HAYASAKA, T., TAKEDA, J., OSAKABE, M. & KURACHI, H. 2013. Ovarian tumors with functioning stroma: a clinicopathologic study with special reference to serum estrogen level, stromal morphology, and aromatase expression. *Int J Gynecol Pathol,* 32**,** 556-61.

KLENOV, V. E. & JUNGHEIM, E. S. 2014. Obesity and reproductive function: a review of the evidence. *Curr Opin Obstet Gynecol,* 26**,** 455-60.

KRISHNA, K. B., STEFANOVIC-RACIC, M., DEDOUSIS, N., SIPULA, I. & O'DOHERTY, R. M. 2016. Similar degrees of obesity induced by diet or aging cause strikingly different immunologic and metabolic outcomes. *Physiol Rep,* 4.

LEROY, J. L., VALCKX, S. D., JORDAENS, L., DE BIE, J., DESMET, K. L., VAN HOECK, V., BRITT, J. H., MAREI, W. F. & BOLS, P. E. 2015. Nutrition and maternal metabolic health in relation to oocyte and embryo quality: critical views on what we learned from the dairy cow model. *Reprod Fertil Dev,* 27**,** 693-703.

LEROY, J. L., VAN SOOM, A., OPSOMER, G., GOOVAERTS, I. G. & BOLS, P. E. 2008. Reduced fertility in high-yielding dairy cows: are the oocyte and embryo in

danger? Part II. Mechanisms linking nutrition and reduced oocyte and embryo quality in high-yielding dairy cows. *Reprod Domest Anim,* 43**,** 623-32.

LEROY, J. L., VANHOLDER, T., MATEUSEN, B., CHRISTOPHE, A., OPSOMER, G., DE KRUIF, A., GENICOT, G. & VAN SOOM, A. 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. *Reproduction,* 130**,** 485-95.

LEROY, J. L. M. R., MEULDERS, B., MOORKENS, K., XHONNEUX, SLOOTMANS, J., DE KEERSMAEKER, L., SMITS, A., PASCOTTINI, O. B. & MAREI, W. F. A. 2023. Maternal metabolic health and fertility: we should not only care about but also for the oocyte! *Reproduction Fertility and Development,* 35**,** 1-18.

LI, J., WANG, S., WANG, B., WEI, H., LIU, X., HAO, J., DUAN, Y., HUA, J., ZHENG, X., FENG, X. & YAN, X. 2018. High-fat-diet impaired mitochondrial function of cumulus cells but improved the efficiency of parthenogenetic embryonic quality in mice. *Anim Cells Syst (Seoul),* 22**,** 243-252.

LIISBERG, U., FAUSKE, K. R., KUDA, O., FJAERE, E., MYRMEL, L. S., NORBERG, N., FROYLAND, L., GRAFF, I. E., LIASET, B., KRISTIANSEN, K., KOPECKY, J. & MADSEN, L. 2016. Intake of a Western diet containing cod instead of pork alters fatty acid composition in tissue phospholipids and attenuates obesity and hepatic lipid accumulation in mice. *J Nutr Biochem,* 33**,** 119-27.

LIN, J., YANG, R., TARR, P. T., WU, P. H., HANDSCHIN, C., LI, S., YANG, W., PEI, L., ULDRY, M., TONTONOZ, P., NEWGARD, C. B. & SPIEGELMAN, B. M. 2005. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. *Cell,* 120**,** 261-73.

LUZZO, K. M., WANG, Q., PURCELL, S. H., CHI, M., JIMENEZ, P. T., GRINDLER, N., SCHEDL, T. & MOLEY, K. H. 2012. High fat diet induced developmental defects in the mouse: oocyte meiotic aneuploidy and fetal growth retardation/brain defects. *PLoS One,* 7**,** e49217.

MAREI, W. F. A., ALVAREZ, M. A., VAN HOECK, V., GUTIERREZ-ADAN, A., BOLS, P. E. J. & LEROY, J. 2017. Effect of nutritionally induced hyperlipidaemia on in vitro bovine embryo quality depends on the type of major fatty acid in the diet. *Reprod Fertil Dev,* 29**,** 1856-1867.

MAREI, W. F. A., SMITS, A., MOHEY-ELSAEED, O., PINTELON, I., GINNEBERGE, D., BOLS, P. E. J., MOERLOOSE, K. & LEROY, J. 2020. Differential effects of high fat diet-induced obesity on oocyte mitochondrial functions in inbred and outbred mice. *Sci Rep,* 10**,** 9806.

MATOBA, S., BENDER, K., FAHEY, A. G., MAMO, S., BRENNAN, L., LONERGAN, P. & FAIR, T. 2014. Predictive value of bovine follicular components as markers of oocyte developmental potential. *Reprod Fertil Dev,* 26**,** 337-45.

MOORKENS, K., LEROY, J., QUANICO, J., BAGGERMAN, G. & MAREI, W. F. A. 2023. How the Oviduct Lipidomic Profile Changes over Time after the Start of an Obesogenic Diet in an Outbred Mouse Model. *Biology (Basel),* 12.

MOORKENS, K., LEROY, J., VERHEYEN, S. & MAREI, W. F. A. 2022. Effects of an obesogenic diet on the oviduct depend on the duration of feeding. *PLoS One,* 17**,** e0275379.

NESTEL, P. J., SCHREIBMAN, P. H. & AHRENS, E. H., JR. 1973. Cholesterol metabolism in human obesity. *J Clin Invest,* 52**,** 2389-97.

PAKIET, A., JAKUBIAK, A., MIERZEJEWSKA, P., ZWARA, A., LIAKH, I., SLEDZINSKI, T. & MIKA, A. 2020. The Effect of a High-Fat Diet on the Fatty Acid Composition in the Hearts of Mice. *Nutrients,* 12.

PANCHAL, S. K. & BROWN, L. 2011. Rodent models for metabolic syndrome research. *J Biomed Biotechnol,* 2011**,** 351982.

PANDEY, S., PANDEY, S., MAHESHWARI, A. & BHATTACHARYA, S. 2010. The impact of female obesity on the outcome of fertility treatment. *J Hum Reprod Sci,* 3**,** 62-7.

PERCIE DU SERT, N., AHLUWALIA, A., ALAM, S., AVEY, M. T., BAKER, M., BROWNE, W. J., CLARK, A., CUTHILL, I. C., DIRNAGL, U., EMERSON, M., GARNER, P., HOLGATE, S. T., HOWELLS, D. W., HURST, V., KARP, N. A., LAZIC, S. E., LIDSTER, K., MACCALLUM, C. J., MACLEOD, M., PEARL, E. J., PETERSEN, O. H., RAWLE, F., REYNOLDS, P., ROONEY, K., SENA, E. S., SILBERBERG, S. D., STECKLER, T. & WURBEL, H. 2020. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol,* 18**,** e3000411.

REVELLI, A., DELLE PIANE, L., CASANO, S., MOLINARI, E., MASSOBRIO, M. & RINAUDO, P. 2009. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics. *Reprod Biol Endocrinol,* 7**,** 40.

REYNOLDS, K. A., BOUDOURES, A. L., CHI, M. M., WANG, Q. & MOLEY, K. H. 2015. Adverse effects of obesity and/or high-fat diet on oocyte quality and metabolism are not reversible with resumption of regular diet in mice. *Reprod Fertil Dev,* 27**,** 716-24.

ROBKER, R. L. 2008. Evidence that obesity alters the quality of oocytes and embryos. *Pathophysiology,* 15**,** 115-21.

SAKLAYEN, M. G. 2018. The Global Epidemic of the Metabolic Syndrome. *Curr Hypertens Rep,* 20**,** 12.

SAMUELSSON, A. M., MATTHEWS, P. A., ARGENTON, M., CHRISTIE, M. R., MCCONNELL, J. M., JANSEN, E. H., PIERSMA, A. H., OZANNE, S. E., TWINN, D. F., REMACLE, C., ROWLERSON, A., POSTON, L. & TAYLOR, P. D. 2008. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. *Hypertension,* 51**,** 383-92.

SILVESTRIS, E., DE PERGOLA, G., ROSANIA, R. & LOVERRO, G. 2018. Obesity as disruptor of the female fertility. *Reprod Biol Endocrinol,* 16**,** 22.

SKAZNIK-WIKIEL, M. E., SWINDLE, D. C., ALLSHOUSE, A. A., POLOTSKY, A. J. & MCMANAMAN, J. L. 2016. High-Fat Diet Causes Subfertility and Compromised Ovarian Function Independent of Obesity in Mice. *Biol Reprod,* 94**,** 108.

SMITS, A., MAREI, W. F. A., DE NEUBOURG, D. & LEROY, J. 2021. Diet normalization or caloric restriction as a preconception care strategy to improve metabolic health and oocyte quality in obese outbred mice. *Reprod Biol Endocrinol,* 19**,** 166.

SMITS, A., MAREI, W. F. A., MOORKENS, K., BOLS, P. E. J., DE NEUBOURG, D. & LEROY, J. 2022. Obese outbred mice only partially benefit from diet normalization or calorie restriction as preconception care interventions to improve metabolic health and oocyte quality. *Hum Reprod,* 37**,** 2867-2884.

SPOONER, H. C., DERRICK, S. A., MAJ, M., MANJARIN, R., HERNANDEZ, G. V., TAILOR, D. S., BASTANI, P. S., FANTER, R. K., FIOROTTO, M. L., BURRIN, D. G., LA FRANO, M. R., SIKALIDIS, A. K. & BLANK, J. M. 2021. High-Fructose, High-Fat Diet Alters Muscle Composition and Fuel Utilization in a Juvenile Iberian Pig Model of Non-Alcoholic Fatty Liver Disease. *Nutrients,* 13.

STANHOPE, K. L. 2016. Sugar consumption, metabolic disease and obesity: The state of the controversy. *Crit Rev Clin Lab Sci,* 53**,** 52-67.

SUPEK, F., BOSNJAK, M., SKUNCA, N. & SMUC, T. 2011. REVIGO summarizes and visualizes long lists of gene ontology terms. *PLoS One,* 6**,** e21800.

SZENDROEDI, J. & RODEN, M. 2009. Ectopic lipids and organ function. *Curr Opin Lipidol,* 20**,** 50-6.

TAPPY, L. & LE, K. A. 2010. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev,* 90**,** 23-46.

TASKINEN, M. R., PACKARD, C. J. & BOREN, J. 2019. Dietary Fructose and the Metabolic Syndrome. *Nutrients,* 11.

TATSUMI, T., TAKAYAMA, K., ISHII, S., YAMAMOTO, A., HARA, T., MINAMI, N., MIYASAKA, N., KUBOTA, T., MATSUURA, A., ITAKURA, E. & TSUKAMOTO, S. 2018. Forced lipophagy reveals that lipid droplets are required for early embryonic development in mouse. *Development,* 145.

TREMELLEN, K., PEARCE, K. & ZANDER-FOX, D. 2017. Increased miscarriage of euploid pregnancies in obese women undergoing cryopreserved embryo transfer. *Reprod Biomed Online,* 34**,** 90-97.

TURATHUM, B., GAO, E. M. & CHIAN, R. C. 2021. The Function of Cumulus Cells in Oocyte Growth and Maturation and in Subsequent Ovulation and Fertilization. *Cells,* 10.

UZBEKOVA, S., ELIS, S., TEIXEIRA-GOMES, A. P., DESMARCHAIS, A., MAILLARD, V. & LABAS, V. 2015. MALDI Mass Spectrometry Imaging of Lipids and Gene Expression Reveals Differences in Fatty Acid Metabolism between Follicular Compartments in Porcine Ovaries. *Biology (Basel),* 4**,** 216-36.

VALCKX, S. D., ARIAS-ALVAREZ, M., DE PAUW, I., FIEVEZ, V., VLAEMINCK, B., FRANSEN, E., BOLS, P. E. & LEROY, J. L. 2014a. Fatty acid composition of the follicular fluid of normal weight, overweight and obese women undergoing assisted reproductive treatment: a descriptive cross-sectional study. *Reprod Biol Endocrinol,* 12**,** 13.

VALCKX, S. D., DE PAUW, I., DE NEUBOURG, D., INION, I., BERTH, M., FRANSEN, E., BOLS, P. E. & LEROY, J. L. 2012. BMI-related metabolic composition of the follicular fluid of women undergoing assisted reproductive treatment and the consequences for oocyte and embryo quality. *Hum Reprod,* 27**,** 3531-9.

VALCKX, S. D. M., VAN HOECK, V., ARIAS-ALVAREZ, M., MAILLO, V., LOPEZ-CARDONA, A. P., GUTIERREZ-ADAN, A., BERTH, M., CORTVRINDT, R., BOLS, P. E. J. & LEROY, J. L. M. R. 2014b. Elevated non-esterified fatty acid concentrations during in vitro murine follicle growth alter follicular physiology and reduce oocyte developmental competence. *Fertility and Sterility,* 102**,** 1769-U607.

VALSESIA, A., SARIS, W. H. M., ASTRUP, A., HAGER, J. & MASOODI, M. 2016. Distinct lipid profiles predict improved glycemic control in obese, nondiabetic patients after a low-caloric diet intervention: the Diet, Obesity and Genes randomized trial. *American Journal of Clinical Nutrition,* 104**,** 566-575.

VAN HOECK, V., LEROY, J. L., ARIAS ALVAREZ, M., RIZOS, D., GUTIERREZ-ADAN, A., SCHNORBUSCH, K., BOLS, P. E., LEESE, H. J. & STURMEY, R. G. 2013. Oocyte developmental failure in response to elevated nonesterified fatty acid concentrations: mechanistic insights. *Reproduction,* 145**,** 33-44.

VAN HOECK, V., RIZOS, D., GUTIERREZ-ADAN, A., PINTELON, I., JORSSEN, E., DUFORT, I., SIRARD, M. A., VERLAET, A., HERMANS, N., BOLS, P. E. & LEROY, J. L. 2015. Interaction between differential gene expression profile and phenotype in bovine blastocysts originating from oocytes exposed to elevated non-esterified fatty acid concentrations. *Reprod Fertil Dev,* 27**,** 372-84.

WARZYCH, E., CIESLAK, A., MADEJA, Z. E., PAWLAK, P., WOLC, A. & LECHNIAK, D. 2014. Multifactorial analysis of the follicular environment is predictive of oocyte morphology in cattle. *J Reprod Dev,* 60**,** 1-8.

WHITTEN, W. K. 1956. Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *J Endocrinol,* 13**,** 399-404.

WHO, W. H. O. 2016. Global Status Report on Noncommunicable Diseases. Geneva: WHO IRIS.

WILLIAMS, L. M., CAMPBELL, F. M., DREW, J. E., KOCH, C., HOGGARD, N., REES, W. D., KAMOLRAT, T., THI NGO, H., STEFFENSEN, I. L., GRAY, S. R. & TUPS, A. 2014. The development of diet-induced obesity and glucose intolerance in C57BL/6 mice on a high-fat diet consists of distinct phases. *PLoS One,* 9**,** e106159.

WINSHIP, A. L., STRINGER, J. M., LIEW, S. H. & HUTT, K. J. 2018. The importance of DNA repair for maintaining oocyte quality in response to anti-cancer treatments, environmental toxins and maternal ageing. *Human Reproduction Update,* 24**,** 119-134.

WITTEMER, C., OHL, J., BAILLY, M., BETTAHAR-LEBUGLE, K. & NISAND, I. 2000. Does body mass index of infertile women have an impact on IVF procedure and outcome? *J Assist Reprod Genet,* 17**,** 547-52.

WU, L. L., DUNNING, K. R., YANG, X., RUSSELL, D. L., LANE, M., NORMAN, R. J. & ROBKER, R. L. 2010. High-fat diet causes lipotoxicity responses in cumulus-oocyte complexes and decreased fertilization rates. *Endocrinology,* 151**,** 5438-45.

WU, L. L., NORMAN, R. J. & ROBKER, R. L. 2011. The impact of obesity on oocytes: evidence for lipotoxicity mechanisms. *Reprod Fertil Dev,* 24**,** 29-34.

YANG, X., WU, L. L., CHURA, L. R., LIANG, X., LANE, M., NORMAN, R. J. & ROBKER, R. L. 2012. Exposure to lipid-rich follicular fluid is associated with endoplasmic reticulum stress and impaired oocyte maturation in cumulus-oocyte complexes. *Fertil Steril,* 97**,** 1438-43.

ZHANG, D., ZHANG, X., ZENG, M., YUAN, J., LIU, M., YIN, Y., WU, X., KEEFE, D. L. & LIU, L. 2015. Increased DNA damage and repair deficiency in granulosa cells are associated with ovarian aging in rhesus monkey. *J Assist Reprod Genet,* 32**,** 1069-78.

CHAPTER 6:
GENERAL DISCUSSION AND CONCLUSIONS

General discussion and conclusions

A summary of the aims and obtained results

The significant impact of metabolic disorders, such as those associated with obesity, on fertility has been demonstrated in various studies (Gesink Law et al., 2007; Wise et al., 2010; Loy et al., 2018; Silvestris et al., 2018). Metabolic disorders are linked with an altered maternal metabolic status which may be reflected in the follicular (Leroy et al., 2005; Robker et al., 2009; Valckx et al., 2012; Valckx et al., 2014a) as well as the oviductal microenvironment (Leese, 1988; Jordaens et al., 2017a). The specific effects of an obesogenic diet on oocytes such as mitochondrial dysfunction, oxidative and endoplasmic reticulum stress and higher fat contents (Igosheva et al., 2010; Wu et al., 2010; Marei et al., 2020), demonstrate the extreme vulnerability of the oocyte and pre-implantation embryo to potential metabolic stressors, ultimately leading to disappointing fertility results. How the oviduct is involved in the link between an obesogenic environment and the potential impact on early embryo development is not known. Furthermore, the timing at which the detrimental effects are detectable after the start of consuming an obesogenic diet, which is typically high in saturated fat and refined sugars (Malesza et al., 2021), has not been investigated yet, however, it may aid in discriminating effects caused by short-term feeding of an obesogenic diet and the impact of the specific dietary components and/or by long-term feeding of the diet and the development of an obese phenotype and the associated disturbed metabolic health. Furthermore, we do not know whether the impact of an obesogenic environment is gradual over time or whether we may expect signs of adaptation. Therefore, the following general hypothesis in the present thesis was formulated:

Short and long-term feeding of a high-fat/high-sugar (HF/HS) diet leads to acute and chronic changes in the micro-environment of the newly formed embryo (i.e. the oviduct) and in the ovary and ovarian cells of mice. For this we investigated whether the mechanisms by which peripheral tissues and cells are affected during the

development of metabolic disorders are also evident at the level of the ovary and oviduct. We especially focused on local changes in oxidative and endoplasmic reticulum (ER) stress levels in oviductal epithelial cells (OECs), inflammatory responses in OECs and serum, mitochondrial stress levels in oocytes, cellular pathways in granulosa cells (GCs) and the lipid content of oocytes and ovarian tissue as well as changes in the lipid profile of serum and the oviductal epithelium.

A multitude of oviductal interventions contributing to reproductive success (Holt and Fazeli, 2016a; Holt and Fazeli, 2016b) demonstrate the importance of this finely regulated oviductal micro-environment for the early embryo. Furthermore, OECs can recognize an excessive increase in saturated fatty acids, as was demonstrated by the production of pro-inflammatory cytokine IL-8, mitochondrial dysfunction and autophagy in bovine OECs in response to *in vitro* exposure to PA (Ohtsu et al., 2017). In addition, in an *in vitro* setting, high FFA concentrations resulted in altered bovine oviductal cell integrity and proliferation as well as sperm binding capacity (Jordaens et al., 2015). Moreover, Jordaens et al. (2020) reported direct embryo toxicity and reduced oviductal ability to support and protect early embryo development after *in vitro* exposure of bovine oviductal cells to elevated NEFA concentrations. Diet-induced hyperlipidemia is known to induce lipotoxicity in non-adipose tissues, which leads to mitochondrial dysfunction, altered cellular metabolism and increased OS levels (Engin and Engin, 2017, Lin et al., 2005). However, such pathophysiological changes in response to consumption of an obesogenic diet at the level of the oviduct *in vivo* have not been reported yet. In addition, whether the oviduct may already acutely be impacted by eating an obesogenic diet and/or on the long-term by the development of the obese phenotype, remained to be investigated. Furthermore, the sensitivity of mice to high-fat (HF) diet induced obesity and the impact on their metabolic profile and oocyte quality is influenced by the mitochondrial and/or genetic background of the mouse model (Marei et al., 2020), indicating that the outcome of diet-induced obesity

(DIO) studies can be significantly different, depending on the strain used. The first aim of this thesis was to obtain more insights in the acute and chronic effects due to an obesogenic diet, specifically related to oxidative and cellular stress and inflammatory responses, in the OECs *in vivo* and the nature and magnitude of these changes over time after different periods of feeding the obesogenic diet to outbred Swiss mice and inbred C57BL/6N mice **(Chapter 3)**. Whether the transcriptomic and time dependent impact that was observed in the first experiment was linked to alterations in the lipidomic profile of the oviduct, remained however unknown. In addition, the lipid profile of ovarian follicular fluid (FF), follicular cells and endometrial epithelial cells in cows and sheep could be influenced by alterations in the dietary fat content and in the ratio between saturated and unsaturated fatty acids (FAs) (Childs et al., 2008; Wonnacott et al., 2010; Zachut et al., 2011). Such changes in the oviduct in response to an obesogenic diet were not illustrated yet in human or relevant animal models. For the second aim of this PhD thesis, we therefore investigated whether feeding an obesogenic diet in outbred Swiss mice alters the lipidomic profile of the oviductal epithelium (OE) and we studied the development of these changes over time while the mice proceeded to consume the obesogenic diet and developed an obese phenotype **(Chapter 4)**. Feeding a HF diet for a long-term period ranging from four to 13 weeks (w) increased the lipid content of oocytes and the lipid profile of GCs in mice (Wu et al., 2010; Reynolds et al., 2015; Marei et al., 2020; Gao et al., 2023). The long-term exposure to the diet as used in these studies makes it however difficult to discriminate between effects caused by long-term feeding and the development of obesity and its associated metabolic alterations or acute effects elicited after short-term feeding of an obesogenic diet. In addition, GCs are known to selectively accumulate saturated fatty acids from the circulation and FF, which may result in different dynamics in response compared to the oocytes (Wonnacott et al., 2010; Zachut et al., 2011). Furthermore, the tissue specific effects within the ovary caused by an obesogenic diet and obesity were not reported yet. Therefore, the third aim investigated in this PhD research was to

examine acute and/or long-term changes in lipid droplet volume and mitochondrial abnormalities in oocytes, neutral lipid content in the ovaries, and transcriptomic changes in the GCs of Swiss mice fed a HF/HS diet for different time periods and describe the development of these changes over time **(Chapter 5)**.

The mouse models used in this PhD research both demonstrated increased adiposity and hypercholesterolemia, which indicates that the mice are sensitive to the HF/HS diet and validates the induction of the required metabolic alterations associated with the development of obesity over time.

With our findings we are the first to show the impact of an obesogenic diet on OECs and to demonstrate the effect of metabolic stress on the oviductal microenvironment *in vivo*. We were able to demonstrate that feeding a HF/HS diet led to a series of transcriptomic changes related to oxidative stress (OS) in the OECs of mice as early as 3 days (d) after the start of the obesogenic diet, thus before the development of the obese phenotype. The acute increase in OS was followed by a cascade of transcriptomic changes related to mitochondrial ROS production and ER stress, and ultimately transcriptomic changes showing a local inflammatory response during long-term feeding of 12w to 16w. The transcriptomic changes were found to be dependent on the genetic background of the mouse model in terms of magnitude and timing **(Chapter 3)**. Next to the transcriptomic profile, the lipidomic profile of the OE also shows strong evidence of the sensitivity of the oviduct to systemic metabolic changes. Already after 3d of HF/HS feeding, a distinct lipidomic profile of the OE was observed compared with the controls, which was demonstrated by increased average peak intensity of all detected signals in the spectra from the OE and by the detection of discriminative masses (DMs) by the ROC analysis of the ion signals. The early lipidomic changes appeared to be variable and reversible but over time, when the mice continued consuming the HF/HS diet, the non-specific overall change in lipid profile of OE progressed to a persistent, more specific and irreversible change of the lipid profile in

the HF/HS OE. Between 1w and 12w of HF/HS feeding the differences in lipid profile were stable. Eventually, after chronic HF/HS exposure of 16w both detected DMs and the average peak intensity were relatively high. The observed differentially abundant lipids are involved in plasma membrane functions, cell signaling, mitochondrial functions and cellular homeostasis **(Chapter 4)**. After feeding a HF/HS diet in Swiss mice for only 24 hours (h), their oocytes already exhibited an acute increase in lipid droplet volume. ER and mitochondrial related pathways appeared to be impacted after as early as 3d and 1w of HF/HS feeding. The effects of the obesogenic diet consumption progressively increased over time, showing increased mitochondrial abnormalities in the oocytes from 8w onwards and different affected pathways, linked to DNA repair, cell signalling, UPRs and many others in the GCs of the mice due to feeding the obesogenic diet and the development of the obese phenotype. The absence of detectable overall changes in lipid content in the ovarian tissue, indicated that the changes observed in the oocytes and GCs were cell specific **(Chapter 5)**. An overview of the obtained results is shown in Figure 1.

The data generated in the three experiments of this PhD research are based on the same set of Swiss mice. This makes it possible to link the observed effects of each experiment.

The changes that were observed in the OECs, OE, GCs and oocytes demonstrated to be dependent on the duration of exposure to the HF/HS diet and they showed a similar overall pattern over time. We demonstrated very acute changes after short-term feeding, even as short as 24h, which appeared to remain stable and then progressively increase after long-term feeding of the HF/HS diet. As such, we were able to discriminate the direct effects due to the consumption of the obesogenic diet from the effects due to the metabolic stress associated with obesity and a potential adaptive response over time.

The transcriptomic changes observed in the OECs illustrated that the sequence of pathophysiological changes that are known to occur at the cellular level in response to diet-induced lipotoxicity are also evident in the oviductal cells, as was demonstrated by the altered gene expression in genes related to (mitochondrial) OS, ER stress and UPRs in the OECs of the HF/HS fed mice. Moreover, the time dependent transcriptomic changes can be linked with the observed lipidomic changes in the OE as well as changes in the ovarian cells. Feeding an obesogenic diet in mice for only 3d to 1w already elicits an acute (mitochondrial) OS and ER stress response in both the OECs and GCs of the mice. The acute altered expression of genes related to ER stress in the OECs can be linked to the observations in the lipid profile of the OE, as the ER is the main organelle for lipid biosynthesis producing the majority of structural phospholipids. The latter comprised almost 50% of the diet-induced lipidomic changes in the OE. Furthermore, the detected DMs in OE were more abundant in the CTRL OE, suggesting that the de novo synthesis of many lipids is hampered in the OE of the HF/HS group. This was evident from as early as 3d up until 16w of feeding. Thus, the distinct lipid profile of the HF/HS OE, potentially due to hampered de novo lipid synthesis, may be linked to the acute ER stress response that was observed in the OECs. Furthermore, prolonged HF/HS diet feeding of the mice resulted in a cascade of transcriptomic changes in the OECs to control mitochondrial ROS production and ER stress. Both the transcriptomic as well as the lipidomic changes in the oviductal epithelium showed a stabilized response between 1w and 12w of feeding, which may indicate an adaptive response of the cells and tissue to the diet-induced hyperlipidaemia. However, chronic HF/HS feeding of 16w ultimately resulted in increased (mitochondrial) OS and ER stress responses in the OECs, as well as persistent changes in different lipids in the OE. The OE showed altered expression of lipids that were assigned to SM at all time points. This may also be linked to the ER stress that was elicited by short and long-term feeding of the HF/HS diet as the backbone of SM is formed by CER which is produced by the ER. Furthermore, the altered expression of lipids assigned to PC may indicate altered PC biosynthesis and thus

influence the cells mechanism to adapt to the accumulation of lipids, thereby impacting cell viability (as explained in chapter 4). This confirms that the OE acts upon the lipotoxicity responses as shown by the increased cellular stress in the OECs observed after 16w of HF/HS feeding. In addition, the persistent upregulation of mitochondrial OS that was seen in the mid and late phase in the OECs of the HF/HS mice can be linked to the altered abundance of PCs and PEs that was detected mainly from 8w of HF/HS feeding onwards, since the shifted PC/PE ratio may result in altered mitochondrial function and energy metabolism in the OE (as was explained in chapter 4). Moreover, as mitochondrial lipids define the physical properties of mitochondrial membranes, any reduction in mitochondrial PE can alter the mitochondrial functions and be linked to profound alteration in mitochondrial morphology. It was also only after 8w of HF/HS diet feeding that a significant increase in mitochondrial abnormalities was detected in the oocytes, which may indicate that the same mechanisms as detected in the oviductal cells, may also apply to the oocytes.

Our findings in the oviduct demonstrate that the obesogenic diet not only affects fertility through reduced oocyte quality but that it may also directly impact early embryo development through an altered oviductal micro-environment. Maternal metabolic stress conditions are, however, not only limited to the oviduct and the ovarian cells. Also, the uterine environment, in which the embryo resides the majority of its developmental time, can be impacted by metabolic disorders (Tripathi et al., 2016). As such, obesity in rats has shown to impact their uteri as they developed insulin resistance during the estrous phase through mechanisms that involve the induction of uterine hypoxia and down-regulation of the insulin receptor gene (Bazzano et al., 2018). Furthermore, metabolic stress causes an impaired uterine inflammatory response in cattle, which may still permit survival of the embryo, but also decreases the endometrial defences to bacterial infections (Sheldon et al., 2018). The uterine environment is

beyond the scope of this thesis, but it is important to mention that the oocyte and growing embryo can be influenced at all different stages of its development starting in the follicle up to the uterus. Although it may seem that the oviduct only forms a small segment in this process, we were able to show evidence of the direct impact of feeding an obesogenic diet and therefore the determinative role that the impact on the oviduct may play in the pathogenesis of subfertility.

To further reflect on the research and experiments conducted in this thesis, some critical topics are discussed below.

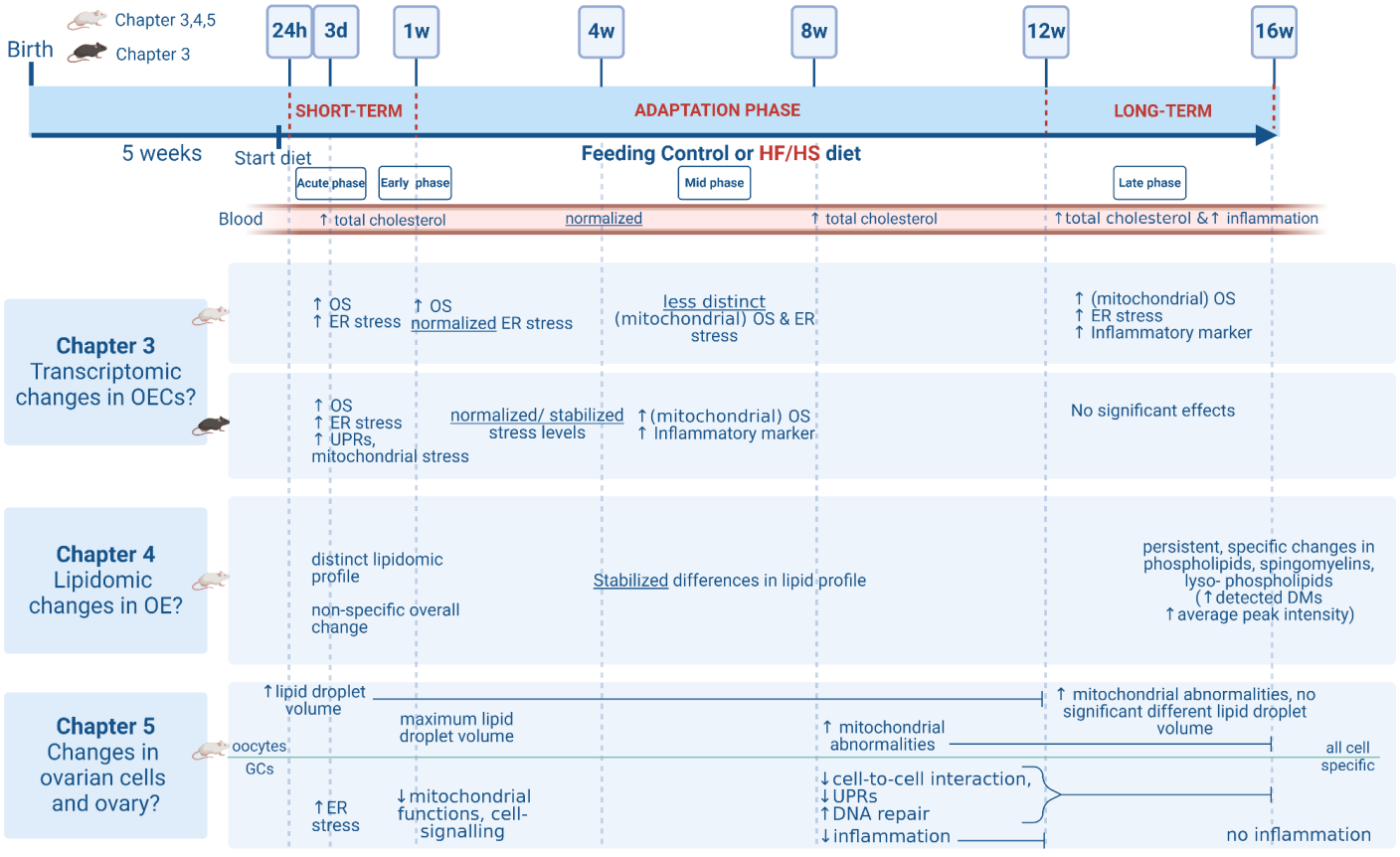


Figure 1: Overview of the obtained results in this PhD thesis regarding the effects on the oviductal epithelium, the ovary and ovarian cells after different durations of feeding an obesogenic diet in mice (made with Biorender). (OEC = Oviductal Epithelial Cell; OE = Oviductal Epithelium; HF/HS = High-fat/high-sugar; OS = Oxidative stress; ER = endoplasmic reticulum; UPR = Unfolded Protein Response; GC = Granulosa Cell)

1. Short-term versus long-term feeding of the obesogenic diet: the time points.

The main focus of this PhD research was to study the development of effects after different periods of consuming an obesogenic diet. By feeding mice the HF/HS diet for 24 hours, 3 days (d), 1 week (w), 4w, 8w, 12w and 16 weeks, we were able to acquire more insights into the acute effects after short-term feeding of the obesogenic diet and the more chronic effects due to long-term feeding of the obesogenic diet and the altered metabolic state associated with the development of obesity in mice. Moreover, the stabilized and less distinct effects that we observed in the time period between short-term and long-term feeding, may indicate an adaptive response of the body to the diet-induced hyperlipidemia. The different durations of feeding in our experiments were carefully selected to coincide with specific metabolic windows of short and long-term exposure based on the study of Williams et al. (2014). They investigated the timeline of the induction of glucose intolerance and inflammation in mice in response to the consumption of a HF diet, compared to low fat (LF) diet fed mice. The researchers demonstrated that 3d of HF diet feeding in mice already resulted in a peak in glucose intolerance and an acute phase response detected in the plasma. After 1w on the diet, the elevated glucose concentration appeared to be relatively improved and no difference in total AUC was detected between the HF and the LF group up until 12w of feeding, indicating that the glucose tolerance remained stable. Additionally, no inflammation markers were detected in that same time period between 1w and 12w. These observations suggest an adaptive phase, which we observed in the oviductal epithelium between the early and late phase of HF/HS feeding as well. Williams et al. (2014) demonstrated that after 12w of HF diet feeding the glucose concentrations showed a 60% increase and gene markers for inflammation appeared in the white adipose tissue (WAT) and the muscle of the mice but not in their liver. Additionally, they compared the HF diet mice to mice fed a HF diet matched to the caloric intake of the LF fed mice (PF) for 3 days or 1 week. They observed that glucose tolerance after 3 days of

HF diet feeding showed that both PF and HF mice had a similar AUC which was higher than the LF mice. By 1 week on the diet the PF mice had a similar AUC to the LF mice and HF mice had a higher AUC than both PF and LF mice. This shows that glucose intolerance at 3 days appears to be due to the composition of the HF diet, but this changes to caloric intake by 1 week, indicating a relatively rapid switch in the mechanism for the induction of insulin insensitivity from dietary fat to overconsumption within this timeframe. Furthermore, data from the PF mice show that the induction of the acute phase response in the HF mice at 3 days appears to be due to the increased caloric intake rather than composition of the HF diet. With these findings, Williams et al. (2014) indicate separate causative mechanisms for the glucose intolerance and the acute phase response at 3 days of HF feeding. Each of the different properties of the diet can thus contribute to the different results. In addition, the second increase in glucose intolerance observed between 12 and 16 weeks of HF diet feeding, coincided with a second wave of inflammation, in the white adipose tissue and muscle, thereby indicating that the impact of the HF/HS diet and obesity on cellular functions depends on the duration of the direct exposure to the dietary components. The findings of Williams et al. (2014) suggest distinct responses to HF feeding, related initially to nutritional challenge and latterly to increased adiposity and increased inflammation in WAT and muscle rather than a gradual increase over time. We were the first now to confirm this development of effects in distinct phases after the start of feeding an obesogenic diet as was demonstrated in this thesis by the time dependent changes that locally occurred in the oviduct and ovarian cells after HF/HS feeding in mice. The time points used by Williams et al. (2014) were also used in our experiments to cover the very acute effects after short-term dietary exposure and the effects after the development of obesity due to long-term dietary exposure. In addition, the 24h time point was included in our study as well, to acquire insights on the effects that may immediately occur in response to feeding a HF/HS diet.

As all three experiments of this PhD research were performed on tissue and cells coming from the same group of mice, a lot of material had to be collected at each sampling time point. To maximise the efficiency and feasibility of the project, specific time points were selected for some of the outcome parameters while still maximizing our chances in detecting effects both after short and long-term feeding. The specific time points were based on previous results and preliminary data analysis.
The different acute and chronic effects, that were previously discussed, reveal a very logical network of molecular interaction and cellular events detected in our experiments thereby validating our experimental set-up with the precisely selected specific time points.

As described before, we are the first to demonstrate the acute and chronic changes and their development over time by obesogenic feeding for very short as well as long-term time periods going up to 16 weeks *in vivo*. Previous research has reported effects on oocyte quality after a very short high NEFA exposure period of 24h during bovine oocyte *in vitro* maturation, in which was demonstrated that the oocyte suffers from the lipotoxic conditions, indicated by less active oxidative metabolism, altered DNA methylation and transcriptomic fingerprints (Desmet et al., 2016) and a slower developmental competence of the blastocysts after fertilization (Marei et al., 2017). In addition, Valckx et al. (2014b) cultured secondary murine follicles for 12 days *in vitro* in the presence of elevated NEFA concentrations. After this slightly longer exposure they reported reduced follicle diameters, substantially reduced oocyte developmental competence and a changed gene expression in granulosa cells of genes related to energy/fatty acid/steroid metabolism, apoptosis, and oxidative stress in the high NEFA-exposed follicles (Valckx et al., 2014b). These studies demonstrate the effects of a direct exposure of the oocyte to lipotoxic conditions *in vitro*, whereas the effects on oocyte quality by exposure to obesogenic conditions *in vivo* after different periods of experimental feeding has never been investigated before and was shown in this PhD

thesis for the first time, together with the different effects in other reproductive tissue, being the oviduct.

The timespan in which the oviductal micro-environment can potentially affect the oocyte and early embryo is limited as the period for oviductal oocyte transfer is only 3 days in mice and 3.5 days in women (Croxatto, 2002). Several *in vitro* embryo culture experiments have indicated that suboptimal metabolite composition of the embryonic micro-environment can decrease embryo developmental potential, quality and transcriptome (Leroy et al., 2017). Furthermore, a recent study from our research group demonstrated that continued in vitro exposure to PA during early embryo development resulted in altered DNA and histone methylation in morulae (Meulders et al., 2023). Optimal levels of DNA methylation and histone modifications during early embryo development are required for key biological processes such as embryonic genome activation and genomic imprinting (van Otterdijk and Michels, 2016). Epigenetic reprogramming during early embryo development is a very dynamic process which makes it a highly vulnerable window for disruption of epigenetic programming due to environmental changes (Lucas, 2013). Improper establishment or erasure of epigenetic marks might be irreversible and may lead to permanently altered gene expression, abnormal development and early onset of disease (Godfrey et al., 2007; Rivera and Ross, 2013). The results of this current PhD thesis demonstrate that the oviductal epithelium and its cells are already very acutely impacted by the consumption of an obesogenic diet and that these changes progressively increase and persist over time. At any specific moment during the dietary exposure window, may it be short or long-term, an oocyte or early embryo may pass through the affected oviductal micro-environment. Even though the exposure in the oviduct to stress conditions is limited in time, the timing is extra critical considering the sensitivity of the early embryo at this point and the importance of the developmental processes that occur in the oviduct. Thus, the passing oocyte and early embryo may be severely impacted by the stress responses and

changed lipid profile of the oviductal cells. Therefore, our findings may explain why subtle nutritional challenges during the preimplantation period, resulted in offspring with a higher risk of developing deleterious phenotypes in adulthood (Velazquez, 2015). However, the impact in HF/HS diet fed mothers on the specific supportive interaction between the oviduct and the zygote was not investigated in this PhD research. Dissecting the specific impact of an adverse oviductal environment in an in vivo experimental set-up is very difficult from a practical point of view. One technique to study this may be through zygote transfer into oviducts from both control and HF/HS fed mothers. Zygote transfer has previously been performed in cattle to examine oviduct-embryo interactions (Maillo et al., 2015).

2. Why is it interesting to compare the C57BL/6N with the Swiss strain when investigating the effects after short and long-term feeding of an obesogenic diet in oviductal epithelial cells?

First of all it is important the address the choice of an *in vivo* research model in this PhD thesis. Previously, *in vitro* research has extensively been used to gain more insights in the process of embryo development, both in normal and altered conditions. Various *in vitro* studies have investigated the impact of metabolic stress on oocyte and embryo quality and development (Leroy et al., 2005; Aardema et al., 2011; Yang et al., 2012; Van Hoeck et al., 2013; Desmet et al., 2016; De Bie et al., 2017; Marei et al., 2017; Meulders et al., 2023). Also oviduct physiology and function and its relation to maternal metabolic health has previously been studied using bovine *in vitro* models (Jordaens et al., 2017b; Hamdi et al., 2019; Banliat et al., 2020; Jordaens et al., 2020; Leal et al., 2022; Mahe et al., 2023). Whereas *in vitro* models allow the researcher to focus on the impact of one specific factor on important pathways in a controlled environment, an *in vivo* model encompasses the full complexity of the animal or human body. As the main focus of this thesis was to detect effects on the oviductal and ovarian cells after short and long-term periods of diet exposure, revealing the acute and chronic effects of the associated metabolic alterations, an *in vivo* model was chosen as it allows us to investigate the complexity and development of effects over time. *In vivo* research investigating metabolic syndrome most commonly uses rodents (Wong et al., 2016). Mice are small, housing them is easy and they have a short gestational period, which makes them an efficient model to study fertility and intergenerational effects. Furthermore, they are suitable to study human diseases because mice and humans have several physiological similarities (Perlman, 2016). Similar to humans, mice progressively gain weight, develop hypercholesterolemia, inflammatory responses, obesity and insulin resistance in response to a HF diet (Reynolds et al., 2015; Boudoures et al., 2016). In addition, mice have a gastro-intestinal system that is comparable to humans (Kleinert et al., 2018) and more importantly for our research, their reproductive

physiology is similar to humans with a comparable oogenesis, oocyte maturation and preimplantation embryo development (Jamsai and O'Bryan, 2011). However, there are also important physiological differences that have to be taken into account. Namely, the period of oocyte recruitment in mice is much shorter compared to women (Clarke, 2017) and mice are poly-ovulatory animals.

It is however important to take into account the increasing evidence showing that the genetic background (inbred/outbred) of mice may influence their sensitivity to a HF diet and can therefore affect the outcome of murine diet-induced obesity studies (Kleinert et al., 2018).

The mouse strain(s) used in this thesis, were carefully selected since the choice of mouse strain has shown to be of crucial importance for the specific research hypothesis that will be investigated. The mouse strain options to use in studies focusing on obesity, the obesogenic diet, associated metabolic health and fertility appear to be endless, going from monogenic knock-out strains to polygenic inbred or outbred strains. Obesity studies often use an ob/ob mouse strain. This strain has mutations in the gene responsible for leptin production, leading to leptin deficiency. This not only results in obesity in the mice, but also hyperglycaemia, glucose intolerance and elevated plasma insulin levels were reported. However, these mice may not be the best choice for fertility studies as they often show to be subfertile (Charles River). Up until now, most research investigating the impact of diet-induced obesity on metabolic health and fertility use the well-established B6 or C57BL6 strain (Champy et al., 2008). This strain is polygenic and its genome was the first to be completely sequenced (Gregory et al., 2002). The B6 strain is an inbred strain, meaning the mice are genetically homogenous with little variation or heterogeneity, which may reduce experimental variability and increase reproducibility. The B6 strain is relatively susceptible to diet-induced obesity (Champy et al., 2008; Collins and Rossi, 2015) as they show to progressively gain weight and develop hyperlipidaemia when fed a HF diet (Spearow and Barkley, 1999; Nicholson

et al., 2010; Wu et al., 2015). Inbred strains often undergo genetic drift and mutations, which results in different sub strains with different susceptibility to diet-induced obesity. The C57BL/6 strain has the C57BL/6J and C57BL/6N sub strains. Feeding a HF diet for only three weeks to the C57BL/6N strain (only 5 SNP differences to the C57BL/6J strain) results in hepatosteatosis, hyperglycaemia and hyperinsulinemia (Kahle et al., 2013) whereas the C57BL/6J strain rarely develops hyperglycaemia when fed an obesogenic diet (Leiter, 2002; Attie and Keller, 2010). The C57BL/6J strain appears to be much more used compared to the C57BL/6N strain, indicated by the ten thousands of entries in Pubmed compared to only thousands of entries that use the C57BL/6N strain (Laboratory, 2019). Even though C57BL/6J mice are often used in research regarding obesity, the choice of sub strain is very important when using it specifically for obesity research. That is because C57BL/6J mice carry a mutation in the Nnt gene. This gene encodes a mitochondrial protein involved in β-cell mitochondrial metabolism, ultimately resulting in impaired glucose-mediated insulin secretion (Freeman et al., 2006). The C57BL/6N mice do not carry this Nnt deletion and may thus be a better model to use in studies regarding the impact of obesity on metabolic health. For that reason the C57BL/6N sub strain was used in this thesis. The C57BL/6 strain may however be less suitable for fertility research as it has shown poor fertility outcomes. This may be due to many generations of inbreeding causing an increased homozygosity of deleterious recessive alleles which directly results in decreased fertility (Ober et al., 1992; Bittles et al., 2002). In addition, inbreeding has shown to increase the risk of spontaneous abortions (Diamond, 1987). Inbred strains also show small litter sizes and cannibalism of pups. Furthermore, the inbred physiology of the strain might make translation to the human (outbred) physiology more difficult. Outbred mice resemble the human physiology better as no two individuals are genetically identical since they are bred to maximize heterozygosity and genetic diversity within a population (Ellacott et al., 2010). This makes extrapolation to the human situation easier or at least more relevant (Chia et al., 2005), but can also cause more experimental variability and thus

more animals may be needed to reach statistical significance. Furthermore, in contrast to inbred mice, the outbred mice appear to be better breeders since they have a higher reproductive performance, lower neonatal mortality, better nurturing behavior and reach sexual maturity earlier (Gill, 1980). It was also recently demonstrated that outbred mice might be a better subject for biomedical research (Tuttle et al., 2018). Commonly used outbred strains are the ICR, CD-1 and Swiss strain. The latter is very fertile and possesses perfect nurturing abilities. Furthermore, the Swiss strain has shown to develop hypercholesterolemia, obesity, hyperinsulinemia and insulin resistance in response to an obesogenic diet, indicating the strain is susceptible to diet-induced obesity (Rosenfeld et al., 2003; Alexenko et al., 2007). As the Swiss strain mimics the genetic diversity in the human population, it might be a more suitable mouse model to use for research on the impact of obesogenic diets and obesity on fertility and metabolic health.

Different studies have investigated the influence of differences in genetic background on reproductive parameters and concluded that the impact on fertility is strain dependent (inbred vs. outbred). This was observed in meiotic maturation rate (Ibanez et al., 2005), parthenogenetic activation (Gao et al., 2004), oocyte developmental competence (Kaleta, 1977) and preimplantation development (Suzuki et al., 1996). Therefore, it was advised to take strain differences into account in the interpretation of reproductive experiments performed with mouse models (Kaleta, 1977; Ibanez et al., 2005). Fetterman et al. (2013) have shown that the bioenergetics and vulnerability to metabolic diseases is modulated by the mitochondrial genetic background (Fetterman et al., 2013). This was tested by cross insertion of mtDNA from cardiomyocytes of B6 mice to cardiomyocytes of outbred C3H/HeN mice. More ROS and a higher MMP was found to be generated by the mitochondria containing the B6 mtDNA compared to the mitochondria containing the C3H/HeN mtDNA**.** In line with these observations, a recent study in our laboratory has shown that the type and magnitude of mitochondrial

dysfunction was different in B6 compared to Swiss oocytes in HF diet mice (Marei et al., 2020). Even though the B6 mice are a commonly used model for DIO studies, it may already exhibit mitochondrial dysfunctions that could influence the sensitivity to a HF/HS diet (Marei et al., 2020). As far as we know, comparing the commonly used C57BL/6 inbred strain with the outbred Swiss strain in such a long-term experimental set-up of 16 weeks has never been done before. It was not known yet whether the genetic background of the used mouse strain would influence the effects over time in the oviductal microenvironment in response to feeding an obesogenic diet and the development of the obese phenotype (investigated in Chapter 3). By comparing the C57BL/6 strain with the Swiss strain, we could provide insights that may aid researchers when designing future experiments.

Our findings confirmed that both strains were metabolically sensitive to the HF/HS diet as was demonstrated by the significant increase in body weight and serum total cholesterol. The increased adiposity in mice after 16w of HF/HS feeding is shown in Figure 2. The weight gain and serum total cholesterol results were similar to previous studies investigating the impact of a HF diet on metabolic health using the inbred B6 mice as they also demonstrated an altered serum profile and significant weight gain (Williams et al., 2014; Boudoures et al., 2016), even already after 3 days of feeding (Williams et al., 2014). Also in Swiss mice significant weight gain as well as an aberrant serum lipid profile, demonstrated by increased serum cholesterol and NEFA, were previously reported after HF diet feeding for four weeks (Simino et al., 2021).
As described in the aims of this PhD thesis we describe the observed changes over time, rather than defining the time point of the onset of obesity after the start of obesogenic feeding, as there is no specific parameter of clear consensus on defining obesity. Many other studies consider differences in total body weight gain as the main parameter to assess the outcome of obesity (Dias et al., 2021). The significant change in body weight and/or blood cholesterol in the current thesis can provide further evidence of the

sensitivity of the mouse model to the obesogenic diet and the development of the expected metabolic changes.

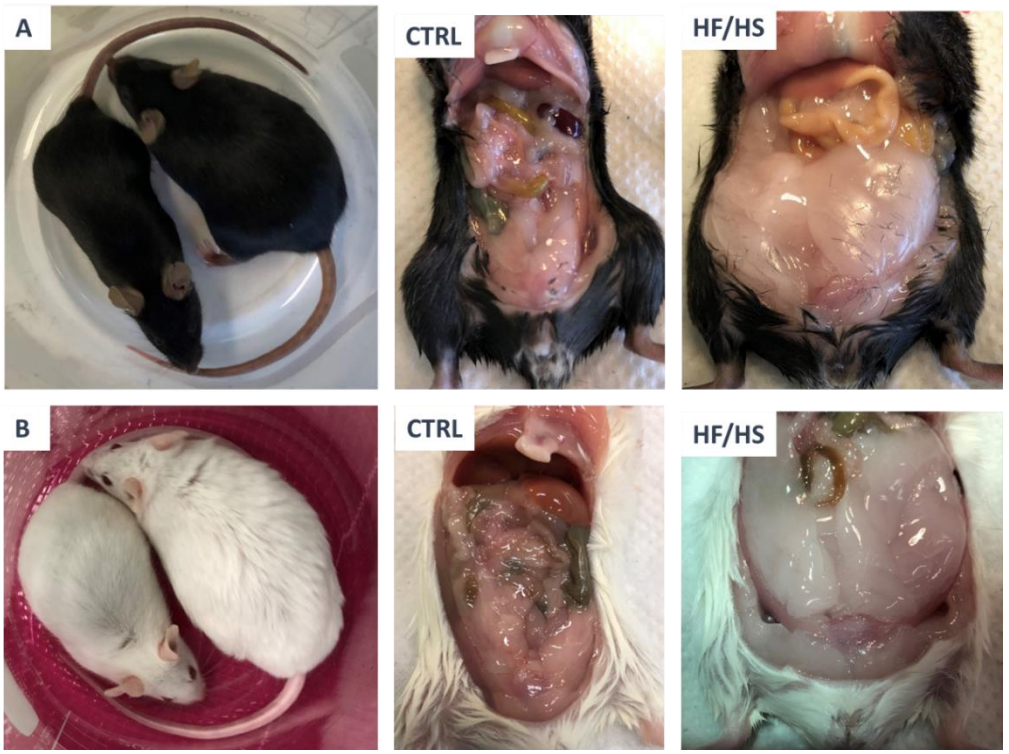


Figure 2. Increased adipose tissue in A. C57BL/6 mice and B. Swiss mice after 16 weeks of HF/HS diet feeding.

Additionally, to the best of our knowledge, we are the first to demonstrate that the changes in gene expression of genes related to OS, mitochondrial stress and ER stress in response to an obesogenic diet were different between C57BL6 mice and Swiss mice and were thus dependent on the mouse strain, as well as on duration of exposure to the diet. The changes detected in the OECs of the HF/HS fed B6 mice were mainly observed during the acute phase (3d) and involved higher levels of mitochondrial OS. Furthermore, the acute increase in cellular stress in the B6 OECs reached levels that appeared to be high enough to already stimulate the expression of UPR genes in the ER and mitochondria. The absence of such concomitant acute increase in ER and

mitochondrial stress in the Swiss OECs, indicated that the level of acute cellular stress was higher in the B6 mice. These relatively higher stress levels in the B6 mice may also have contributed to the earlier (12w) initiation of inflammatory responses in the HF/HS B6 mice than in the Swiss mice (16w). The higher (mitochondrial) sensitivity to metabolic stress in the acute phase and the lack of response in the late phase in the B6, which is contrasting to the responses seen in Swiss mice, can thus be explained by the observations of Fetterman et al. (2013) and Marei et al. (2020) as described before. Based on their observations, we may assume that the OECs of the B6 mice are more vulnerable to mitochondrial OS and therefore show higher levels of mitochondrial and ER stress. However, according to that reasoning, upregulated expression of mitochondrial *PRDX3* should also be expected in the HF/HS B6 OECs, but was not observed in our study at any of the timepoints. The failure of upregulation of PRDX3 may have however contributed to the increased mitochondrial stress levels. Further research in this field is thus necessary in order to study the molecular mechanism involved. In addition, the mitochondrial ultrastructure and lipid droplet volume in the oocytes of the B6 mice was also assessed in this PhD research. These results were not published, but also illustrated strain dependent differences. The higher mitochondrial stress level in the HF/HS B6 mice is also evident in their oocytes as they exhibited a significant increase in the proportion of mitochondrial abnormalities already after 4 weeks of HF/HS feeding (Fig. 3). In the HF/HS Swiss oocytes a significantly increased proportion of mitochondrial abnormalities was only evident from 8w onwards (as described in Chapter 5). In the study of Marei et al. (2020) B6 control oocytes already exhibited very high rates of mitochondrial abnormalities and this was suggested to be genetically driven or strain-specific. In addition, the lipid droplet volume in the B6 oocytes of the current PhD research was significantly increased after 1w on the HF/HS diet after which a significant difference between CTRL and HF/HS B6 oocytes could only be detected again at 12w and 16w of feeding (Fig. 4A). The lipid droplet volume in the B6 oocytes thus demonstrates a different pattern in the changes over time in response

to the obesogenic diet compared to the lipid droplet volume in Swiss oocytes (described in chapter 5). Furthermore, the lipid droplet distribution pattern in the oocytes appeared to be strain dependent, regardless of the diet (Fig. 4B), as was also observed by Marei et al. (2020). As such, in the B6 oocytes lipid droplets were found aggregated in large clusters, whereas the lipid droplets in Swiss oocytes were evenly distributed throughout the ooplasm and were either not clustered or formed very small aggregates. These observations confirmed the previous findings of Marei et al. (2020) on lipid droplet distribution in oocytes of B6 and Swiss mice.

Czajkowska et al. (2020) have reported that the impact of maternal aging on oocyte quality, which was assessed as its ability to generate a certain pattern of Ca^{2+} oscillations in response to fertilization, differs depending on the genetic background of female mice. Their study showed that the outbred C57BL/6 oocytes were affected by the advanced reproductive age, whereas Swiss oocytes were less affected by the female advanced reproductive age. Thus, the lack of dietary effects on gene expression at the late phase in the B6 mice may be due to the advanced reproductive age of the mice at that time point.

The maximal feeding period in our experimental set-up was 16 weeks. Therefore, the age of the mice at this time point in the experiment should be taken into account. The reproductive life span of a mouse starts at age 4 to 7 weeks until 6-8 months (Fox, 2019). After 16 weeks of feeding, the mice had reached the age of 21 weeks (± 5 months) at the time of sample collection. Therefore, a possible impact of increasing age on the functionality and quality of the oviducts and ovaries cannot be ruled out. This was however taken into account in our experimental set-up by starting the control and HF/HS diet feeding at the youngest age possible. Furthermore, the data of the different outcome parameters was analyzed using univariate ANOVA tests or Kruskal Wallis tests with Bonferroni correction (serum cytokines, gene expression OECs) on the control groups of different time points to check for potential aging effects. No significant impact of age was detected at any of the outcome parameters in our experiments. Although

not performed during this PhD research, another way to control for aging effects, could be to measure steroid hormone levels as aging is accompanied by decreasing levels of circulating sex steroid hormones estrogen, progesterone (Sherman et al., 1976; Velarde, 2014; Fiacco et al., 2019).

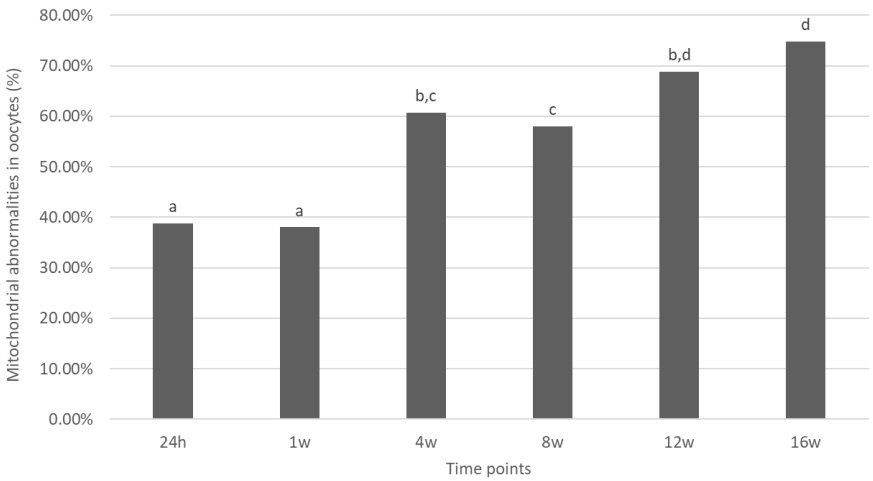


Figure 3. **Proportions of mitochondria with abnormal ultrastructure in oocytes of HF/HS fed C57BL/6 mice per time point (using TEM).** A significant difference between the HF/HS groups of different time points is indicated with letters a, b, c and d (P<0.05).

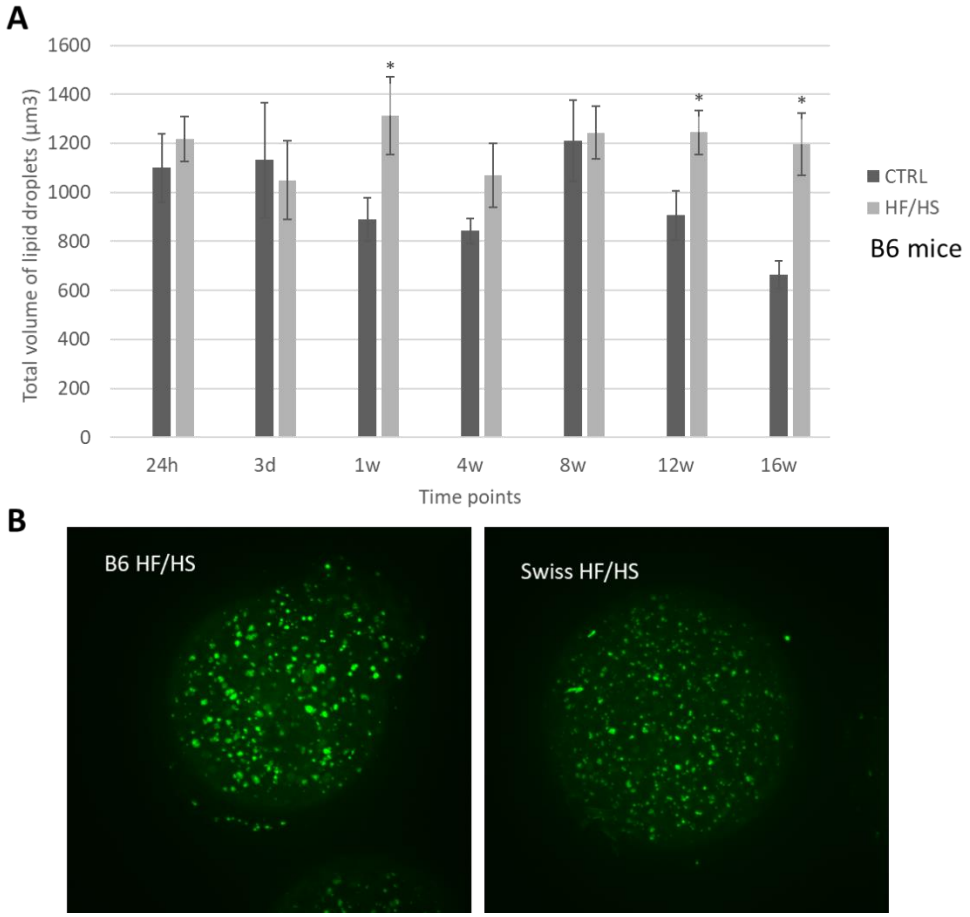


Figure 4. Assessment of lipid droplet volume in oocytes collected from mice fed either a control or a HF/HS diet for different durations (from 24h up to 16weeks). **A**. Bar chart showing means ± SEM from 12 to 21 oocytes per dietary treatment (1 to 4 oocytes per mouse), collected from 4 to 5 **B6** mice per treatment per time point. A significant difference between HF/HS and control within a time point is indicated by an asterisk (*). **B**. Representative confocal images of a B6 (left) and Swiss (right) oocyte showing a different distribution pattern of lipid droplets. Each confocal image is a z-stack projection of 40 x 1 µm steps.

By comparing both strains we were thus in line with earlier data and we could demonstrate that the sensitivity to cellular stress and inflammation in response to a HF/HS diet and obesity in the oviduct is dependent on the genetic background of the mouse model. Additionally, our findings confirm that strain differences should be considered when interpreting reproductive experiments (Kaleta, 1977). Our results

should further build on awareness about the importance of choosing the correct mouse strain when designing mouse models to study human fertility.

3. Why was a HF/HS diet used to study the effects of an obesogenic diet in the oviduct and ovarian cells?

High energy dense food, diets high in fat and the consumption of sugar-rich drinks are known to compose the major dietary determinants linked to obesity (Abiri et al., 2023). Fast food is very energy dense and characterized by high levels of saturated fat, fructose and a high glycemic index (Guthrie et al., 2002) and it is part of the typical Western diet, which is low in fruits and vegetables and high in fat and sodium. The Western diet is also characterized by large portion sizes, a high amount of calories and excess sugar (Wartella EA, 2010). The excess sugar accounts for about 14% to 17% of the daily caloric intake which is above the recommended level of 10% of the total caloric intake, according to the WHO guidelines. Sugar-sweetened beverages constitute 47% of these added sugars (Clemente-Suarez et al., 2023) and their worldwide consumption has increased in parallel with the obesity pandemic (Schulze et al., 2004; Malik et al., 2010). Furthermore, insulin resistance, intrahepatic lipid accumulation, and hypertriglyceridemia have all been associated with the consumption of fructose and other sugar-sweetened beverages and may contribute to the development of type 2 diabetes and cardiovascular diseases in the long-term (Taskinen et al., 2019). Additionally, the Western diet is also high in saturated and trans fats, which not only contribute to more calories, but were also reported to increase low-density lipoproteins leading to atherosclerosis (Rakhra et al., 2020). Dietary fat is a strong predictor of weight gain (Sherwood et al., 2000). Increased fast food intake is associated with an increase in BMI and development of insulin resistance (Pereira et al., 2005). It is therefore currently recommended that dietary fat has to be reduced, from the present 35–45% of the total energy content in most Western diets, to below 30% (Health, 2000). Furthermore, the composition of the fatty acids in the diet also plays an important role

in the development of obesity. A typical fast food meal consists of 85% of the recommended daily fat intake and even 73% of the recommended saturated fat intake. The effect on increase in body fat and impaired insulin sensitivity was more pronounced after feeding a diet high in saturated fat than a poly-unsaturated fatty acid (PUFA) rich diet as was shown in animal models (Botchlett et al., 2016; Botchlett and Wu, 2018).

The name Western diet indicates it is the diet typically consumed in America and the western societies. However, already in 1997, Drewnowski and Popkin were the first to report evidence of a global dietary convergence to the Western diet (Drewnowski and Popkin, 1997). Over the past two decades increasing evidence has shown the transformation of the food systems of developing countries from traditional to western type diets, which forms a great concern (Azzam, 2021). As the consumption of a western type diet no longer only occurs in western countries, the naming might thus not be accurate anymore. Therefore, in this thesis we referred to the typical Western diet as the obesogenic diet, or HF/HS diet.

DIO studies have been using both HF diets and HF/HS diets to induce obesity in their mouse model as both diets have shown significant impact on metabolic health that is similar to the effects reported in obese women. Both diets result in increased serum lipid concentrations, hyperinsulinemia, insulin resistance, impaired glucose tolerance and hepatic steatosis (Panchal and Brown, 2011). Previous studies in our research group have used both HF diet and HF/HS diet in mice and although no direct comparison was made between the two diets, the effects on metabolic health and oocyte quality were found to be very similar (Smits et al., 2021; Smits et al., 2022). However, even if it does induce most symptoms of human metabolic syndrome in rodents, the HF diet may not be the best diet to use as it does not mimic the diet that results in metabolic syndrome and the associated complications as the human diet is much more complex than a HF diet alone. Therefore, to make our experiments more representative for the obesogenic conditions, a HF/HS diet was used. Mice in the control group were exposed to a matched control diet from Sniff Diets (Soest, Germany), containing 10 kJ% fat and 7%

sucrose. The control diet is lower in saturated fatty acids (CTRL 5; HF/HS 6) compared to the HF/HS diet. Using the matched control diet ensured that differences between both experimental groups are only due to differences in fat and sugar content. For the HF/HS diet the DIO - 60 kJ% fat and 9.4% sucrose diet from Sniff Diets (Soest, Germany) was used in combination with drinking water with a final fructose concentration of 20%. The fat source in the HF/HS diet comes from beef tallow which was chosen over lard. Beef tallow has shown to increase plasma lipid, insulin and leptin concentrations and leads to hepatic steatosis. Diets that contain lard as a fat source also increased plasma triglyceride, free fatty acid and insulin levels as well as liver triglycerides. Furthermore, beef tallow contains more saturated fatty acids compared to lard. Next to the high level of fat, carbohydrates were needed to mimic the western type diet more closely. The most common carbohydrates are fructose and sucrose (Panchal and Brown, 2011; Wong et al., 2016). In the experiments of this thesis fructose was chosen to add to the drinking water of the mice. High fructose consumption has been linked to the development of metabolic syndrome (Pereira et al., 2005; Aydin et al., 2014). Unlike glucose, fructose has shown to lead to increased body weight, adiposity, hypertriglyceridemia, hypertension, hyperlipidemia, glucose intolerance and decreased insulin sensitivity in animal models (Schulze et al., 2004; Lê and Tappy, 2006; Johnson et al., 2007; Tran et al., 2009; Tappy and Le, 2010). Fructose increases caloric intake as it reduces satiety (Pereira et al., 2005). Additionally, based on the knowledge on fructose metabolism, it is considered to be superior in inducing metabolic syndrome compared to glucose or starch. Diet-induced obesity in rodent models has traditionally been obtained by the use of fructose and fat rich dietary components (Panchal and Brown, 2011; Crescenzo et al., 2015). Fructose has also been used in various studies investigating the effects on reproduction. For example, feeding a 60% fructose diet to female mice for six weeks resulted in a 30% decrease in pregnancy rates (Saben et al., 2016). Another study investigated the impact of a high fructose diet on the reproductive organs in female rats and reported longer estrous cycles, relatively bigger ovaries that

possessed more corpora lutea than control ovaries and uterine sections with a well-developed stratum vasculare between inner and outer myometrial layers and differences in the number of endometrial glands compared to the controls (Ko et al., 2017). The oviduct was not included in their study.

Diets containing both high levels of fat and fructose can cause many effects such as increased body weight, increased plasma concentrations of triglycerides, cholesterol, FFAs and leptin, hyperinsulinemia, insulin resistance, impaired glucose tolerance, increased abdominal fat deposition, hepatic steatosis and inflammation. When comparing metabolic alterations in rats on either a high fat diet, a high fat-high glucose diet or a high fat-high fructose diet, all three diets caused an increase in fasting plasma glucose and insulin levels, but the high fat-high fructose diet elicited the greatest increase (Moreno-Fernandez et al., 2018). Additionally, mice on a high fat-high fructose diet showed a higher caloric intake and gained more weight compared to mice fed a high fat diet only (Della Vedova et al., 2016).

Previous research in our laboratory demonstrated the metabolic sensitivity of mice to a HF diet (Marei et al., 2020). We were able to confirm this with the HF/HS diet as we observed a significant increase in body weight and serum total cholesterol concentration in the experiments of this thesis, thereby demonstrating that the mice were metabolically sensitive to the high dietary saturated fat and fructose content. Also from the detected development of the tissue and cell specific changes in this thesis, we may conclude that the use of a HF/HS diet was an efficient obesogenic diet to use in our experiments.

It is evident that the impact of feeding an obesogenic diet should be an important focus in research as it has become a global phenomenon with significant impacts on human health. The consumption of an obesogenic diet is not only linked to obesity but a whole range of other chronic diseases and inflammation processes, such as type 2 diabetes, cardiovascular disease and certain cancers (reviewed by (Clemente-Suarez et al., 2023).

Mice that were fed an obesogenic diet were found to have higher levels of inflammation and sepsis resulting in more severe disease and worse outcomes, compared to mice fed a standard fiber-rich diet (Napier et al., 2019). Furthermore, consumption of an obesogenic diet during pregnancy affects gene expression and epigenetics by altering nutrient availability, hormonal levels, environmental cues, cellular signaling pathways, DNA methylation, histone modification and many other factors, which could ultimately limit an offspring's brain function for life (Cinquina et al., 2020). The impact of an obesogenic diet on reproduction through its effects on oocyte quality has been demonstrated in various studies (Robker, 2008; Wu et al., 2010; Luzzo et al., 2012; Marei et al., 2020). Therefore, investigating the direct dietary effects of a HF/HS diet on health but with a specific focus on the reproductive tract and cells, should be of continuing interest.

Additionally, as the worldwide consumption of an obesogenic diet has increased enormously it may be interesting to shortly touch on the different factors that contribute to people choosing this type of diet. As such, income and education are essential social factors influencing dietary choices (Yuan et al., 2016; Burrows et al., 2017). Higher educated people with higher income are more likely to consume healthier diets that include more fruit, vegetables, whole grains and lean proteins (Burrows et al., 2017). Moreover, lower-income individuals and those with less education were shown to be more likely to have poor dietary habits and had a higher risk for chronic diseases such as type 2 diabetes, cardiovascular disease and obesity (Clemente-Suarez et al., 2023). Dietary factors can also be influenced by social norms as people are often influenced by the behavior of those around them, including family, friends, and peers (Clemente-Suarez et al., 2023). It has been described that social norms related to healthy eating were positively associated with fruit and vegetable intake among adolescents (Lally et al., 2011). Additionally, the food environment, including the availability and accessibility of healthy food options, also plays a role in shaping dietary

choices. People living in areas with limited access to healthy food options, less accessibility to food or food stores, are more likely to have poor dietary habits with lower intakes of fruits and vegetables and higher intakes of sugar-sweetened beverages compared to those living in areas with better food access (Cooksey Stowers et al., 2020), again leading to a higher risk for obesity and type 2 diabetes (Caspi et al., 2012).

4. Lessons learnt from the MALDI MSI experiments

Setting up a protocol for the MALDI Mass Spectrometry Imaging (MALDI MSI) analysis to study the lipidomic profile of the mouse oviduct appeared to be challenging and time consuming. Even though the process of sample preparation for MALDI MSI imaging comprises a lot of steps which all contributed to different issues, we were still able to obtain very interesting results. The MALDI MSI experiments have taught us that there are some factors to take into consideration, therefore we will shortly touch on some of our experiences from the 'trial and error' process. Prior to MALDI-imaging analysis, the oviducts were straightened during sample collection. This was especially important in order to be able to obtain cross-sections of the oviductal tissue. Even though tissue sections were made using a cryostat in -20°C, the small size of the samples caused them to melt very quickly and thereby return back to their coiled structure which made obtaining cross-sections very difficult. When the tissue sections were embedded in water, which is commonly used, they would melt quickly. Next to the issue regarding going back to their coiled structure, the melting also resulted in diffusion of the lipids in the tissue, which could influence the results. Therefore, carboxymethylcellulose (CMC) was used as it is a more viscous embedding material (Nelson et al., 2013), which helps in keeping the linear shape of the oviducts and causes less diffusion of lipids in the tissue. On the other hand, the viscosity of the CMC medium caused the tissue sections to stick to the blade of the cryostat. Therefore proper cleaning of the blade with 70% EtOH was needed between every section. Before matrix sublimation, the tissue sections needed to be properly fixated on to slides, which is usually done by

thawing. During this thawing step the CMC would however form liquid droplets next to the tissue which can again cause lipid diffusion. This could be prevented by drying the slides using a vacuum dryer in which the liquid droplets evaporated very quickly. Furthermore, when the aim is to detect small molecules, such as lipids, sublimation of the matrix is better than spraying it as sublimation covers the whole slide at once thereby pulling the lipid molecules quicker into the matrix and out of the tissue.

5. Limitations of the study

When studying the oviduct physiology in a mouse model it is important to take into account its specific structure. In contrast to the curved human oviduct, the mouse oviduct is coiled and contains about 11 turns which are supported by connective tissue, called the mesosalpinx (Behringer, 2012). Obtaining oviductal tissue sections for MALDI imaging as well as collecting OECs were both complicated by the coiled structure and required straightening of the murine oviduct (Fig. 5). This was done by carefully cutting the mesosalpinx between the coils.

Another point to take into account when working with a murine model is their small body size and thereby the limited availability of certain biological material. For example, in order to collect a sufficient volume of blood to assess the different serum parameters, the mice had to be sacrificed. This would not be an issue when larger animal models, like the cow, are used. To collect the oviducts and ovaries in our experiments, the mice had to be sacrificed anyway, which made a linear comparison through repeated measurements within the same animal over the different time periods of feeding impossible.

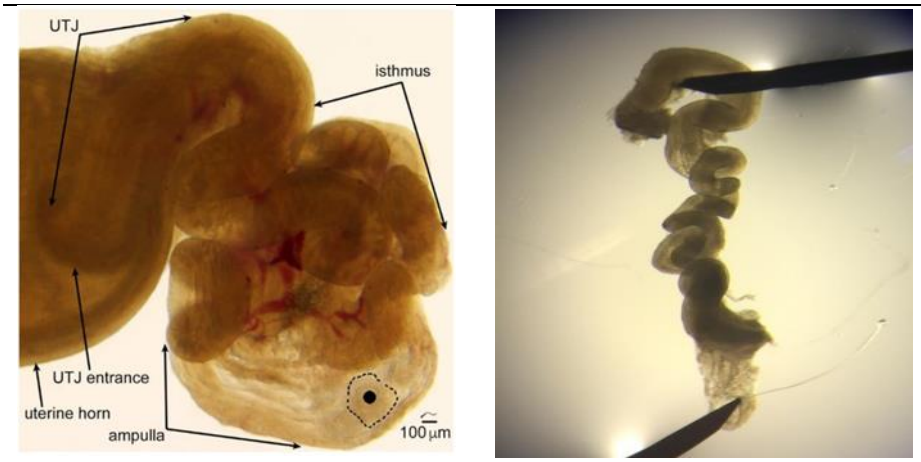


Figure 5. Left: picture of a coiled mouse oviduct from (Chang and Suarez, 2010). Right: Partially straightened murine oviduct by cutting the mesosalpinx (picture taken in our own lab).

Our experimental set-up required the collection of a large amount of research material at each time point. We collected the whole reproductive system of each mouse after which the needed cells were immediately collected from one ovary and oviduct and the tissue samples from the other ovary and oviduct. To keep this practically and logistically feasible, a relatively low number of animals was used in each dietary group (5 mice / treatment / time point). For the serum cytokine analysis and gene expression analysis numbers were limited to 3 replicates (i.e. from 3 mice/ treatment/ timepoint) (chapter 3). From a practical point of view, this was more feasible during sample analysis as this way all samples could be included in each assay (performed in 96 well plates), thereby excluding inter-assay variation. In addition, some samples had to be excluded due to incorrect snap freezing of the OECs at collection. Nevertheless, very interesting and biologically relevant differences between the dietary groups were demonstrated. These differences validated one another as they formed a very logical network of molecular interaction and cellular events.

6. How can future research further improve the insights?

Some extra measurements and analysis could be done to further improve the insights of this PhD research. As such, it might be interesting to test extra parameters of metabolic health in the serum of mice. Adiposity-associated dyslipidemia is characterized by low HDL cholesterol levels and an increase in LDL-cholesterol levels (Stadler and Marsche, 2020). Therefore, analyzing serum HDL- and LDL-cholesterol concentrations separately may help us to further substantiate the link between changes in metabolic health in response to the obesogenic diet and the local changes that were observed in the oviduct in response to the obesogenic diet. To further learn about the development of the effects over time due to the altered maternal metabolic state, it might also be interesting to investigate the changes, regarding lipid profile or immunohistochemical changes, in the liver of mice after HF/HS feeding for different time periods. Furthermore, as the production of adipokines by the adipose tissue significantly increases when lipolysis increases (Arner, 2005), it might also be interesting to measure adipokine levels over time. With these additional measurements and analyses a better link can be made between the locally initiated changes at the level of the oviduct or ovary and the systemic changes after different duration of feeding.

Analyzing the expression of extra marker genes related to inflammatory markers and lipid biosynthesis can complete the whole 'picture' on changes in gene expression in OECs after exposure to the HF/HS diet. To gain more insights into the changes in functionality of the OECs, proteomic analysis could be performed. This way the observed changes in gene expression can be linked to changes in the proteome of the OECs, which may indicate whether the functionality of these cells is affected due to the HF/HS diet.

As discussed in Chapter 4, it has been reported that the molecular weight (MW) of lipid ions in the bovine uterus and oviduct is linked to embryo receptivity and fertility. To

further elucidate the impact of the obesogenic diet and obesity on the functionality of the OE, we could supplement our findings in the lipidomic profile with further investigation in the potential role of specific lipids and their MW in the oviductal epithelium under obesogenic conditions.

With the observations in this PhD research, we gained insights on how much time it actually takes for an obesogenic diet to elicit different changes in the body. This can be seen as the 'wash-in' period of the obesogenic diet and can increase awareness around the, already acute, impact that the consumption of such diet may have. These insights may be used to further improve preconception care strategies and clinical advice. On the other hand, it would be very interesting to follow-up this PhD research with a 'wash-out' study, in which it will be investigated how much time it takes for the different effects that developed in response to long-term feeding of an obesogenic diet, to be alleviated or improved when terminating the long-term consumption of that diet. Previous research in our laboratory has investigated the impact of different preconception care strategies, such as switching to a control diet, after 7 weeks of feeding an obesogenic diet (Smits et al., 2021; Smits et al., 2022). However, the impact of switching to a control diet after long-term consumption of an obesogenic diet of up to 16 weeks, has not been studied before, but can be very interesting as we demonstrated various effects in the oviductal and ovarian microenvironment that occurred after more than 7 weeks of obesogenic feeding. The Centers for Disease Control and Preventions reported in 2018 that about a third of American adults eats fast food on any given day (Fryar D., 2017). In a society in which such large proportion of the population has a diet that is dominated by obesogenic fast food, there will be an increasing need for strategies that can rescue the chronic impact of long-term obesogenic conditions. The findings of a wash out study may provide insights in the different durations of the effects to 'wash-out' of the body and could be used to further

substantiate preconception guidelines and find strategies to rescue the affected oviductal and ovarian microenvironment.

Finally, it was not assessed whether the functionality of the oviduct is impacted by the consumption of an obesogenic diet over time. It would therefore be interesting to investigate this by studying the oviductal ability to support embryo development after different durations of HF/HS feeding. Such research can be performed using an *in vitro* model in which oviductal epithelial cells are isolated and cultured after different periods of feeding the obesogenic diet. The OEC monolayer can then be co-cultured with zygotes, after which morula and blastocyst rates and quality can be assessed to check whether the oviductal ability to support embryo development is impacted by the obesogenic diet. Previous research in our laboratory demonstrated that a polarized cell culture (PCC) is a preferred cell culture system because of the possibility to expose the cells via two compartments as seen *in vivo* and the use of cells that maintain their natural characteristics more closely (Jordaens et al., 2015). Another option would be to use an *ex vivo* approach in which zygotes are cultured that were collected from mothers that have been fed an obesogenic diet for different time periods, after which embryo developmental competence and the quality of morulae and blastocysts are assessed. Another way to investigate the impact on the oviductal ability to support embryo development is using an *in vivo* approach, in which embryos, produced by *in vitro* maturation and fertilization, are transferred to oviducts of mothers that consumed either a control or an obesogenic diet for different time periods. Afterwards, the number of embryos that were able to develop to the blastocyst stage can be recorded by flushing the oviduct and uterus. A similar set-up has previously been described in a bovine study to examine the direct effect of lactation on the ability of the reproductive tract of postpartum dairy cows to support early embryo development (Maillo et al., 2012). Furthermore, embryo transfer has been used in mouse studies before (Mahabir et al., 2018; Lamas et al., 2020).

CONCLUSIONS

This thesis investigated the changes in the oviductal microenvironment and the ovary and ovarian cells after different time periods of feeding an obesogenic diet using an *in vivo* mouse model.

- We are the first to investigate the impact of an obesogenic diet on the oviductal microenvironment in a time dependent manner. The different durations of feeding allowed a clear distinction of acute responses due to short-term obesogenic feeding, and chronic changes observed after long-term feeding and the development of the obese phenotype and the associated unhealthy metabolic phenotype. We provided a deep insight into a network of sequential events that could demonstrate how the oviductal epithelial cells can quicky sense metabolic stress in a matter of only 3 days, and how such local metabolic stress can evolve over time.
- We could also demonstrate important differences in cellular responses between the inbred and outbred mouse model which further highlights the importance of choosing physiologically relevant mouse models in other studies and which should aid in correct interpretation and comparisons of results of similar studies performed in other laboratories.
- The oviductal epithelium showed distinct differences in its lipidomic profile already at 3 days and 1 week of HF/HS diet feeding. After remaining relatively stable, marked lipidomic changes became evident again after prolonged feeding up to 12 and 16 weeks. The differentially abundant lipids appeared to be involved in plasma membrane functions, cell signalling, mitochondrial functions and cellular homeostasis.
- The lipidomic alterations detected in the oviductal epithelium were not only due to the direct accumulation of the excess dietary fat in the OECs, but also

due to a reduction in de novo synthesis of several lipid classes, most likely due to ER dysfunction.

- Ovarian cells can sense and react to systemic diet-induced hyperlipidaemia. Short-term feeding of the obesogenic diet for only 24h till 1w results in acute changes in the lipid content of oocytes and impacted ER and mitochondrial functions in granulosa cells. These effects progressively increased over time which was demonstrated by increased mitochondrial abnormalities in oocytes and different affected pathways, linked to DNA repair, cell signalling, UPRs and many others in the granulosa cells. These responses have shown to be cell specific and can directly impact oocyte quality and early embryo development, ultimately leading to reduced fertility.

With the findings of this thesis, we are the first to show strong evidence of the sensitivity of the oviduct and oviductal epithelium to systemic metabolic changes at both the transcriptomic and lipidomic levels *in vivo* (Chapter 3 and 4). We demonstrated that the oviduct and oviductal cells can sense and respond to systemic diet-induced hyperlipidaemia, which illustrates that the mechanism by which the obesogenic diet impacts fertility is not only mediated through reduced oocyte quality but might also directly impact early embryo development in the oviduct, potentially leading to long-term effects on foetal development, pregnancy success and postnatal health through epigenetic alterations. In addition, exposure to an obesogenic diet resulted in cell specific changes in oocytes and granulosa cells (Chapter 5), which can directly affect oocyte quality and early embryo development, eventually leading to reduced fertility. We believe that such deep analysis and novel insights can be useful for many researchers and form an important fundament for further studies that focus on protecting and enhancing oocyte and embryo development during the very early stages under diet-induced metabolic stressed conditions.

REFERENCES

AARDEMA, H., VOS, P. L., LOLICATO, F., ROELEN, B. A., KNIJN, H. M., VAANDRAGER, A. B., HELMS, J. B. & GADELLA, B. M. 2011. Oleic acid prevents detrimental effects of saturated fatty acids on bovine oocyte developmental competence. *Biol Reprod,* 85**,** 62-9.

ABIRI, B., VALIZADEH, M., NASREDDINE, L. & HOSSEINPANAH, F. 2023. Dietary determinants of healthy/unhealthy metabolic phenotype in individuals with normal weight or overweight/obesity: a systematic review. *Crit Rev Food Sci Nutr,* 63**,** 5856-5873.

ALEXENKO, A. P., MAO, J., ELLERSIECK, M. R., DAVIS, A. M., WHYTE, J. J., ROSENFELD, C. S. & ROBERTS, R. M. 2007. The contrasting effects of ad libitum and restricted feeding of a diet very high in saturated fats on sex ratio and metabolic hormones in mice. *Biol Reprod,* 77**,** 599-604.

ARNER, P. 2005. Human fat cell lipolysis: biochemistry, regulation and clinical role. *Best Pract Res Clin Endocrinol Metab,* 19**,** 471-82.

ATTIE, A. D. & KELLER, M. P. 2010. Gene co-expression modules and type 2 diabetes. *Results Probl Cell Differ,* 52**,** 47-56.

AYDIN, S., AKSOY, A., AYDIN, S., KALAYCI, M., YILMAZ, M., KULOGLU, T., CITIL, C. & CATAK, Z. 2014. Today's and yesterday's of pathophysiology: biochemistry of metabolic syndrome and animal models. *Nutrition,* 30**,** 1-9.

AZZAM, A. 2021. Is the world converging to a 'Western diet'? *Public Health Nutr,* 24**,** 309-317.

BANLIAT, C., LE BOURHIS, D., BERNARDI, O., TOMAS, D., LABAS, V., SALVETTI, P., GUYONNET, B., MERMILLOD, P. & SAINT-DIZIER, M. 2020. Oviduct Fluid Extracellular Vesicles Change the Phospholipid Composition of Bovine Embryos Developed In Vitro. *Int J Mol Sci,* 21.

BAZZANO, M. V., SARRIBLE, G. B., MARTINEZ, N., DE ASTRADA, M. B. & ELIA, E. M. 2018. Obesity alters the uterine environment before pregnancy. *Journal of Nutritional Biochemistry,* 62**,** 181-191.

BEHRINGER, S. A. 2012. Mouse Oviduct Development. *In:* KUBIAK, J. (ed.) *Mouse Development.* 10.1007/978-3-642-30406-4_14 ed. Berlin Heidelberg: Springer-Verlag.

BITTLES, A. H., GRANT, J. C., SULLIVAN, S. G. & HUSSAIN, R. 2002. Does inbreeding lead to decreased human fertility? *Ann Hum Biol,* 29**,** 111-30.

BOTCHLETT, R., LI, H., GUO, X., QI, T., ZHAO, J., ZHENG, J., WOO, S. L., PEI, Y., LIU, M., HU, X., CHEN, G., GUO, T., YANG, S., LI, Q., XIAO, X., HUO, Y. & WU, C. 2016. Glucose and Palmitate Differentially Regulate PFKFB3/iPFK2 and Inflammatory Responses in Mouse Intestinal Epithelial Cells. *Sci Rep,* 6**,** 28963.

BOTCHLETT, R. & WU, C. 2018. Diet Composition for the Management of Obesity and Obesity-related Disorders. *J Diabetes Mellit Metab Syndr,* 3**,** 10-25.

BOUDOURES, A. L., CHI, M., THOMPSON, A., ZHANG, W. & MOLEY, K. H. 2016. The effects of voluntary exercise on oocyte quality in a diet-induced obese murine model. *Reproduction,* 151**,** 261-70.

BURROWS, T. L., WHATNALL, M. C., PATTERSON, A. J. & HUTCHESSON, M. J. 2017. Associations between Dietary Intake and Academic Achievement in College Students: A Systematic Review. *Healthcare (Basel),* 5.

CASPI, C. E., SORENSEN, G., SUBRAMANIAN, S. V. & KAWACHI, I. 2012. The local food environment and diet: a systematic review. *Health Place,* 18**,** 1172-87.

CHAMPY, M. F., SELLOUM, M., ZEITLER, V., CARADEC, C., JUNG, B., ROUSSEAU, S., POUILLY, L., SORG, T. & AUWERX, J. 2008. Genetic background determines metabolic phenotypes in the mouse. *Mamm Genome,* 19**,** 318-31.

CHANG, H. & SUAREZ, S. S. 2010. Rethinking the relationship between hyperactivation and chemotaxis in mammalian sperm. *Biol Reprod,* 83**,** 507-13.

CHIA, R., ACHILLI, F., FESTING, M. F. & FISHER, E. M. 2005. The origins and uses of mouse outbred stocks. *Nat Genet,* 37**,** 1181-6.

CHILDS, S., HENNESSY, A. A., SREENAN, J. M., WATHES, D. C., CHENG, Z., STANTON, C., DISKIN, M. G. & KENNY, D. A. 2008. Effect of level of dietary n-3 polyunsaturated fatty acid supplementation on systemic and tissue fatty acid concentrations and on selected reproductive variables in cattle. *Theriogenology,* 70**,** 595-611.

CINQUINA, V., CALVIGIONI, D., FARLIK, M., HALBRITTER, F., FIFE-GERNEDL, V., SHIRRAN, S. L., FUSZARD, M. A., BOTTING, C. H., POULLET, P., PISCITELLI, F., MATE, Z., SZABO, G., YANAGAWA, Y., KASPER, S., DI MARZO, V., MACKIE, K., MCBAIN, C. J., BOCK, C., KEIMPEMA, E. & HARKANY, T. 2020. Life-long epigenetic programming of cortical architecture by maternal 'Western' diet during pregnancy. *Mol Psychiatry,* 25**,** 22-36.

CLARKE, H. 2017. Control of Mammalian Oocyte Development by Interactions with the Maternal Follicular Environment. *Results Probl Cell Differ,* 63**,** 17-41.

CLEMENTE-SUAREZ, V. J., BELTRAN-VELASCO, A. I., REDONDO-FLOREZ, L., MARTIN-RODRIGUEZ, A. & TORNERO-AGUILERA, J. F. 2023. Global Impacts of Western Diet and Its Effects on Metabolism and Health: A Narrative Review. *Nutrients,* 15.

COLLINS, G. G. & ROSSI, B. V. 2015. The impact of lifestyle modifications, diet, and vitamin supplementation on natural fertility. *Fertil Res Pract,* 1**,** 11.

COOKSEY STOWERS, K., JIANG, Q., ATOLOYE, A., LUCAN, S. & GANS, K. 2020. Racial Differences in Perceived Food Swamp and Food Desert Exposure and Disparities in Self-Reported Dietary Habits. *Int J Environ Res Public Health,* 17.

CRESCENZO, R., BIANCO, F., COPPOLA, P., MAZZOLI, A., CIGLIANO, L., LIVERINI, G. & IOSSA, S. 2015. The effect of high-fat--high-fructose diet on skeletal muscle mitochondrial energetics in adult rats. *Eur J Nutr,* 54**,** 183-92.

CROXATTO, H. B. 2002. Physiology of gamete and embryo transport through the fallopian tube. *Reprod Biomed Online,* 4**,** 160-9.

CZAJKOWSKA, K., WALEWSKA, A., ISHIKAWA, T., SZCZEPANSKA, K. & AJDUK, A. 2020. Age-related alterations in fertilization-induced Ca2+ oscillations depend on the genetic background of mouse oocytesdagger. *Biol Reprod,* 103**,** 986-999.

DE BIE, J., MAREI, W. F. A., MAILLO, V., JORDAENS, L., GUTIERREZ-ADAN, A., BOLS, P. E. J. & LEROY, J. 2017. Differential effects of high and low glucose concentrations during lipolysis-like conditions on bovine in vitro oocyte quality, metabolism and subsequent embryo development. *Reprod Fertil Dev,* 29**,** 2284-2300.

DELLA VEDOVA, M. C., MUNOZ, M. D., SANTILLAN, L. D., PLATEO-PIGNATARI, M. G., GERMANO, M. J., RINALDI TOSI, M. E., GARCIA, S., GOMEZ, N. N., FORNES, M. W., GOMEZ MEJIBA, S. E. & RAMIREZ, D. C. 2016. A Mouse Model of Diet-Induced Obesity Resembling Most Features of Human Metabolic Syndrome. *Nutr Metab Insights,* 9**,** 93-102.

DESMET, K. L., VAN HOECK, V., GAGNE, D., FOURNIER, E., THAKUR, A., O'DOHERTY, A. M., WALSH, C. P., SIRARD, M. A., BOLS, P. E. & LEROY, J. L. 2016. Exposure of bovine oocytes and embryos to elevated non-esterified fatty acid concentrations: integration of epigenetic and transcriptomic signatures in resultant blastocysts. *BMC Genomics,* 17**,** 1004.

DIAMOND, J. M. 1987. Causes of death before birth. *Nature,* 329**,** 487-8.

DIAS, M. D. E., DOS REIS, S. A., DA CONCEIÇAO, L. L., SEDIYAMA, C. M. N. D., PEREIRA, S. S., DE OLIVEIRA, L. L., PELUZIO, M. D. G., MARTINEZ, J. A. & MILAGRO, F. I. 2021. Diet-induced obesity in animal models: points to consider and influence on metabolic markers. *Diabetology & Metabolic Syndrome,* 13.

DREWNOWSKI, A. & POPKIN, B. M. 1997. The nutrition transition: new trends in the global diet. *Nutr Rev,* 55**,** 31-43.

ELLACOTT, K. L., MORTON, G. J., WOODS, S. C., TSO, P. & SCHWARTZ, M. W. 2010. Assessment of feeding behavior in laboratory mice. *Cell Metab,* 12**,** 10-7.

FETTERMAN, J. L., ZELICKSON, B. R., JOHNSON, L. W., MOELLERING, D. R., WESTBROOK, D. G., POMPILIUS, M., SAMMY, M. J., JOHNSON, M., DUNHAM-SNARY, K. J., CAO, X., BRADLEY, W. E., ZHANG, J., WEI, C. C., CHACKO, B., SCHURR, T. G., KESTERSON, R. A., DELL'ITALIA, L. J., DARLEY-USMAR, V. M., WELCH, D. R. & BALLINGER, S. W. 2013. Mitochondrial genetic background modulates bioenergetics and susceptibility to acute cardiac volume overload. *Biochem J,* 455**,** 157-67.

FIACCO, S., WALTHER, A. & EHLERT, U. 2019. Steroid secretion in healthy aging. *Psychoneuroendocrinology,* 105**,** 64-78.

FOX, G. F. K. 2019. Mouse Breeding Colony Management. *Division of Laboratory Animal Resources.* Research University of Kentucky.

FREEMAN, H., SHIMOMURA, K., HORNER, E., COX, R. D. & ASHCROFT, F. M. 2006. Nicotinamide nucleotide transhydrogenase: a key role in insulin secretion. *Cell Metab,* 3**,** 35-45.

FRYAR D., J. P. 2017. Fast food consumption among adults in the United States, 2013-2018. Centers for Disease Controle and Prevention (CDC), National Center for Health Statistics.

GAO, J., LIU, M., LIU, J., SHI, P., CUI, H., ZHAO, S., ZHANG, X. & TAO, C. 2023. Effect of high-fat diet on the lipid profile of ovarian granulosa cells and female reproduction in mice. *PLoS One,* 18**,** e0287534.

GAO, S., CZIRR, E., CHUNG, Y. G., HAN, Z. & LATHAM, K. E. 2004. Genetic variation in oocyte phenotype revealed through parthenogenesis and cloning: correlation with differences in pronuclear epigenetic modification. *Biol Reprod,* 70**,** 1162-70.

GESINK LAW, D. C., MACLEHOSE, R. F. & LONGNECKER, M. P. 2007. Obesity and time to pregnancy. *Hum Reprod,* 22**,** 414-20.

GILL, T. J. 1980. The use of randomly bred and genetically defined animals in biomedical research. *Am J Pathol,* 101**,** S21-32.

GODFREY, K. M., LILLYCROP, K. A., BURDGE, G. C., GLUCKMAN, P. D. & HANSON, M. A. 2007. Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease. *Pediatr Res,* 61**,** 5R-10R.

GREGORY, S. G., SEKHON, M., SCHEIN, J., ZHAO, S., OSOEGAWA, K., SCOTT, C. E., EVANS, R. S., BURRIDGE, P. W., COX, T. V., FOX, C. A., HUTTON, R. D., MULLENGER, I. R., PHILLIPS, K. J., SMITH, J., STALKER, J., THREADGOLD, G. J., BIRNEY, E., WYLIE, K., CHINWALLA, A., WALLIS, J., HILLIER, L., CARTER, J., GAIGE, T., JAEGER, S., KREMITZKI, C., LAYMAN, D., MAAS, J., MCGRANE, R., MEAD, K., WALKER, R., JONES, S., SMITH, M., ASANO, J., BOSDET, I., CHAN, S., CHITTARANJAN, S., CHIU, R., FJELL, C., FUHRMANN, D., GIRN, N., GRAY, C., GUIN, R., HSIAO, L., KRZYWINSKI, M., KUTSCHE, R., LEE, S. S., MATHEWSON, C., MCLEAVY, C., MESSERVIER, S., NESS, S., PANDOH, P., PRABHU, A. L., SAEEDI, P., SMAILUS, D., SPENCE, L., STOTT, J., TAYLOR, S., TERPSTRA, W., TSAI, M., VARDY, J., WYE, N., YANG, G., SHATSMAN, S., AYODEJI, B., GEER, K., TSEGAYE, G., SHVARTSBEYN, A., GEBREGEORGIS, E., KROL, M., RUSSELL, D., OVERTON, L., MALEK, J. A., HOLMES, M., HEANEY, M., SHETTY, J., FELDBLYUM, T., NIERMAN, W. C., CATANESE, J. J., HUBBARD, T., WATERSTON, R. H., ROGERS, J., DE JONG, P. J., FRASER, C. M., MARRA, M., MCPHERSON, J. D. & BENTLEY, D. R. 2002. A physical map of the mouse genome. *Nature,* 418**,** 743-50.

GUTHRIE, J. F., LIN, B. H. & FRAZAO, E. 2002. Role of food prepared away from home in the American diet, 1977-78 versus 1994-96: changes and consequences. *J Nutr Educ Behav,* 34**,** 140-50.

HAMDI, M., SANCHEZ-CALABUIG, M. J., RODRIGUEZ-ALONSO, B., BAGES ARNAL, S., ROUSSI, K., STURMEY, R., GUTIERREZ-ADAN, A., LONERGAN, P. & RIZOS, D. 2019. Gene expression and metabolic response of bovine oviduct epithelial cells to the early embryo. *Reproduction,* 158**,** 85-94.

HEALTH, N. I. O. 2000. The Practical Guide: Identification, Evaluation and Treatment of Overweight and Obesity in Adults.

HOLT, W. V. & FAZELI, A. 2016a. Sperm selection in the female mammalian reproductive tract. Focus on the oviduct: Hypotheses, mechanisms, and new opportunities. *Theriogenology,* 85**,** 105-12.

HOLT, W. V. & FAZELI, A. 2016b. Sperm Storage in the Female Reproductive Tract. *Annu Rev Anim Biosci,* 4**,** 291-310.

IBANEZ, E., ALBERTINI, D. F. & OVERSTROM, E. W. 2005. Effect of genetic background and activating stimulus on the timing of meiotic cell cycle progression in parthenogenetically activated mouse oocytes. *Reproduction,* 129**,** 27-38.

IGOSHEVA, N., ABRAMOV, A. Y., POSTON, L., ECKERT, J. J., FLEMING, T. P., DUCHEN, M. R. & MCCONNELL, J. 2010. Maternal diet-induced obesity alters mitochondrial activity and redox status in mouse oocytes and zygotes. *PLoS One,* 5**,** e10074.

JAMSAI, D. & O'BRYAN, M. K. 2011. Mouse models in male fertility research. *Asian J Androl,* 13**,** 139-51.

JOHNSON, R. J., SEGAL, M. S., SAUTIN, Y., NAKAGAWA, T., FEIG, D. I., KANG, D. H., GERSCH, M. S., BENNER, S. & SANCHEZ-LOZADA, L. G. 2007. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am J Clin Nutr,* 86**,** 899-906.

JORDAENS, L., ARIAS-ALVAREZ, M., PINTELON, I., THYS, S., VALCKX, S., DEZHKAM, Y., BOLS, P. E. & LEROY, J. L. 2015. Elevated non-esterified fatty acid concentrations hamper bovine oviductal epithelial cell physiology in three different in vitro culture systems. *Theriogenology,* 84**,** 899-910.

JORDAENS, L., VAN HOECK, V., DE BIE, J., BERTH, M., MAREI, W. F. A., DESMET, K. L. J., BOLS, P. E. J. & LEROY, J. 2017a. Non-esterified fatty acids in early luteal bovine oviduct fluid mirror plasma concentrations: An ex vivo approach. *Reprod Biol,* 17**,** 281-284.

JORDAENS, L., VAN HOECK, V., MAILLO, V., GUTIERREZ-ADAN, A., MAREI, W. F. A., VLAEMINCK, B., THYS, S., STURMEY, R. G., BOLS, P. E. J. & LEROY, J. 2017b. Maternal metabolic stress may affect oviduct gatekeeper function. *Reproduction,* 153**,** 759-773.

JORDAENS, L., VAN HOECK, V., PINTELON, I., THYS, S., BOLS, P. E. J., MAREI, W. F. A. & LEROY, J. 2020. Altered embryotrophic capacities of the bovine oviduct under elevated free fatty acid conditions: an in vitro embryo--oviduct co-culture model. *Reprod Fertil Dev,* 32**,** 553-563.

KAHLE, M., HORSCH, M., FRIDRICH, B., SEELIG, A., SCHULTHEISS, J., LEONHARDT, J., IRMLER, M., BECKERS, J., RATHKOLB, B., WOLF, E., FRANKE, N., GAILUS-DURNER, V., FUCHS, H., DE ANGELIS, M. H. & NESCHEN, S. 2013. Phenotypic comparison of common mouse strains developing high-fat diet-induced hepatosteatosis. *Mol Metab,* 2**,** 435-46.

KALETA, E. 1977. Influence of genetic factors on the fertilization of mouse ova in vitro. *J Reprod Fertil,* 51**,** 375-81.

KLEINERT, M., CLEMMENSEN, C., HOFMANN, S. M., MOORE, M. C., RENNER, S., WOODS, S. C., HUYPENS, P., BECKERS, J., DE ANGELIS, M. H., SCHURMANN, A., BAKHTI, M., KLINGENSPOR, M., HEIMAN, M., CHERRINGTON, A. D., RISTOW, M., LICKERT, H., WOLF, E., HAVEL, P. J., MULLER, T. D. & TSCHOP, M. H. 2018. Animal models of obesity and diabetes mellitus. *Nat Rev Endocrinol,* 14**,** 140-162.

KO, E. A., KIM, H. R., KIM, Y. B., KIM, H. S. & LEE, S. H. 2017. Effect of High Fructose Corn Syrup (HFCS) Intake on the Female Reproductive Organs and Lipid Accumulation in Adult Rats. *Dev Reprod,* 21**,** 151-156.

LABORATORY, T. J. 2019. *Here's What You Need To Know about the C57BL/6 Substrains.* [Online]. [Accessed].

LALLY, P., BARTLE, N. & WARDLE, J. 2011. Social norms and diet in adolescents. *Appetite,* 57**,** 623-7.

LAMAS, S., FRANQUINHO, F., MORGADO, M., MESQUITA, J. R., GARTNER, F. & AMORIM, I. 2020. C57BL/6J and B6129F1 Embryo Transfer: Unilateral and Bilateral Transfer, Embryo Number and Recipient Female Background Control for the Optimization of Embryo Survival and Litter Size. *Animals (Basel),* 10.

LÊ, K. A. & TAPPY, L. 2006. Metabolic effects of fructose. *Current Opinion in Clinical Nutrition and Metabolic Care,* 9**,** 469-475.

LEAL, C. L. V., CANON-BELTRAN, K., CAJAS, Y. N., HAMDI, M., YARYES, A., MILLAN DE LA BLANCA, M. G., BELTRAN-BRENA, P., MAZZARELLA, R., DA SILVEIRA, J. C., GUTIERREZ-ADAN, A., GONZALEZ, E. M. & RIZOS, D. 2022. Extracellular vesicles from oviductal and uterine fluids supplementation in sequential in vitro culture improves bovine embryo quality. *J Anim Sci Biotechnol,* 13**,** 116.

LEESE, H. J. 1988. The formation and function of oviduct fluid. *J Reprod Fertil,* 82**,** 843-56.

LEITER, E. H. 2002. Mice with targeted gene disruptions or gene insertions for diabetes research: problems, pitfalls, and potential solutions. *Diabetologia,* 45**,** 296-308.

LEROY, J. L., VANHOLDER, T., MATEUSEN, B., CHRISTOPHE, A., OPSOMER, G., DE KRUIF, A., GENICOT, G. & VAN SOOM, A. 2005. Non-esterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. *Reproduction,* 130**,** 485-95.

LEROY, J. L. M. R., DE BIE, J., JORDAENS, L., DESMET, K., SMITS, A., MAREI, W. F. A., BOLS, P. E. J. & VAN HOECK, V. 2017. Negative energy balance and metabolic stress in relation to oocyte and embryo quality: an update on possible pathways reducing fertility in dairy cows. *Animal Reproduction,* 14**,** 497-506.

LOY, S. L., CHEUNG, Y. B., SOH, S. E., NG, S., TINT, M. T., ARIS, I. M., BERNARD, J. Y., CHONG, Y. S., GODFREY, K. M., SHEK, L. P., TAN, K. H., LEE, Y. S., TAN, H. H., CHERN, B. S. M., LEK, N., YAP, F., CHAN, S. Y., CHI, C. & CHAN, J. K. Y. 2018. Female adiposity and time-to-pregnancy: a multiethnic prospective cohort. *Hum Reprod,* 33**,** 2141-2149.

LUCAS, E. 2013. Epigenetic effects on the embryo as a result of periconceptional environment and assisted reproduction technology. *Reprod Biomed Online,* 27**,** 477-85.

LUZZO, K. M., WANG, Q., PURCELL, S. H., CHI, M., JIMENEZ, P. T., GRINDLER, N., SCHEDL, T. & MOLEY, K. H. 2012. High fat diet induced developmental defects in the mouse: oocyte meiotic aneuploidy and fetal growth retardation/brain defects. *PLoS One,* 7**,** e49217.

MAHABIR, E., VOLLAND, R., LANDSBERGER, A., MANZ, S., NA, E., URBAN, I. & MICHEL, G. 2018. Reproductive Performance after Unilateral or Bilateral Oviduct Transfer of 2-Cell Embryos in Mice. *J Am Assoc Lab Anim Sci,* 57**,** 110-114.

MAHE, C., PRANOMPHON, T., REYNAUD, K., LAFFONT, L., MEYLHEUC, T., SCHOEN, J., MERMILLOD, P. & SAINT-DIZIER, M. 2023. Sperm-fluid-cell interplays in the bovine oviduct: glycosaminoglycans modulate sperm binding to the isthmic reservoir. *Sci Rep,* 13**,** 10311.

MAILLO, V., GAORA, P. O., FORDE, N., BESENFELDER, U., HAVLICEK, V., BURNS, G. W., SPENCER, T. E., GUTIERREZ-ADAN, A., LONERGAN, P. & RIZOS, D. 2015. Oviduct-Embryo Interactions in Cattle: Two-Way Traffic or a One-Way Street? *Biol Reprod,* 92**,** 144.

MAILLO, V., RIZOS, D., BESENFELDER, U., HAVLICEK, V., KELLY, A. K., GARRETT, M. & LONERGAN, P. 2012. Influence of lactation on metabolic characteristics and embryo development in postpartum Holstein dairy cows. *J Dairy Sci,* 95**,** 3865-76.

MALESZA, I. J., MALESZA, M., WALKOWIAK, J., MUSSIN, N., WALKOWIAK, D., ARINGAZINA, R., BARTKOWIAK-WIECZOREK, J. & MADRY, E. 2021. High-Fat, Western-Style Diet, Systemic Inflammation, and Gut Microbiota: A Narrative Review. *Cells,* 10.

MALIK, V. S., POPKIN, B. M., BRAY, G. A., DESPRES, J. P. & HU, F. B. 2010. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation,* 121**,** 1356-64.

MAREI, W. F. A., DE BIE, J., MOHEY-ELSAEED, O., WYDOOGHE, E., BOLS, P. E. J. & LEROY, J. 2017. Alpha-linolenic acid protects the developmental capacity of bovine cumulus-oocyte complexes matured under lipotoxic conditions in vitro. *Biol Reprod,* 96**,** 1181-1196.

MAREI, W. F. A., SMITS, A., MOHEY-ELSAEED, O., PINTELON, I., GINNEBERGE, D., BOLS, P. E. J., MOERLOOSE, K. & LEROY, J. 2020. Differential effects of high fat diet-induced obesity on oocyte mitochondrial functions in inbred and outbred mice. *Sci Rep,* 10**,** 9806.

MEULDERS, B., MAREI, W. F. A., XHONNEUX, I., BOLS, P. E. J. & LEROY, J. 2023. Effect of lipotoxicity on mitochondrial function and epigenetic programming during bovine in vitro embryo production. *Sci Rep,* 13**,** 21664.

MORENO-FERNANDEZ, S., GARCES-RIMON, M., VERA, G., ASTIER, J., LANDRIER, J. F. & MIGUEL, M. 2018. High Fat/High Glucose Diet Induces Metabolic Syndrome in an Experimental Rat Model. *Nutrients,* 10.

NAPIER, B. A., ANDRES-TERRE, M., MASSIS, L. M., HRYCKOWIAN, A. J., HIGGINBOTTOM, S. K., CUMNOCK, K., CASEY, K. M., HAILESELASSIE, B., LUGO, K. A., SCHNEIDER, D. S., SONNENBURG, J. L. & MONACK, D. M. 2019. Western diet regulates immune status and the response to LPS-driven sepsis independent of diet-associated microbiome. *Proc Natl Acad Sci U S A,* 116**,** 3688-3694.

NELSON, K. A., DANIELS, G. J., FOURNIE, J. W. & HEMMER, M. J. 2013. Optimization of whole-body zebrafish sectioning methods for mass spectrometry imaging. *J Biomol Tech,* 24**,** 119-27.

NICHOLSON, A., REIFSNYDER, P. C., MALCOLM, R. D., LUCAS, C. A., MACGREGOR, G. R., ZHANG, W. & LEITER, E. H. 2010. Diet-induced obesity in two C57BL/6 substrains with intact or mutant nicotinamide nucleotide transhydrogenase (Nnt) gene. *Obesity (Silver Spring),* 18**,** 1902-5.

OBER, C., ELIAS, S., KOSTYU, D. D. & HAUCK, W. W. 1992. Decreased fecundability in Hutterite couples sharing HLA-DR. *Am J Hum Genet,* 50**,** 6-14.

OHTSU, A., TANAKA, H., SENO, K., IWATA, H., KUWAYAMA, T. & SHIRASUNA, K. 2017. Palmitic acid stimulates interleukin-8 via the TLR4/NF-kappaB/ROS pathway and induces mitochondrial dysfunction in bovine oviduct epithelial cells. *Am J Reprod Immunol,* 77.

PANCHAL, S. K. & BROWN, L. 2011. Rodent models for metabolic syndrome research. *J Biomed Biotechnol,* 2011**,** 351982.

PEREIRA, M. A., KARTASHOV, A. I., EBBELING, C. B., VAN HORN, L., SLATTERY, M. L., JACOBS, D. R., JR. & LUDWIG, D. S. 2005. Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. *Lancet,* 365**,** 36-42.

PERLMAN, R. L. 2016. Mouse models of human disease: An evolutionary perspective. *Evol Med Public Health,* 2016**,** 170-6.

RAKHRA, V., GALAPPATHTHY, S. L., BULCHANDANI, S. & CABANDUGAMA, P. K. 2020. Obesity and the Western Diet: How We Got Here. *Mo Med,* 117**,** 536-538.

REYNOLDS, K. A., BOUDOURES, A. L., CHI, M. M., WANG, Q. & MOLEY, K. H. 2015. Adverse effects of obesity and/or high-fat diet on oocyte quality and metabolism are not reversible with resumption of regular diet in mice. *Reprod Fertil Dev,* 27**,** 716-24.

RIVERA, R. M. & ROSS, J. W. 2013. Epigenetics in fertilization and preimplantation embryo development. *Prog Biophys Mol Biol,* 113**,** 423-32.

ROBKER, R. L. 2008. Evidence that obesity alters the quality of oocytes and embryos. . *Pathophysiology,* 15**,** 115-121.

ROBKER, R. L., AKISON, L. K., BENNETT, B. D., THRUPP, P. N., CHURA, L. R., RUSSELL, D. L., LANE, M. & NORMAN, R. J. 2009. Obese women exhibit differences in ovarian

metabolites, hormones, and gene expression compared with moderate-weight women. *J Clin Endocrinol Metab,* 94**,** 1533-40.

ROSENFELD, C. S., GRIMM, K. M., LIVINGSTON, K. A., BROKMAN, A. M., LAMBERSON, W. E. & ROBERTS, R. M. 2003. Striking variation in the sex ratio of pups born to mice according to whether maternal diet is high in fat or carbohydrate. *Proc Natl Acad Sci U S A,* 100**,** 4628-32.

SABEN, J. L., ASGHAR, Z., RHEE, J. S., DRURY, A., SCHEAFFER, S. & MOLEY, K. H. 2016. Excess Maternal Fructose Consumption Increases Fetal Loss and Impairs Endometrial Decidualization in Mice. *Endocrinology,* 157**,** 956-68.

SCHULZE, M. B., MANSON, J. E., LUDWIG, D. S., COLDITZ, G. A., STAMPFER, M. J., WILLETT, W. C. & HU, F. B. 2004. Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. *JAMA,* 292**,** 927-34.

SHELDON, I. M., CRONIN, J. G., POSPIECH, M. & TURNER, M. L. 2018. Symposium review: Mechanisms linking metabolic stress with innate immunity in the endometrium. *J Dairy Sci,* 101**,** 3655-3664.

SHERMAN, B. M., WEST, J. H. & KORENMAN, S. G. 1976. The menopausal transition: analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles of older women. *J Clin Endocrinol Metab,* 42**,** 629-36.

SHERWOOD, N. E., JEFFERY, R. W., FRENCH, S. A., HANNAN, P. J. & MURRAY, D. M. 2000. Predictors of weight gain in the Pound of Prevention study. *Int J Obes Relat Metab Disord,* 24**,** 395-403.

SILVESTRIS, E., DE PERGOLA, G., ROSANIA, R. & LOVERRO, G. 2018. Obesity as disruptor of the female fertility. *Reprod Biol Endocrinol,* 16**,** 22.

SIMINO, L. A. P., PANZARIN, C., TORSONI, M. A., IGNACIO-SOUZA, L. M., MILANSKI, M. & TORSONI, A. S. 2021. Maternal resistance to diet-induced obesity partially protects newborn and post-weaning male mice offspring from metabolic disturbances. *J Dev Orig Health Dis,* 12**,** 660-670.

SMITS, A., MAREI, W. F. A., DE NEUBOURG, D. & LEROY, J. 2021. Diet normalization or caloric restriction as a preconception care strategy to improve metabolic health and oocyte quality in obese outbred mice. *Reprod Biol Endocrinol,* 19**,** 166.

SMITS, A., MAREI, W. F. A., MOORKENS, K., BOLS, P. E. J., DE NEUBOURG, D. & LEROY, J. 2022. Obese outbred mice only partially benefit from diet normalization or calorie restriction as preconception care interventions to improve metabolic health and oocyte quality. *Hum Reprod,* 37**,** 2867-2884.

SPEAROW, J. L. & BARKLEY, M. 1999. Genetic control of hormone-induced ovulation rate in mice. *Biol Reprod,* 61**,** 851-6.

STADLER, J. T. & MARSCHE, G. 2020. Obesity-Related Changes in High-Density Lipoprotein Metabolism and Function. *Int J Mol Sci,* 21.

SUZUKI, O., ASANO, T., YAMAMOTO, Y., TAKANO, K. & KOURA, M. 1996. Development in vitro of preimplantation embryos from 55 mouse strains. *Reprod Fertil Dev,* 8**,** 975-80.

TAPPY, L. & LE, K. A. 2010. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev,* 90**,** 23-46.

TASKINEN, M. R., PACKARD, C. J. & BOREN, J. 2019. Dietary Fructose and the Metabolic Syndrome. *Nutrients,* 11.

TRAN, L. T., YUEN, V. G. & MCNEILL, J. H. 2009. The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. *Molecular and Cellular Biochemistry,* 332**,** 145-159.

TRIPATHI, S. K., FARMAN, M., NANDI, S., KUMAR, V. G. & GUPTA, P. S. P. 2016. Oviductal and uterine fluid analytes as biomarkers of metabolic stress in ewes (

). *Small Ruminant Research,* 144**,** 225-228.

TUTTLE, A. H., PHILIP, V. M., CHESLER, E. J. & MOGIL, J. S. 2018. Comparing phenotypic variation between inbred and outbred mice. *Nat Methods,* 15**,** 994-996.

VALCKX, S. D., ARIAS-ALVAREZ, M., DE PAUW, I., FIEVEZ, V., VLAEMINCK, B., FRANSEN, E., BOLS, P. E. & LEROY, J. L. 2014a. Fatty acid composition of the follicular fluid of normal weight, overweight and obese women undergoing assisted reproductive treatment: a descriptive cross-sectional study. *Reprod Biol Endocrinol,* 12**,** 13.

VALCKX, S. D., DE PAUW, I., DE NEUBOURG, D., INION, I., BERTH, M., FRANSEN, E., BOLS, P. E. & LEROY, J. L. 2012. BMI-related metabolic composition of the follicular fluid of women undergoing assisted reproductive treatment and the consequences for oocyte and embryo quality. *Hum Reprod,* 27**,** 3531-9.

VALCKX, S. D., VAN HOECK, V., ARIAS-ALVAREZ, M., MAILLO, V., LOPEZ-CARDONA, A. P., GUTIERREZ-ADAN, A., BERTH, M., CORTVRINDT, R., BOLS, P. E. & LEROY, J. L. 2014b. Elevated non-esterified fatty acid concentrations during in vitro murine follicle growth alter follicular physiology and reduce oocyte developmental competence. *Fertil Steril,* 102**,** 1769-76 e1.

VAN HOECK, V., LEROY, J. L., ARIAS ALVAREZ, M., RIZOS, D., GUTIERREZ-ADAN, A., SCHNORBUSCH, K., BOLS, P. E., LEESE, H. J. & STURMEY, R. G. 2013. Oocyte developmental failure in response to elevated nonesterified fatty acid concentrations: mechanistic insights. *Reproduction,* 145**,** 33-44.

VAN OTTERDIJK, S. D. & MICHELS, K. B. 2016. Transgenerational epigenetic inheritance in mammals: how good is the evidence? *FASEB J,* 30**,** 2457-65.

VELARDE, M. C. 2014. Mitochondrial and sex steroid hormone crosstalk during aging. *Longev Healthspan,* 3**,** 2.

VELAZQUEZ, M. A. 2015. Impact of maternal malnutrition during the periconceptional period on mammalian preimplantation embryo development. *Domest Anim Endocrinol,* 51**,** 27-45.

WARTELLA EA, L. A., BOON CS 2010. *Overview of health and diet in America,* Washington (DC), National Academies Press (US).

WILLIAMS, L. M., CAMPBELL, F. M., DREW, J. E., KOCH, C., HOGGARD, N., REES, W. D., KAMOLRAT, T., THI NGO, H., STEFFENSEN, I. L., GRAY, S. R. & TUPS, A. 2014. The

development of diet-induced obesity and glucose intolerance in C57BL/6 mice on a high-fat diet consists of distinct phases. *PLoS One,* 9**,** e106159.

WISE, L. A., ROTHMAN, K. J., MIKKELSEN, E. M., SORENSEN, H. T., RIIS, A. & HATCH, E. E. 2010. An internet-based prospective study of body size and time-to-pregnancy. *Hum Reprod,* 25**,** 253-64.

WONG, S. K., CHIN, K. Y., SUHAIMI, F. H., FAIRUS, A. & IMA-NIRWANA, S. 2016. Animal models of metabolic syndrome: a review. *Nutr Metab (Lond),* 13**,** 65.

WONNACOTT, K. E., KWONG, W. Y., HUGHES, J., SALTER, A. M., LEA, R. G., GARNSWORTHY, P. C. & SINCLAIR, K. D. 2010. Dietary omega-3 and -6 polyunsaturated fatty acids affect the composition and development of sheep granulosa cells, oocytes and embryos. *Reproduction,* 139**,** 57-69.

WU, L. L., DUNNING, K. R., YANG, X., RUSSELL, D. L., LANE, M., NORMAN, R. J. & ROBKER, R. L. 2010. High-fat diet causes lipotoxicity responses in cumulus-oocyte complexes and decreased fertilization rates. *Endocrinology,* 151**,** 5438-45.

WU, L. L., RUSSELL, D. L., WONG, S. L., CHEN, M., TSAI, T. S., ST JOHN, J. C., NORMAN, R. J., FEBBRAIO, M. A., CARROLL, J. & ROBKER, R. L. 2015. Mitochondrial dysfunction in oocytes of obese mothers: transmission to offspring and reversal by pharmacological endoplasmic reticulum stress inhibitors. *Development,* 142**,** 681-91.

YANG, X., WU, L. L., CHURA, L. R., LIANG, X., LANE, M., NORMAN, R. J. & ROBKER, R. L. 2012. Exposure to lipid-rich follicular fluid is associated with endoplasmic reticulum stress and impaired oocyte maturation in cumulus-oocyte complexes. *Fertil Steril,* 97**,** 1438-43.

YUAN, M., CHEN, W., TENG, B. & FANG, Y. 2016. Occupational Disparities in the Association between Self-Reported Salt-Eating Habit and Hypertension in Older Adults in Xiamen, China. *Int J Environ Res Public Health,* 13.

ZACHUT, M., ARIELI, A. & MOALLEM, U. 2011. Incorporation of dietary n-3 fatty acids into ovarian compartments in dairy cows and the effects on hormonal and behavioral patterns around estrus. *Reproduction,* 141**,** 833-40.

SUMMARY

Maternal metabolic disorders associated with the consumption of an obesogenic (high fat/high sugar, HF/HS) diet are strongly linked with reduced fertility in women. The focus of the studies presented in this thesis was to determine the temporal changes that occur in the ovarian and oviductal microenvironment upon starting HF/HS diet feeding in mice. In **Chapter 1,** we provide a literature review on obesity and the obesogenic diet-induced metabolic changes and dynamics that lead to systemic metabolic stress and metabolic disease. We describe the involved mechanisms such as dyslipidaemia, oxidative stress and inflammation. The causative link between maternal metabolic health and subfertility is discussed, including the well-documented direct detrimental effects of the metabolic alterations on oocyte quality and ovarian cell functions. These changes appear to be different depending on the used mouse strain. In addition, the function and importance of the oviductal microenvironment, where fertilization and early embryo development take place is described. Furthermore, we discussed the dynamics and the progressive nature of the diet-induced metabolic alterations in the blood, muscles and liver, and defined the gaps in knowledge about the onset and progression of diet-induced effects on the reproductive tissues.

In **Chapter 2**, the specific aims of the thesis are overviewed. We first investigated the impact of feeding an obesogenic diet for different time periods on cellular stress markers, at the gene expression level, in the oviductal epithelial cells of outbred and inbred mice (CHAPTER 3). Concomitant changes in live body weight, and blood cholesterol and cytokine concentrations were also described. Next, we wanted to study whether feeding an obesogenic diet for different time periods would also influence the lipidomic profile of the oviductal epithelium. This was investigated *in situ* using MALDI-Imaging Lipidomics in an outbred mouse model (CHAPTER 4). Finally, to check the temporal changes in the ovarian follicle microenvironment, the impact on granulosa cell transcriptomics (RNAseq analysis) and oocyte quality (lipid content and mitochondrial

features) were examined after different time periods of HF/HS diet feeding in outbred mice (CHAPTER 5).

The impact of obesity and an obesogenic diet on the oviductal microenvironment is much less characterized compared to the impact on the follicular environment and oocyte quality. Furthermore, the onset and duration of changes after the start of an obesogenic diet were unclear. In addition, in diet-induced obesity studies the inbred C57BL/6N strain is most commonly used, however, an outbred strain (Swiss mice) is more pathophysiologically relevant to the human (outbred) physiology, which might facilitate translation to the human population. The sensitivity to obesogenic diet-induced obesity and its influence on the metabolic profile and oocyte quality have been shown to be dependent on the genetic background of the mouse model. In **Chapter 3**, we investigated the impact of feeding an obesogenic diet for different time periods on the transcriptome of oviductal epithelial cells (OECs) in Swiss compared to C57BL/6N mice, specifically focusing on genes involved in oxidative and cellular stress levels and inflammatory responses. We could demonstrate, for the first time, that feeding an obesogenic diet for a short period of only three days resulted in acute systemic changes and acute local oxidative stress or mitochondrial stress effects on OECs, evident already before the development of an obese phenotype. The acute effects in the OECs initiated a cascade of transcriptomic changes to control mitochondrial ROS production and endoplasmic reticulum (ER)-stress. However, prolonged, long-term feeding resulted in a persistent upregulation of (mitochondrial) oxidative stress and ER-stress, with ultimate signs of local and systemic inflammation. Furthermore, different responses to the obesogenic diet were observed between Swiss and C57BL/6N mice.

Since fatty acids play an important role as structural membrane components, in cell signalling and cell-to-cell interactions, alterations to the lipid composition of the oviduct may reduce its ability to support early embryo development, which may have long-

lasting consequences. Therefore, in **Chapter 4**, we studied the dynamics and the nature of changes in the lipidomic profile of the oviductal epithelium upon and during obesogenic diet feeding over time in an outbred Swiss mouse model. We demonstrated that feeding the obesogenic diet resulted in acute changes in the lipid profile in the oviductal epithelium already after 3 days on the obesogenic diet. These remarkable effects are in line with the transcriptomic changes described in chapter 3. The changes in the lipid profile progressively increased and became more persistent after long-term obesogenic diet feeding. Functional annotation revealed a differential abundance of phospholipids, sphingomyelins and lysophospholipids in particular.

In the ovary, it is known that the impact of lipotoxicity, caused by an obesogenic diet, on oocyte quality is mediated by oxidative stress, ER stress and mitochondrial dysfunction. However, the nature and magnitude of these changes may be dependent on the duration of feeding (and thus the development of the obesogenic phenotype), which remained to be investigated. Therefore, in **Chapter 5**, we investigated the impact of feeding an obesogenic diet for different time periods on the ovary and ovarian cells of outbred Swiss mice, with a focus on dynamic changes in granulosa cell transcriptomics and oocyte quality. We reported that feeding an obesogenic diet resulted in acute changes in the lipid content of oocytes and impacted endoplasmic reticulum and mitochondrial functions in granulosa cells already after short-term feeding of only 24 hours till 1 week. The effects progressively increased over time after prolonged obesogenic diet feeding, with increased mitochondrial abnormalities in oocytes and different affected pathways, linked to DNA repair, cell signalling, UPRs and many other GO annotation biological terms in the granulosa cells.

Finally, in **Chapter 6**, the current findings were summarized and discussed in the context of previous existing literature. We conclude that we are the first to show strong evidence of the sensitivity of the oviduct and oviductal epithelium to very short-term changes in the dietary composition, both at the transcriptomic and lipidomic levels *in*

vivo. The different durations of feeding allowed a clear distinction of acute responses and later changes after long-term feeding and after the development of the obese phenotype, and the associated unhealthy metabolic phenotype. We provided a deep insight into a network of sequential events that could demonstrate how the OECs can quickly sense metabolic stress in a matter of only three days, and how such local metabolic stress can evolve over time. Furthermore, we demonstrated important differences in cellular responses between the inbred C57BL/6N strain and outbred Swiss strain which further highlights the importance of choosing physiologically relevant mouse models in other studies. Our findings in the oviduct illustrate that the mechanism by which the obesogenic diet impacts fertility is not only mediated through reduced oocyte quality but might also directly impact early embryo development in the oviduct, potentially leading to long-term effects on foetal development, pregnancy success and postnatal health through epigenetic alterations. In addition, exposure to an obesogenic diet resulted in cell specific changes in oocytes and granulosa cells, already after 24 hours to 1w, which can directly affect oocyte quality and early embryo development, eventually leading to reduced fertility.

We believe that the deep analysis and novel insights of this PhD thesis can be useful for many researchers and form an important fundament for further studies that focus on protecting and enhancing oocyte and embryo development during the very early stages under diet-induced metabolic stressed conditions. Understanding the magnitude and nature of reproductive cell dysfunction after different exposure periods to HF/HS diet is crucial to develop more efficient preconception care intervention strategies to enhance fertility under diet-induced metabolic stress conditions.

SAMENVATTING

Maternale metabole stoornissen worden geassocieerd met de consumptie van een obesogeen (vet- en suikerrijk) dieet en worden sterk gelinkt aan verminderde vruchtbaarheid in vrouwen. De focus van de studies die beschreven worden in deze thesis was de dynamiek en progressie te bepalen van de effecten in het ovariële en oviductale micromilieu na de start van het voeden van het vet- en suikerrijk dieet in muizen. In **Hoofdstuk 1** voorzien we een literatuurstudie over obesitas en de obesogeen dieet geïnduceerde veranderingen en dynamiek die leiden tot systemische metabole stress en verminderde metabole gezondheid. We beschrijven de betrokken mechanismen zoals dyslipidemie, oxidatieve stress en inflammatie. Het oorzakelijk verband tussen maternale metabole gezondheid en subfertiliteit wordt beschreven, alsook de uitgebreid gedocumenteerde directe nadelige effecten van metabole wijzigingen op eicel kwaliteit en ovariële cel functies. Deze veranderingen blijken verschillend te zijn afhankelijk van het gebruikte muizen ras. Verder worden de functie en het belang van het oviductale micromilieu, waar bevruchting en de vroege embryonale ontwikkeling plaatsvinden, beschreven. We bespreken ook de dynamiek en progressieve karakter van de dieet geïnduceerde metabole veranderingen in het bloed, spieren en lever en we bepalen het gebrek aan kennis over de start en progressie van dieet geïnduceerde effecten op de reproductieve weefsels.

In **Hoofdstuk 2** worden de specifieke doelstellingen van de thesis beschreven. Eerst onderzochten we de impact van een obesogeen dieet na verschillende tijdsperioden op cellulaire stress merkers, op gen expressie niveau in de oviductale epitheelcellen van outbred and inbred (inteelt) muizen (HOOFDSTUK 3). We beschreven ook de gelijktijdige veranderingen in lichaamsgewicht en cholesterol en cytokine concentraties in het bloed. Vervolgens wilden we bestuderen of het voeden van een obesogeen dieet voor verschillende tijdsperioden ook het lipidomisch profiel van het oviductaal epitheel zou beïnvloeden. Dit werd onderzocht *in situ* doormiddel van MALDI-Imaging

Lipidomics in een outbred muis model (HOOFDSTUK 4). Om de tijdsafhankelijke veranderingen in het ovarieel folliculair micromilieu te bestuderen, werd de impact op granulosa cell transcriptomics (RNA seq analyse) en eicel kwaliteit (vet gehalte en mitochondriale kenmerken) onderzocht na verschillende tijdsperioden van het voeden van een vet- en suikerrijk dieet in outbred muizen (HOOFDSTUK 5).

De invloed van obesitas en een obesogeen dieet op het oviductaal micromilieu werd tot nu toe veel minder beschreven in vergelijking met de impact op het folliculair milieu en eicel kwaliteit. Verder was de start en duur van veranderingen na de start van een obesogeen dieet nog onduidelijk. Het inteelt C57BL/6N muizen ras wordt het meest gebruikt in dieet-geïnduceerde obesitas studies. Echter, een outbred muizen ras (Swiss muizen) is pathofysiologisch meer relevant voor de humane (outbred) fysiologie wat vertaling naar de humane populatie makkelijker zou kunnen bevorderen. De gevoeligheid aan obesogeen dieet geïnduceerde obesitas en diens invloed op het metabole profiel en eicel kwaliteit blijken afhankelijk te zijn van de genetische achtergrond van het muis model. In **Hoofdstuk 3** onderzochten we de invloed van het voeden van een obesogeen dieet voor verschillende tijdsperioden op het transcriptoom van de oviductale epitheelcellen (OECs) in Swiss muizen vergeleken met C57BL/6N muizen, waarbij we specifiek focusten op genen die betrokken zijn in oxidatieve en cellulaire stress en inflammatoire reacties. We waren de eersten die konden aantonen dat het voeden van het obesogeen dieet voor een korte periode van slechts drie dagen reeds resulteerde in acute systemische veranderingen en acute lokale oxidatieve of mitochondriale stress effecten op de OECs, die duidelijk werden reeds vóór de ontwikkeling van een obees fenotype. De acute effecten in de OECs initieerden een cascade van transcriptomische veranderingen om mitochondriale ROS productie en endoplasmatisch reticulum (ER) stress onder controle te houden. Echter, langdurig voeden resulteerde in een aanhoudende opregulering van (mitochondriale) oxidatieve stress en ER-stress, wat uiteindelijk leidde tot lokale en systemische tekenen

van inflammatie. De reacties op het obesogeen dieet waren verschillend tussen Swiss en C57BL/6N muizen.

Aangezien vetzuren een belangrijke rol spelen als structurele membraan componenten, in cel signalering en cel-tot-cel interacties, zouden veranderingen in de lipiden samenstelling van het oviduct diens bekwaamheid om de ontwikkeling van het vroege embryo te ondersteunen, kunnen verminderen, wat langdurige gevolgen zou kunnen hebben. Daarom hebben we in **Hoofdstuk 4** de dynamiek en aard van veranderingen in het lipidoom profiel van het oviductale epitheel bestudeerd vanaf en tijdens het voeden van een obesogeen dieet doorheen de tijd in een outbred Swiss muismodel. We toonden aan dat 3 dagen voeden van een obesogeen dieet reeds resulteerde in acute veranderingen in het lipiden profiel van het oviductaal epitheel. Deze opmerkelijke effecten liggen in lijn met de transcriptomische veranderingen die beschreven werden in hoofdstuk 3. De veranderingen in het lipiden profiel vermeerderden geleidelijk en werden steeds hardnekkiger na een lange termijn van het voeden van het obesogeen dieet. Functionele annotatie onthulde vooral een verschillende abundantie van fosfolipiden, sfingomyelines en lyso-fosfolipiden.

Het werd reeds beschreven dat de invloed van lipotoxiciteit, veroorzaakt door een obesogeen dieet, op het ovarium, bewerkstelligd wordt oxidatieve stress, ER-stress en mitochondriale dysfunctie. Echter, de aard en omvang van deze veranderingen zou afhankelijk kunnen zijn van de duur het voeden (en dus de ontwikkeling van het obesogene fenotype). Dat laatste werd echter nog niet onderzocht. Daarom bestudeerden we in **Hoofdstuk 5** de invloed van het voeden van een obesogeen dieet voor verschillende tijdsperioden op het ovarium en de ovariële cellen van outbred Swiss muizen, waarbij we focusten op dynamische veranderingen in granulosa cel transcriptomics en eicel kwaliteit. We toonden aan dat het voeden van een obesogeen dieet voor een korte periode van slechts 24 uur tot 1 week, resulteerde in acute

veranderingen in het lipidengehalte van eicellen en in ER en mitochondriale functies van de granulosa cellen. De effecten vermeerderen geleidelijk doorheen de tijd na aanhoudend voeden van het obesogeen dieet, wat gekenmerkt werd door een verhoogde proportie aan mitochondriale abnormaliteiten in de eicellen en verschillende aangetaste pathways, gerelateerd aan DNA herstel, cel signalering, UPRs en vele andere GO geannoteerde biologische veranderingen in de granulosa cellen.

Tot slot warden in **Hoofdstuk 6** de bevindingen van dit doctoraatsonderzoek samengevat en besproken in het licht van de bestaande literatuur. Hieruit kunnen we concluderen dat we de eersten waren om sterk bewijs te tonen voor de gevoeligheid van het oviduct en oviductaal epitheel aan zeer korte-termijn veranderingen in het dieet, zowel op transcriptoom als lipidoom niveau *in vivo*. De verschillende tijdsperioden van het voeden maakten het mogelijk om een duidelijk onderscheid te maken tussen acute reacties en latere veranderingen na lange termijn voeden en de ontwikkeling van het obees fenotype en het geassocieerde ongezonde metabole fenotype. We voorzagen een dieper inzicht in een netwerk van opeenvolgende gebeurtenissen die konden aantonen hoe snel de OECs de metabole stress konden voelen, na slechts 3 dagen, en hoe zo'n lokale metabole stress zich kan ontplooien doorheen de tijd. Verder toonden we belangrijke verschillen in cellulaire reacties tussen het inteelt C57BL/6N en het outbred Swiss muizen ras, wat het belang van de keuze van fysiologisch relevante muismodellen in andere studies extra benadrukt. Onze bevindingen in het oviduct illustreren dat een obesogeen dieet de vrouwelijke vruchtbaarheid niet enkel beïnvloedt doormiddel van verminderde eicel kwaliteit, maar dat het ook een directe invloed op de vroege embryonale ontwikkeling in het oviduct zou kunnen hebben, wat mogelijks leidt tot lange termijns-effecten op de foetale ontwikkeling, succes op zwangerschap en postnatale gezondheid via epigenetische veranderingen. Verder resulteerde het voeden van en obesogeen dieet in cel specifieke veranderingen in eicellen en granulosa cellen, reeds na 24 uur tot 1

week, die rechtstreeks de eicel kwaliteit en vroege embryonale ontwikkeling kunnen aantasten, wat uiteindelijk leidt tot verminderede vruchtbaarheid.

De diepgaande analyse en nieuwe inzichten van deze PhD thesis kunnen van belang zijn voor veel onderzoekers en vormen een belangrijk fundament voor toekomstige studies met een focus op het beschermen en verbeteren van de eicel en embryonale ontwikkeling tijdens de allereerste stadia in dieet-geïnduceerde metabool gestresseerde condities. Het begrijpen van de omvang en aard van de reproductieve cel dysfunctie na verschillende tijdsperioden van blootstelling aan een vet- en suikerrijk dieet is van cruciaal belang om meer efficiënte preconceptie zorg interventie strategieën te ontwikkelen en zo de vruchtbaarheid in dieet-geïnduceerde metabole stress condities te verbeteren.

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BIBLIOGRAPHY

INTERNATIONAL PEER REVIEWED PUBLICATIONS

A Smits, WFA Marei, **K Moorkens**, PEJ Bols, D De Neubourg, JLMR Leroy, Obese outbred mice only partially benefit from diet normalization or calorie restriction as preconception care interventions to improve metabolic health and oocyte quality, *Human Reproduction*, Volume 37, Issue 12, December 2022, Pages 2867–2884,

Moorkens K, Leroy JLMR, Verheyen S, Marei WFA (2022). Effects of an obesogenic diet on the oviduct depend on the duration of feeding. *PLoS ONE* 17(9): e0275379.

K Moorkens, JLMR Leroy, J Quanico, G Baggerman, WFA Marei, MALDI-TOF lipidomic imaging of the oviduct after short and long-term exposure to an obesogenic diet in outbred mice, Abstracts 37th Annual Meeting of the Association of Embryo Technology in Europe (AETE), Anim. Reprod. 2022.;19(2 spe):e22209

Leroy JLMR, Meulders B, **Moorkens K**, Xhonneux I, Slootmans J, De Keersmaeker L, Smits A, Bogado Pascottini O, Marei WFA. Maternal metabolic health and fertility: we should not only care about but also for the oocyte! *Reprod Fertil Dev*. 2022. Dec;35(2):1-18

Moorkens, K.; Leroy, J.L.M.R.; Quanico, J.; Baggerman, G.; Marei, W.F.A. (2023). How the Oviduct Lipidomic Profile Changes over Time after the Start of an Obesogenic Diet in an Outbred Mouse Model. *Biology*, 12, 1016.

INTERNATIONAL ORAL PRESENTATIONS

Smits A, Marei WFA, **Moorkens K**, Mohey-Elsaeed O, Pintelon I, Moerloose K, Ginneberge D, Leroy JLMR (2019). Effect of a long-term high fat diet on metabolic health and oocyte quality of an outbred (Swiss) vs. inbred (C57BL/6N) mouse strain. *Flanders TrAining network LifE Sciences* (*f-TALES)*, Ghent, Belgium, 17-18/09/2019

Moorkens K, Verheyen S, Bols PEJ, Leroy JLMR, Marei WFA (2021). Temporal changes in the oviduct following the introduction of an obesogenic diet in outbred mice: a focus

on transcriptomic changes. *Association of Embryo Technology in Europe (AETE)*, Virtual Online meeting, 06/09/2021. Runner up for student competition.

Moorkens K, Leroy JLMR, Quanico J, Baggerman G, Marei WFA (2022). MALDI-TOF lipidomic imaging of the oviduct after short and long-term exposure to an obesogenic diet in outbred mice. *Association of Embryo Technology in Europe (AETE)*, Utrecht, The Netherlands, 15-16/09/2022

POSTER PRESENTATIONS

Smits A, Marei WFA, **Moorkens K**, Mohey-Elsaeed O, Pintelon I, Moerloose K, Ginneberge D, Leroy JLMR (2019). Effect of a long-term high fat diet on metabolic health and oocyte quality of an outbred (Swiss) vs. inbred (C57BL/6N) mouse strain. *Flanders TrAining network LifE Sciences* (*f-TALES)*, Ghent, Belgium, 17-18/09/2019

Moorkens K, Verheyen S, Bols PEJ, Leroy JLMR, Marei WFA (2021). Temporal changes in the oviduct following the introduction of an obesogenic diet in outbred mice: a focus on transcriptomic changes. *Association of Embryo Technology in Europe (AETE)*, Virtual Online meeting, 06/09/2021

Moorkens K, Leroy JLMR, Bols PEJ, Thys S, Marei WFA (2021). How long does it take for an obesogenic diet to impact oocyte quality? Insights from an outbred mouse model. *Belgian Society for Reproductive Medicine (BSRM)*, Brussels, Belgium, 26-27/11/2021

Moorkens K, Leroy JLMR, Quanico J, Baggerman G, Marei WFA (2022). MALDI-TOF lipidomic imaging of the oviduct after short and long-term exposure to an obesogenic diet in outbred mice. *Association of Embryo Technology in Europe (AETE)*, Utrecht, The Netherlands, 15-16/09/2022

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