Alternative methods and animal models for research in perinatal development

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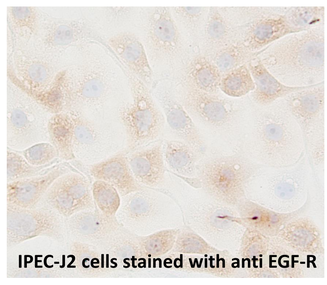
Alternatives to animal use in experiments

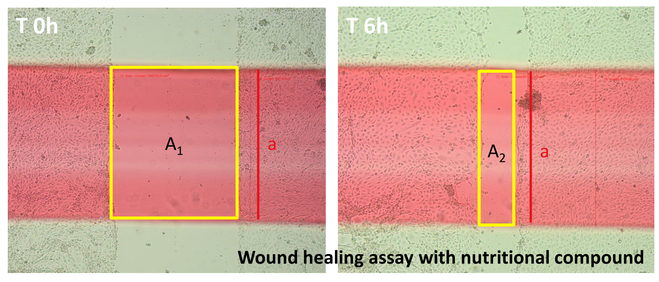
We aim to contribute to the **3Rs** by developing *in vitro* models, including the use of **cell culture** to model a.o. **pig** intestinal oxidative stress and angiogenesis and by investigating **biomarkers** for **disease and toxicity** in **porcine primary cell cultures**. One pathological condition that we focus on is **intra-uterine growth restriction (IUGR**), a condition where the neonate does not reach its full genetic growth potential. Additionally, we investigate the potential of **embryonic stem cells** for developmental toxicity testing. These methods will **reduce and partially replace** animals used for scientific purposes.


In vitro assay in a 96 well plate


IPEC-J2 cells stained with anti EGF-R

Research questions:

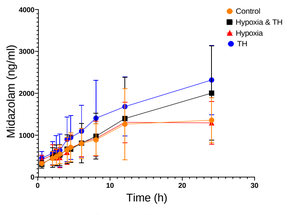
- Is the angiogenetic potential of pig umbilical cord endothelial cells different in IUGR? Influence of sex?
- Can *in vitro* biomarkers reduce animal use in nonclinical safety studies of drug candidates?
- Can embryonic stem cell-based assays replace conventional *in vivo* developmental toxicity testing for pharmaceuticals?



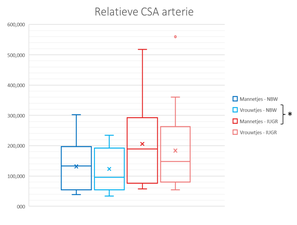
Wound healing assay with nutritional compound

The (mini)pig as a translational model


Minipig under anaesthesia with catheters




PAS staining small intestine




H&E staining umbilical cord

We investigate the pharmacokinetics and safety of drugs (**pediatric drug development**) used in **neonatal intensive care** units in a neonatal (**mini)pig** model under special conditions, such as asphyxia and hypothermia, as this provides critical information for neonatologists. Also, since immature and less developed neonatal pigs show similar disorders as premature and low birth weight babies, our group is interested in examining the translational value of the **neonatal IUGR domestic** piglet. We study the molecular mechanisms underlying their higher vulnerability to disorders during childhood and adulthood.

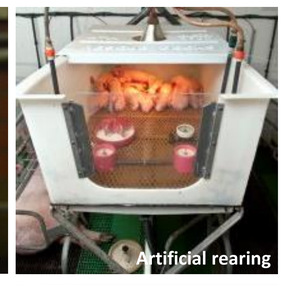
Research questions:

- Which covariates in a disease condition influence drug exposure in neonates?
- Do intra-uterine growth-restricted piglets phenotypically mimic IUGR infants?
- Does biological sex determine the effect of early-life stress on the intestinal barrier function?
- Can we validate and identify biomarkers for safety and efficacy in minipigs with human translatability?

The neonatal pig in pig production

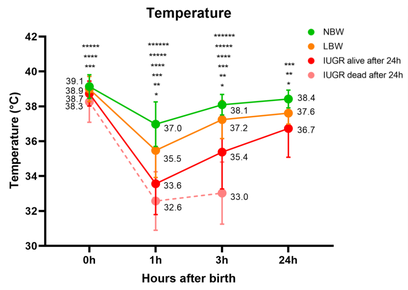
Intra-uterine growth restriction harms neonatal survival, growth, and animal welfare. The male IUGR piglet suffers higher morbidity and mortality. We aim to detail the phenotype of the IUGR piglet (performance, morphology, behavior) and link this description to changes in **intestinal development and vascularization**. In addition, we assess the effects of different strategies **(artificial rearing, nutritional supplements, etc.)** on the pig's resilience and development.


IUGR piglet with normal littermate


Artificial rearing

Research question:

- Are there differences in early life survival potential between IUGR piglets, low birth weight (LBW) piglets and their normal birth weight littermates? Does sex play a role?
- What is the impact of artificial rearing on the development of the IUGR piglet?




Drenching a LBW piglet

Frequently used techniques

- PCR, qPCR, enzyme activity, LC-MS, etc.
- *In vivo* and *ex vivo* functionality assays, gut permeability measurements, etc.
- Field studies: observations, (longitudinal) sampling (blood, feces, urine, saliva, tissue), etc.
- Immunohistochemistry, image analysis, stereology, western blot, ELISA, shotgun and targeted LC-MS/MS, etc.
- Cell culture: IPEC-J2 cell lines (o.a. ROS production and wound healing), primary cell culture, embryonic stem cells, etc.

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