

## The effect of non-esterified fatty acids during *in vitro* fertilisation on the developmental competence and outgrowth of bovine blastocysts

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We showed that deviating metabolic conditions in the oocyte's micro-environment during maturation associated with e.g. negative energy balance (NEB) in dairy cows have significant carry over effects on day 7 embryo quality and physiology. Biochemical changes occurring in metabolic disorders are also reflected in oviductal fluid where fertilization and early embryo development occur. Both processes may be negatively affected due to these changes. Therefore, we hypothesized that exposure to elevated NEFA concentrations, associated with NEB, during fertilization affects this process and further embryonic development. Bovine oocytes were matured following standard procedures. Mature oocytes were fertilized under 4 conditions: 1) standard lab conditions (CON), 2) solvent control (ethanol concentration as in condition 3 and 4) (SOL), 3) physiological NEFA conditions (mixture of 23  $\mu$ M palmitic acid (PA), 28  $\mu$ M stearic acid (SA) and 21  $\mu$ M oleic acid (OA)) (BAS) and 4) lipolytic NEFA conditions (mixture of 230  $\mu$ M PA, 280  $\mu$ M SA and 210  $\mu$ M OA) (HI NEFA). After 24h, presumptive zygotes were cultivated for 9 days following standard procedures. Blastocysts were transferred to growth medium for extended culture and subsequent outgrowth analysis. Cleavage rate tended to be lower in HI NEFA group compared to SOL group ( $P=0,087$ ). However, a higher proportion of HI NEFA zygotes showed 2-cell block (24,8%) as compared to the CON group (6,9%) ( $P=0,001$ ), SOL group (11,5%) ( $P=0,016$ ) and BAS group (13,1%) ( $P=0,057$ ). Blastocyst rate was significantly decreased in BAS (36,7%) ( $P=0,007$ ) and HI NEFA (36,6%) ( $P=0,024$ ) group as compared to CON group (54,3%). Average ( $P=0,053$ ) and net growth ( $P=0,091$ ) during the outgrowth tended to decrease in HI NEFA group compared to CON group. In conclusion, exposure to elevated NEFA concentrations during fertilization has no obvious effect on the fertilization process itself. However, further embryonic development and outgrowth potential seem to be affected.