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EFFECT OF NON-ESTERIFIED FATTY ACIDS DURING SPERM CAPACITATION OR IVF ON DEVELOPMENTAL COMPETENCE OF BOVINE OOCYTES

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Deviating metabolic conditions, present in dairy cows suffering negative energy balance (NEB), are reflected in the follicular and oviductal fluid (Leroy *et al.* (2015), RFD 27: 693-703). Elevated non-esterified fatty acid (NEFA) concentrations, associated with NEB, during *in vitro* maturation and culture have significant carry over effects on embryo quality and physiology (Van Hoeck *et al.* (2014), ARS 149: 19-29). Moreover, the oviduct plays an important role in sperm storage and selection, regulation of sperm motility and capacitation (Holt *et al.* (2010), MRD 77:934-943). This implicates that fertilization can be influenced by alterations in oviductal fluid composition. Therefore, we hypothesized that exposure of sperm cells to elevated (NEB-like) NEFA concentrations shortly before and during IVF can affect fertilization and further embryonic development.

To differentiate between possible effects on both spermatozoa and oocytes, two experiments were conducted. Bovine oocytes were matured following standard procedures. In experiment 1, mature oocytes were fertilized under 4 conditions: 1) standard lab conditions (CONT), 2) solvent control (SOLV), 3) physiological NEFA conditions (mixture of 23 µM palmitic acid (PA), 28 µM stearic acid (SA) and 21 µM oleic acid (OA)) (BAS-NEFAs) or 4) lipolytic NEFA conditions (mixture of 230 µM PA, 280 µM SA and 210 µM OA) (HIGH-NEFAs). In experiment 2, spermatozoa were pre-exposed for 4h under conditions CONT, SOLV, BAS-NEFAs or HIGH-NEFAs, then washed and used for IVF of mature oocytes in FA-free media. After 24h, presumptive zygotes were cultivated in serum-free medium until day 8 and developmental competence was assessed. Development was analyzed using binary logistic regression.

In experiment 1, cleavage rate tended to be lower in the HIGH-NEFAs group compared to the SOLV group (P=0.087). A significantly higher proportion of HIGH-NEFAs zygotes showed 2-cell block (24.8%) compared to CONT (6.9%; P=0.001), SOLV (11.5%; P=0.016) and BAS-NEFAs (13.1%; P=0.057) zygotes. Blastocyst rate was significantly decreased in the BAS-NEFAs (36.7%; P=0.007) and HIGH-NEFAs (36.6%; P=0.024) compared to the CONT group (54.3%). In experiment 2, no differences in developmental competence were observed among treatments.

In conclusion, exposure to elevated NEFA concentrations during IVF has no obvious effect on the fertilization process itself since cleavage rate is not significantly affected. However, further embryonic development is hampered due to NEFA exposure during fertilization. NEFAs have no influence on the fertilizing capacity of pre-exposed sperm suggesting that NEFA-induced reduction in developmental competence is through alterations in oocyte quality but not through affecting sperm quality. More research is ongoing to investigate underlying mechanisms.

Keywords: bovine oocyte, sperm, IVF, development