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FFAR4 is involved in docosahexaenoic acid effects on oocyte developmental potential during in vitro maturation.

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Besides affecting uterine environment, a direct effect of n-3 poly-unsaturated fatty acids (PUFA) on the oocyte could enhance fertility. We previously showed that docosahexaenoic acid (DHA, C22:6 n-3, Sigma), when provided during in vitro maturation (IVM), improved oocyte developmental competence through possible effects on cytoplasm but not nuclear maturation and without affecting lipid metabolism gene expression in cumulus cells (CC) (Oseikria et al *Theriogenology* 85:1625-1634, 2016). DHA could act through several mechanisms of action: i.e. via surface fatty acid receptors (free fatty acid receptor 1 or 4, FFAR1 and 4) or sensors involving PPAR or NFkB pathways; via changes in composition of cell membrane phospholipids; via production of eicosanoids... The aim of the present work was to investigate whether the FFAR4 was involved in the DHA effects previously reported on oocyte quality. We therefore investigated the effect of a specific agonist of the FFAR4, TUG-891, on embryo development after IVF. The response of surrounding CC to DHA or TUG treatment was also studied by gene expression analyses.

Oocyte cumulus complexes were collected from slaughtered cows. The protein FFAR4 was first localized by immunohistochemistry, by using a customized antibody produced specifically against the bovine protein. FFAR4 is expressed in CC and localized close to the cellular membrane, as expected.

After 22h IVM with or without DHA 1 µM or TUG 1 and 5 µM oocytes were subjected to in vitro fertilization (IVF) and in vitro development in modified synthetic oviduct fluid supplemented with 10% fetal calf serum for 7 days. At day 7, both blastocyst and expanded blastocyst rates were significantly increased with either DHA 1µM or TUG 1 or 5 µM (logistic regression, $p < 0.05$).

In order to decipher the DHA mechanisms linked to oocyte developmental competence, we then investigated the common pathways of DHA and TUG actions. Microarray hybridization of CC after 4h IVM in the presence or absence of 1µM DHA was performed (n=4 samples per condition). A customized 60K bovine microarray (Agilent technology) including 97.4% of Ensembl *Bos taurus* transcripts was used (GEO accession: GPL21724). Only 14 differentially expressed genes varied more than two-fold and were enriched in gene ontologies related to regulation of translation, RNA splicing and spliceosome formation, oxidation/reduction, actin cytoskeleton organization and vesicle-mediated transport.

The kinetic of expression of these genes is currently characterized by qRT-PCR analysis on CC samples at 0, 4, 10 and 24h IVM with or without DHA 1 µM, TUG 1 or 5 µM.

Altogether the IVF data suggest that DHA exert its effect partly through FFAR4 on oocyte developmental competence. Also, we are studying the common transcriptomic modulation between DHA and TUG to provide insights on its detailed mechanism of action.

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