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A268E Embryology, Developmental Biology and Physiology of Reproduction

### **Addition of omega-3 DHA during *in vitro* maturation affected embryo development**

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**Keyword:** bovine oocytes, *in vitro* maturation, omega-3 DHA.

Several studies have suggested a positive effect of n-3 poly-unsaturated fatty acids (PUFA) on bovine reproduction. Indeed, n-3 PUFA reduced prostaglandin secretion in uterine environment, thus providing more favorable conditions for embryo development. Other studies suggested a direct effect of n-3 PUFA on the oocyte that could enhance fertility. In the present study, we aimed at investigating *in vitro* the effect of docosahexaenoic acid (DHA, C22:6 n-3, Sigma, Saint-Quentin Fallavier, France) on bovine oocyte maturation and developmental competence. Oocyte cumulus complexes (OCC) were collected from slaughtered cows. In first experiment, *in vitro* maturation (IVM) with DHA 1, 10 and 100  $\mu$ M was performed (n=3 replicates, 50-60 OCC per condition). After IVM, oocyte viability was assessed using Live/DEAD staining and then meiotic stages were determined by using Hoechst staining after oocyte fixation. Neither difference in viability nor in maturation rate was observed after IVM between control and treated oocytes whatever the DHA concentration. 83.1% of mature oocytes in control IVM and 78.9%; 84.0%; and 84.0% in presence of DHA at 1, 10, 100  $\mu$ M, respectively, were observed. In second experiment (n=5 replicates, 50-60 OCC per condition), after 26h IVM with or without DHA 1, 10 and 100  $\mu$ M, oocytes were subjected to parthenogenetic activation (ionomycin 5  $\mu$ M, 5 min and 6DMAP 2 mM, 4h). Oocytes were then *in vitro* developed in modified synthetic oviduct fluid supplemented with 1% estrus cow serum for 7 days. Cleavage rate and a number of blastomers were assessed in resulting embryos at day 2 post activation. Cleavage rate significantly increased after IVM with DHA 1 $\mu$ M (84.3%) but significantly decreased with 100 $\mu$ M DHA (66.2%) as compared to control (76.0%) embryos (Chi-square test p=0.02). Moreover, the percentage of embryos that progressed further than 4 cells at day 2 was significantly higher (p=0.02) in the presence of 1 and 10  $\mu$ M DHA (40.8% and 40.4%, respectively) than in control (31.2%) and with DHA 100  $\mu$ M (22.2%). At day 7, embryos from DHA 1  $\mu$ M-treated oocytes encountered more cells than those from control and other DHA groups (10 and 100  $\mu$ M). Altogether these data suggest that a low dose of DHA (1 $\mu$ M) during IVM might improve oocyte developmental competence through possible effect on cytoplasm but not nuclear maturation. Also, we confirmed that a high dose of DHA (100 $\mu$ M) is deleterious for oocyte developmental potential.