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A287E Support biotechnologies: Cryopreservation and cryobiology, diagnosis through imaging, molecular biology, and “omics”

### **Enriched n-3 polyunsaturated fatty acid diet modified oocyte lipid composition and may influence oocyte quality in Prim Holstein dairy cows**

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Administration of long chain n-3 polyunsaturated fatty acid (n-3 PUFA) diet to dairy cows may impact oocyte quality (Elis et al, *Animal Reproduction Science* 164:121, 2016). Addition of docosahexaenoic acid (C22:6 n-3) during IVM led to higher blastocyst rate after IVF (Oseikria et al, *Theriogenology* 85(9):1625, 2016) and significantly changed oocyte lipid content (Elis et al, *J Ovarian Res* 10(1):74, 2017). The present objective was to compare lipid content of the oocytes from the dairy cows supplemented with n-3 or n-6 PUFA-enriched diet. Oocyte-cumulus complexes were aspirated by OPU after hormonal ovarian stimulation, from 18 primiparous Holstein dairy cows after 3 or 9 weeks of supplementation with 1% dry matter of either n-3 PUFAs (n = 9, micro encapsulated fish oil, OMG750®) or n-6 PUFA (n = 9, micro encapsulated soy oil, OMG Soy®) (Kemin). N-3 PUFA level in plasma and follicular fluid was measured after 2, 5 and 7 weeks of supplementation. Immature oocytes from n-3 and n-6 diet groups (60 and 61 oocytes, respectively) were denuded from CC and analyzed individually using an UltrafleXtreme MALDI-TOF/TOF instrument in positive reflector mode, with DHAP matrix. Lipid spectral profiles (3000 shots per spectra) were acquired for each oocyte. M/z peaks were detected in the range of 160 to 1000 m/z. Values of the normalized peak heights (NPH) were quantified and compared between the two groups by t-test with Benjamini-Hochberg correction. Multivariate Principal Component Analysis (PCA) was performed using differential NPHs. Lipids were identified by high-resolution mass spectrometry LC-MS or by direct infusion combined to top-down MS/MS analyses, and annotated according to Lipid maps database. Concentration of eicosapentaenoic acid (C20:5 n-3) and total n-3 PUFA significantly increased in n-3 group, after 2 weeks of diet in plasma, and after 5 weeks in follicular fluid, as compared to n-6 group. Body weight and milk production did not differ. Lipid profiles of the oocytes showed significant difference between n-3 and n-6 diets (97 up-regulated and 91 down-regulated peaks, P < 0.05, fold change > 2). PCA allowed clear discrimination of n-3 and n-6 groups. 40 differential peaks were identified (496-827 m/z); among them 12 phosphatidylcholines (PC), 3 phosphatidylethanolamines (PE, C36), 2 sphingomyelins (SM, C35) and lyso-phosphatidylcholine LPC 22:4 were more abundant in n-3 oocytes, whereas 14 PC, PE 30:0, SM 34:1, two LPC (16:0 and 18:0) and two triglycerides (46:1, 47:1) were more abundant in n-6 group. These variations indicated profound changes in composition of several lipid classes from oocyte membrane and intracellular pool, occurring after only few weeks of n-3 or n-6 PUFA dietary supplement. These cellular lipid changes may influence oocyte capacity to develop better blastocysts after IVF in n-3 supplemented cows (see Elis et al, AETE 2018), and highlight the importance of identifying beneficial oocyte lipid profile to improve embryo biotechnologies issues.

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