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Follicular and systemic levels of IL-6, lipid metabolites, and oxidative stress index during the non-breeding season in mares

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The application of trans-vaginal OPU and ICSI is well established for the commercial equine IVP. These assisted reproductive techniques are especially applied during the non-breeding season of the mare. Interestingly, no research data are available concerning the biochemical composition of the follicular fluid (FF) in small and medium-sized follicles routinely aspirated during OPU and how this is correlated with the serum composition. This study aimed to measure the FF concentrations of interleukin-6 (IL-6), total cholesterol (CHOL), triglycerides (TG), non-esterified fatty acids (NEFA), reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), and oxidative stress index (OSI) in relation to follicle size, and to investigate a possible association with their systemic concentrations during the OPU-ICSI (non-breeding) season in mares. These parameters are representing the maternal inflammatory, metabolic, and oxidative stress status. At slaughterhouse, serum (n=12) and FF of small (SF; 5-10 mm in diameter, n=10), medium (MF; >10-20 mm in diameter, n=11), and large (LF; >20-30 mm in diameter, n=4) follicles were sampled from 12 apparently healthy mares. An enzymatic-colorimetric assay was used (CHOL2 and TRIGL kits; Roche Diagnostics, and NEFA F5 Kit; DiSys Diagnostic Systems, Germany) to assess the concentrations of CHOL, NEFA, and TG. For IL-6 estimation, equine kit (Nori[®], Genorise Scientific, USA) was used. Concentrations of d-ROMs and BAP were identified using photometric Diacron[®] kits (Diacron International, Italy). One-way ANOVA was used to compare means between the different follicle categories, while t-test was conducted to compare between the systemic and follicular concentrations of each variable per follicle category. Pearson correlation coefficients were tested. P value <0.05 was considered significant. Concentrations of all variables did not show significant differences between follicle classes. Concentration of IL-6 did not differ significantly between serum (60.1±1.9 pg/mL), SF (72.8±9.5 pg/mL), MF (65.4±3.5 pg/mL), and LF (60.2±0.6 pg/mL). Concentrations of CHOL, TG, and NEFA, respectively were higher in serum (96.0±5.9 mg/dL, 45.2±6.1 mg/dL and 12.1±2.1 mg/dL) compared to SF (67.0±7.9 mg/dL, 19.3±1.9 mg/dL and 5.3±0.4) and MF (46.3±2.6 mg/dL, 15.5±1.3 mg/dL and 6.1±0.5). Concentrations of CHOL were higher in serum compared to LF (44.7±4.2 mg/dL, 16.7±1.6 mg/dL and 6.8±1.2 mg/dL). Concentrations of d-ROMs in serum (138.7±8.8 Carratelli units; UCARR) were markedly higher than SF (57.6±7.1 UCARR), MF (67.2±9.0 UCARR), and LF (42.0±11.2 UCARR). The higher concentration of BAP in serum (5571.6±619.9 µmol/L) was only significant when compared to MF (2521.8±464.4 µmol/L), but not to SF (4658.0±1116.1 µmol/L) and LF (2244.6±563.9 µmol/L). There was a strong positive association (r=0.8, P <0.01) between levels of IL-6 in serum and MF. Also, values of CHOL, d-ROMs, and OSI in serum were positively correlated with those measured in MF (P <0.05). Taken together, follicular size is not associated with alterations in the studied biochemical components during the non-breeding season of mares. There is a crosstalk between serum and follicular fluid composition. In addition, changes in the blood composition associated with maternal health (IL-6 and OSI) or diet (CHOL and NEFA) during the commercial OPU-ICSI season in mares may lead to an altered oocytes microenvironment, which may affect oocyte quality. More studies are recommended to check the influence of maternal health on the oocyte developmental capacity and subsequent embryo quality in mares.

Keywords: follicular fluid metabolism, maternal health, OPU/ICSI

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