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Composition of proteins present in dog oocyte by mass spectrometry

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The efficiency of in vitro maturation (IVM) of canine oocytes is still very low compared to that found in other mammalian species. Small proportions of canine oocytes completed nuclear maturation after being cultured for 48 to 72 h in vitro. In dogs, mass spectrometry have been used as a viable tool to identify markers involved in oocyte quality, as the analysis of the components present in the follicular fluid [1]. This study aimed to characterize protein composition within canine oocytes. The ovaries were opportunistically obtained from 30 bitches undergoing routine ovariohysterectomy (OSH). After OSH, the ovaries were immersed in sterile 0.9% NaCl solution and immediately transported to the laboratory at 4°C. In the laboratory, the ovaries were sliced repeatedly to release of cumulus-oocyte complexes (COCs). Only grade 1 were selected and cultured in HEPES-buffered TCM 199 medium supplemented with 26mM sodium bicarbonate, 1.5mM sodium pyruvate, 0.6mM cysteine, 0.03U/mL hCG, 0.5μg/mL FSH, 20mg/mL E2 and 10ng/mL EGF for 72 h at 38.5°C and 5% CO2 in air in a humidified atmosphere. After IVM, oocytes were denuded, washed three times in PBS, and placed in tubes containing lysis buffer (20mM TRIS, 150mM NaCl, 1.0 EGTA, 1.0mM EDTA, 2.5mM sodium phosphate, 1.0mM β-glicerophosphate, 1.0mM Na3VO4, 1.0mg/mL leupeptin and 1.0mM PMSF). The samples were frozen in liquid nitrogen and sonicated (probe 3.0-mm, five times during 25s, 10% amplitude and 1 minute interval)). Cell extracts were stores at -80°C until mass spectrometry analysis (LC-MS/MS) in the Q-ToF equipment (Bruker Daltonics) and MASCOT bioinformatics tool for the identification of the major proteins. We identified 13 major proteins: Zona pellucida 2 glycoprotein, Zona pellucida C protein, Complement C3, Tudor and KH domain containing protein isoform X5, Major vault protein, Astacin-like metallo endopeptidase isoform X1, Rab proteins geranyltransferase component A1 isoform X2, Actin cytoplasmic 1, Centrosomal protein, Epithelial keratin 10, Cationic trypsin, Vimentin. Researchers reported that the K10 keratin acts as a negative modulator of cell cycle progression involving changes in phosphoinositide 3-kinase (PI-3K) signal transduction pathway [2].The knowledge of these proteins is essential to understand what keeps the oocyte in germinal vesicle in bitches. Our results provide the first analysis of protein composition within the canine oocytes. The findings lay important foundation for future studies aiming at formulating a suitable culture medium that will improve the IVM rates in this species.