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Folliculogenesis and morphometry of oocyte and follicle in different ages queens (*Felis Catus*)

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The aim of this study was to analyze the follicle and oocytes morphometry from different follicular classes. The ovaries of 17 queens in anestrus were classified into three groups: Young (0–1 year), Adults (1–6 years) and Older (>6 years). The ovaries were fixed in paraformaldehyde 5%, embedding in paraffin and staining with haematoxylin-eosin. For morphological analysis the tissue sections were photographed by microscope (Olympus BX61) and classified as primordial, unilaminar primary, multilaminar primary, secondary and pre-ovulatory follicles. A total of 1039 follicles were measured and the parameters utilized were: diameter (μm), area (μm2) and perimeter (μm). The statistical used were ANOVA and the means were compared by Tukey test and medians using the Kruskal-Wallis test followed by Dunn's multiple comparisons ($p < 0.05$). In young queens primordial follicles there were increased in the mean diameter, area and perimeter of follicle (45.16 μm, 1941 μm^2 and 157.24 μm) and oocytes (40.55 μm, 1320.4 μm^2 and 129.90 μm) when compared to adults (Follicles: 41.51 μm, 1652.4 μm^2 e 145.56 μm and in oocytes: 37.57 μm, 1134.3 μm^2 and 120.58 μm). A biphasic pattern of follicle and oocyte growth was observed through linear regression. Before antrum formation, follicle (x) and oocyte (y) size were positively and linearly correlated ($y = 0.304x + 25.01$, $r^2 = 0.72$), although after antrum formation a negative correlation were found ($y = 0.007x + 98.00$, $r^2 = 0.00$). The queen offers many benefits as a model of ovarian folliculogenesis, and may be useful in preserving of endangered animals.

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Cryopreservation of ovine *in vitro* produced embryos using centrifugation and cytocalasin D

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The cryosurvival of ovine *in vitro* produced embryos are still low, thus preventing its routine transfer. Attempts have been made to override this problem by decreasing embryos lipid content or protecting their structure with cytoskeletal stabilizers. In this study we used embryo mechanical delipidation through centrifugation in the presence or absence of cytocalasin D testing its effect on embryo quality and cryosurvival. Mature ovine oocytes (n = 1146) were inseminated using fresh semen of a Merino ram. After assessing cleavage, embryo development proceeded until the blastocyst stage. Prior to vitrification, embryos were randomly distributed to the following groups: control (n = 20), without treatment; centrifugation (n = 18), blastocysts were centrifuged at 15 000 ***g***; cytochalasin D (n = 20), embryos were treated with 5 μg/ml cytocalasin D; centrifugation + cytocalasin D (n = 17), embryos were treated with both centrifugation and cytocalasin D. Embryos integrity and re-expansion were assessed post-warming and after 3 h of culture. Post-warming integrity rate was lowest ($p = 0.04$) in embryos of centrifugation group. No differences were identified among groups for re-expansion rates. A possible role of cytocalasin D in protecting mechanical damage of centrifuged embryos during cryopreservation was identified. However this stabilizer alone did not improve the quality of warmed embryos when compared to control.

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Effect of zona pellucida removing of MII bovine oocytes on *in vitro* fertilization

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Some sperm treatments can induce changes on the sperm membrane. After sex-sorting and freezing/thawing, spermatozoa showed an earlier capacitation process and acrosome reaction (AR) after incubation (Moce, 2006, Theriogenology, 66, 929–936). With the aim to synchronise the interaction between gametes, the effect of zona pellucida (ZP) removal of MII bovine oocytes and sperm AR induction on *in vitro* fertilization parameters with non-sorted sperm, was studied. COCs from slaughterhouse ovaries were *in vitro* matured (IVM) for 22–24 h at 38.5°C in 5% CO_2. After IVM, COCs were divided into two groups: Control (IVM intact COCs) and ZP free (MII oocytes without cumulus cells were treated with a 0.5% pronase solution). For IVF, frozen semen was centrifuged on a bovipure gradient. For the ZP free group, sperm was previously incubated in a 400 nM ionomicyn solution for 15 min. IVF was performed in fertitalp medium for 18 h. In five replicates, a total of 243 and 161 COCs for control and ZP free group respectively were used. Results were analysed by Chi-square test. A pronase treatment and sperm AR induction did not improve fertilization efficiency on bovine oocytes. We observed a higher rate of non-fertilized oocytes in the ZP free group in comparison to the control group (50.3% vs. 28.8%; $p < 0.05$). Moreover, from fertilized embryos, the ZP free group presented a higher rate of polyspermy than the control group (17.5% vs. 6.9%; $p < 0.05$). (MAS was supported by Subprograma RyC and European Soc Fund).

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Ultrastructural study of *in vivo* produced ovine embryos: characterization of secretory vesicles

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The ultrastructure of *in vivo* produced ovine embryos at the morula, early blastocyst and late blastocyst stages was evaluated using transmission electron microscopy. *In vivo* produced embryos (n = 14) were obtained from Portuguese Black Merino ewes that were superovulated during Spring. Embryos were recovered by abdominal laparotomy under general anaesthesia, on day 6 or 7 after sponge removal. Embryos were processed for electronic microscopy observation and ultrathin sections (700A) were observed in a transmission electron microscope JEOL 100CXII. Embryonic cells presented with intact intercellular junctions, numerous mitochondria, smooth endoplasmic reticulum cisternae and light vesicles. Polyribosomes, rough endoplasmic reticulum cisternae, secondary lysosomes, Golgi complexes and lipid droplets were also observed in the cytoplasm. The nucleus was well defined with one or more reticular nucleolus. The most noticeable aspect of the cytoplasm of ovine embryos was the presence of abundant cytoplasmic small light vesicles with heterogeneous contents. These light vesicles seem associated to small cisternae of Golgian and endoplasmic reticulum origin and their labile membrane enabled them to rapidly coalesce into medium size vesicles that began to engulf mitochondria and lipid droplets, forming giant vacuoles mostly filled with fat. Incomplete matured secretory vesicles were observed to exocytate into the perivitelline space of morulae, whereas fully matured secretory vesicles appeared only in trophectoderm cells, being exocytated into the blastocoelic cavity.