

Morphofunctional aspects of *in vitro* matured oocytes from prepubertal and adult sheep

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The aim of this study was to analyze the morphofunctional aspects of prepubertal and adult sheep oocytes subjected to *in vitro* maturation (IVM). The structural and ultrastructural morphology was examined by light and transmission electron microscopy (LM and TEM) while the metabolic competence, as determined by the distribution of active mitochondria, was assessed by confocal microscopy (CM). Cumulus-oocyte complexes underwent IVM in standard condition for 24 h. Half of the mature metaphase II (MII) stage oocytes were processed for LM and TEM observations. The other half was subjected to immunostaining with MitoTracker Red (to stain mitochondria with functionally active membrane potential), SNARF-1 (a pH sensitive fluoroprobe), Hoechst 33342 (to label DNA) and analyzed by CM. Immature germinal vesicle (GV) stage oocytes, retrieved at 0 h IVM, were used as controls.

By LM and TEM all the oocytes were regularly rounded, covered by microvilli and surrounded by an intact zona pellucida. Numerous rounded, oval or hooded mitochondria appeared either isolated or grouped in the ooplasm. The GV was usually rounded in prepubertal oocytes. In the adult, the GV often appeared flattened against the oolemma, with a crescent-shaped outline (an early sign of meiotic resumption). Scattered cortical granules (CGs) were rarely found in the ooplasm of both prepubertal and adult GV oocytes. After 24 h of IVM, CGs became abundant and distributed in a single row under the oolemma, particularly in adult oocytes. CM showed a homogeneous fine-to-granular mitochondrial distribution in prepubertal GV and MII oocytes. In adult GV oocytes, the mitochondrial distribution pattern was granular while in MII oocytes mitochondria were arranged in heterogeneous clusters.

Thus, both prepubertal and adult oocytes completed maturation after 24 h in culture and showed an overall good preservation after IVM. However, the diverse distribution patterns of mitochondria in prepubertal oocytes reflect their low developmental competence.

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