THE SPERM ASTER NUCLEATION AND MICROTUBULE ELONGATION IN *IN VITRO* FERTILIZED SHEEP ZYGOTES

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Successful fertilization process and embryo development relies on functional centrioles/centrosomes which have been confirmed to be paternally inherited in most farm animals, including sheep.[2,3,4] Shortly after fertilization, the sperm proximal centriole typically nucleates microtubular aster that function as microtubule organizing center and ensure paternal and maternal genomes merging.[5,6,7] At the moment, there are no data on the timing and dynamics of sperm aster organization in sheep. In this study, we have traced the fate of sperm centriole after fertilization to evaluate the timing of the sperm microtubular aster nucleation in early sheep zygotes. To this extend, we have imaged sperm aster nucleation at different post-fertilization moments throughout α-tubulin immunofluorescence in early *in vitro* fertilized sheep oocytes. To visualize the process of sperm aster nucleation as well as microtubules elongation, sheep oocytes were subjected to *in vitro* maturation (IVM) for 24 h followed by *in vitro* fertilization (IVF). IVF was performed in 50 µl drops of *synthetic oviductal fluid* (SOF) with estrus sheep serum and 16 µM isoproterenol, covered by mineral oil. In a preliminary experiment, we have established that spermatozoon takes almost 3 hours to complete the fertilization and to enter the oocyte. Fertilization has been arrested at different timing after sperm-egg co-culture (from 4 up to 7 hours) and then presumptive zygotes have been removed from *zona pellucida*, fixed and examined with anti-α-tubulin immunofluorescence under confocal microscopy. We have observed that the sperm centriole initiates sperm aster nucleation within 1 hour post-fertilization (4h from sperm-egg co-culture) and that microtubules elongation takes place approximately 3 hours post-insemination. Future investigations will aim to identify which sperm centriole contributes to embryonic inheritance.

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