

REDUCING THE TIME OF SPERMATOZOA/OOCYTE INTERACTION IMPROVES EMBRYONIC DEVELOPMENT IN SHEEP

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In vitro fertilization (IVF) is a well-established Assisted Reproductive Technique (ART) routinely applied in human infertility treatment and in large animals, both to boost reproductive performances of selected genotypes, and also for basic research. Despite its value, IVF has seen very little progress in the last two decades, and relies to established paradigms, like overnight sperm-egg co-incubation. However, the long exposure to the relatively high spermatozoa concentration in a dish increases the risk of polyspermy and could be detrimental for early embryonic development. In our work we have identified the time window within which the fertilization occurs in order to refine the procedure and reduce sperm-egg co-incubation, comparing embryo development after short (sIVF) and overnight spermatozoa/oocyte co-incubation (o/nIVF). A total of 144 in vitro matured sheep oocytes were co-incubated with spermatozoa in IVF medium [synthetic oviductal fluid (SOF) with 20% oestrus sheep serum and 16 μM isoproterenol]. Then, small batches of oocytes were collected every 30 minutes to check for the presence of a fertilizing spermatozoon. To assess that, cumulus cells were removed and presumptive fertilized oocytes were fixed and stained with propidium iodide for nuclei and Pisum sativum agglutinin for zona pellucida (ZP) detection respectively. Pronuclear formation (PN) and embryo development were evaluated after 16 hours (PN), 24 hours (2 cells) and 7 days of culture (blastocyst). We found that spermatozoa engaged with the ZP not earlier than 90 minutes and penetrated the oocytes within 3-4 hours after IVF. A trend for a lower polyspermic fertilization (>2PN) was detected in sIVF (7.7%) vs o/nIVF (18.4%). Likewise, cleavage and blastocyst rate were higher after sIVF compared to o/n-IVF (2-cells: 38.6% vs 23.8%; blastocyst: 18.1% vs 9.5%; sIVF vs o/nIVF). This work demonstrates that 4 hours spermatozoa/oocyte interaction are sufficient to achieve fertilization, reduce polyspermy and improve early embryonic development in sheep.