Short spermatozoa–oocyte co-incubation improves outcomes of IVF in sheep

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The assisted reproductive technique IVF is routinely applied in humans and large animals, both to boost reproductive performance and also for basic research. Despite its value, IVF has seen very little progress in the last two decades and relies on established paradigms, such as overnight sperm–egg co-incubation. However, the long exposure of oocytes to spermatozoa in a dish increases the risk of polyspermy and could be detrimental for early stages of embryonic development. We identified a time window within which fertilization occurs, in order to reduce the length of sperm–egg co-incubation and optimize the procedure, comparing polyspermy rate and embryo development after short (shIVF) and overnight (o/nIVF) spermatozoa–oocyte co-incubation. A total of 666 *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). First, small batches of oocytes were collected every 30 min to check for the presence of a fertilizing spermatozoon. To assess this, 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. Then, pronuclear formation (PN) and embryo development were evaluated after 16 h (PN), 24 h (2 cells), and 7 days of culture (blastocyst). The oocytes that were not cleaved at 24 h were stained for DNA content with Hoechst 33342. Furthermore, we evaluated embryo quality by counting cells of 8-day blastocysts after differential staining of inner cell mass (ICM) and trophectoderm (TE). We found that spermatozoa reach the ZP no earlier than 90 min from the beginning of co-incubation and achieve fertilization within 4 h. Polyspermic fertilization (>2PN) was lower in shIVF (6.5%) than in o/nIVF (17.8%; $P = 0.006$). This proportion of polyspermy was maintained between groups in noncleaved oocytes at 24 h from fertilization. Likewise, cleavage and blastocyst rate were higher in shIVF compared with the o/n-IVF group (2-cells: 48.3% vs. 31.6%, $P = 0.001$; blastocyst: 29.4% vs. 20.5%, $P = 0.046$, respectively). Differential staining of blastocysts revealed no significant difference in cell number between the blastocysts of the two groups. This work demonstrates that 4 h of sperm-egg interaction are sufficient to achieve fertilization, reduce polyspermy, and improve the rate of embryos reaching blastocyst stage without compromising embryo quality.