MONITORING THE MICROTUBULE NUCLEATION DYNAMICS OF SPERM CENTRIOLE AFTER IFV AND ICSI IN SHEEP ZYGOTES

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Intracytoplasmic sperm injection (ICSI) is an assisted reproduction (ART) technique that is less efficient in ruminant, comparing to other species. In mammals, the spermatozoa proximal centriole nucleates the microtubule and generates the functional cell centriole of the resulting organism. Our study aimed to investigate eventual difference in the centriole microtubular nucleation in ICSI fertilized oocytes, comparing to control In Vitro Fertilized ones (IVF). In fact, we made the hypothesis that the tail severing step achieved in our ICSI protocol through applying a few piezo pulses, might mechanically damage the proximal centriol. On this basis, Sheep oocytes were in vitro maturated (IVM) for 24 h then were injected by piezo-pulsed spermatozoa, chemically activated by 5 min of incubation with 5 mg/ml ionomycin, washed in H199 for 5 min and cultured in 50 µl drops of Synthetic Oviductal Fluid (SOF) with estrus sheep serum and 16 µM isoproterenol, covered by mineral oil. Fertilization has been arrested around 5h after ICSI, and the presumptive zygotes were processed for immunological detection of tubulin. Zona Pellucida (ZP) was removed with a combined treatment of acid Tyrode and trypsin and zygotes were then fixed with 4% paraphormaldehyde (pH7.2) and permeabilized by 0.5% Triton X-100, for 20 min each. Microtubular nucleation was assessed with anti-α-tubulin immunofluorescence under confocal microscopy. No difference was noticed in the dynamics and timing of sperm microtubular aster nucleation, that started around 5h post ICSI (5h30). Therefore, we conclude that abnormal microtubular nucleation by the centriole is not responsible for the low development of ICSI fertilized sheep oocytes.