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Conferences papers

Conference abstracts

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A156 Folliculogenesis, Oogenesis and Superovulation

Inhibition of meiosis resumption in bovine oocytes to be used in the intrafollicular transfer of immature oocyte (TIFOI)

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In the intrafollicular transfer of immature oocytes (TIFOI) the oocytes are aspirated from the donors and transferred to the "ovulators". Considering that removal of the oocyte from the follicular environment causes the spontaneous meiosis resumption, it is necessary to inhibit this process to avoid oocytes aging. The present study aimed to evaluate nuclear maturation kinetics in oocytes kept in different manipulation medium to be used in TIFOI. COCs were obtained from slaughterhouse ovaries and all manipulation and selection was performed in follicular fluid (FF). After selection, a group of COCs was placed directly into the IVM (control), the others were transferred to an eppendorf containing 500µl of Follicular Fluid (T1), Follicular aspiration solution consisting of PBS supplemented with 1% fetal calf serum and 0,02% heparin (T2) and Follicular aspiration solution supplemented with 500Mm of IBMX [(nonspecific phosphodiesterase inhibitor) (T3)]. The COCs remained for three hours in the different medium at 36^{o} C. After that period, oocytes were transferred to IVM medium and kept for 22h. At 0, 9, and 22 h of IVM, sample of oocyte from all groups were removed for meiotic stage evaluation. Only for the control groups, a sample of oocytes was also removed at 12 h, which was used to confirm the efficiency of the treatments, regarding to meiosis retention. At each time point COCs were mechanically denuded, fixed for 48 hours in ethanol and acetic acid and stained with lacmoid (45%). The evaluation of meiotic stage was carried out under a phase contrast microscope (Nikon Eclipse E200, 1000X) and the oocytes were classified as: germinal vesicle (GV), germinal vesicle break down (GVBD); metaphase I (MI), anaphase I (AI), telophase I (TI) and metaphase II (MII). Nuclear maturation data were analyzed by Chi-square test ($P<0.05$). We evaluated a total of 293 COCs for the control group at 0 (n=63), 9 (n=76), 12 (n=76) and 22h (n=78). For the other groups a total of 587 oocytes were distributed to T1 (n=214), T2 (n=179) and T3 (n=194). At 0h 98.4% of the oocytes were at GV stage. After 9h of IVM, T1 (75.7%) and T3 (82.4%) presented most of oocytes in GVBD, similar to the control group (72.4%). In T2 only 11.8% were in GVBD, which was lower ($P<0.05$) than the other treatments. This group at 9 h had 86% of the oocytes in MI, which was similar to the 72.4% observed for the control group at 12 h. At 22h most of oocytes from all the groups reached the MII stage, with no difference ($P> 0.05$) among them. The results suggest that oocytes can remain for 3 hours in Follicular Fluid or Aspiration Solution with IBMX and proceed with nuclear maturation without affecting the oocytes. Therefore, both are eligible to be used as handling medium before TIFOI procedures.

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