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Conferences papers

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A115 OPU-IVP and ET

Embryos production from bovine oocytes matured *in vivo* using intrafollicular transfer of immature oocytes (TIFOI)

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Intrafollicular transfer of immature oocytes (TIFOI) provides an entirely *in vivo* environmental condition and may be a good alternative to produce embryos in cattle. However, several aspects of the technique have yet to be established. The aim of this study was to evaluate if the time in which the oocytes remain in the follicle after the TIFOI is enough to allow the oocytes a complete maturation, fertilization and embryo development. Ovulators cows were submitted to a standard estrus synchronization protocol, using a vaginal progesterone device, estradiol benzoate and prostaglandin. On D8, the progesterone device was removed and 52 hours later animals with had a dominant follicles greater than 10 mm was subjected to TIFOI A total of 1297 imature oocytes were obtained from slaughterhouse ovaries, in which 609 were used for TIFOI and the remainder was used as control. In the control group, the oocytes were placed in IVM and IVF was performed at 12, 16 and 22h of culture. For TIFOI 30 to 50 oocytes were transferred to ovulator cow, which at 12 h post-injection were recovered by ovum pick up (OPU). The recovered oocytes were divided into three groups: one was submitted to IVF immediately after aspiration (12h after TIFOI), and the remaining oocytes were placed in IVM for another 4 and 10 hours prior to IVF (16 and 22h after TIFOI). Oocytes and sperm were co-incubated for 12 h and the possible zygotes were transferred to culture drops, where they remained until day 7 (D7). Treatments and number of oocytes per treatment were: control 12h (n=223); control 16h (n=229); control 22h (n=236); TIFOI 12h (n=239); TIFOI 4h (n=185); TIFOI 22h (n=185). The cleavage (D2) rate, blastocyst rates (D6 and D7) and apoptotic index in D7 embryos (Terminal deoxinucleotidyl transferase dUTP nick end labeling [TUNEL]) were evaluated. The cleavage and blastocyst rate data were analyzed by Chi-square test ($P<0.05$). The total number of cells, apoptotic index and ratio between the two were analyzed by ANOVA and Tukey's test ($P<0.05$). The blastocysts rate on D7 was similar ($P>0.05$) among the control groups (control 12h=35.4%, control 16h=36.7% and control 22h=40.7%), as well as among TIFOI groups (TIFOI 12h=18.8%, TIFOI 16h=17.3% and TIFOI 22h=21.6%). The blastocysts rate on D7 was higher ($P<0.05$) in the control groups than in the TIFOI groups. Regarding the total cells number, there was no difference ($P>0.05$) among all groups. The percentage of apoptotic cells in the TIFOI 22h group (4.1%) differed ($P<0.05$) only from the control 12h (7.2%) and control 16h (7.1%) and did not differ from the others ($P>0.05$) (control 22h=6.3%, TIFOI 12h=5.1%, TIFOI 16h=6.3%). Despite the lower embryonic development, observed in the TIFOI group, it can be concluded that the time of 12 hours is sufficient for the oocytes to be ready to be fertilized and to develop to blastocyst stage. It seems that the time after the injection to fertilization is not the main obstacle for the embryonic development.