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# Nuclear maturation of bovine oocytes submitted to the *in vivo* maturation using intrafollicular transfer of immature oocytes (IFIOT) system

**Luzia Renata Oliveira Dias[1], Otavio Augusto Faria[2], Felippe Manoel Costa Caixeta[1], Sarah de Andrade Dias Rodrigues[1], José Felipe Warmling Sprícigo[3], Margot Alves Nunes Dode[3]**

[1]Universidade de Brasília, Brasília, DF; [2]Médico Veterinário Colaborador, Brasília, DF; [3]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF.

This study aimed to evaluate the kinetics of nuclear maturation of bovine oocytes submitted to the in vivo maturation using intrafollicular transfer of immature oocytes (IFIOT) system. To do that, ovulatory cows were previously synchronized. On day 0 (D0) the animals received an intravaginal progesterone-releasing device (1 g), and an injection (i.m.) of 2 mg Estradiol Benzoate. On D8, the progesterone implants were removed and an injection of Prostaglandin F2α analog (0.150 mg d-Cloprostenol) was made (i.m.). On D9 a 1 mg of Estradiol Benzoate (i.m.) was administered and on day 10 oocytes were injected into a ≥10 mm diameter follicle together with an injection of a gonadotrophin releasing hormone (GnRH) analogue - Buserelin. A total of 890 grade 1 and 2 oocytes obtained from slaughterhouse ovaries were used, being 417 for TIFOI and the remainder for Control. In the control group the oocytes were placed in IVM and removed at 0, 8, 12 and 16h. For TIFOI, 30 oocytes per ovulatory cow were used, which at 8, 12 and 16h post-injection were recovered by ovum pick up (OPU). Treatments and number of oocytes evaluated by treatment were: Control 0h (n=51); 8h Control (n=60); Control 12h (n=60); Control 16h (n=38); TIFOI 8h (n=79); TIFOI 12h (n=88); TIFOI 16h (n=7). Oocytes from all groups were denuded by and are fixed for further evaluation of nuclear maturation by lacmoid stain. Oocytes were classified according to meiotic stage in: germinal vesicle (GV), germinal vesicle break (GVB), metaphase I (MI), anaphase I (AI), telophase I (TI), metaphase II (MII) and abnormal. Data were analyzed by Chi-square test ($P<0.05$). At 0h, before maturation or injection, 96.1% of the oocytes were found at GV stage. At 8 h, most of the oocytes of the Control group were in MI stage (76.6%), while TIFOI 8h group presented a greater percentage ($P<0.05$) of oocytes in stage (97.5%). The percentage of oocytes in MI at 12h was similar ($P>0.05$) between the Control (81.7%) and TIFOI 12h (73.9%) and both presented oocytes at more advanced stages of meiosis (Control=TI 13.3%, MII 1.7%, TIFOI=AI 4.5%, TI 7.9%). Control group 16h had oocytes abnormal (2.6%), in MI (52.6%), AI (18.4%), TI (21.1%) and MII (5.3%). In the TIFOI 16h group at the time of aspiration the majority of the animals had already ovulated and, therefore, a small number of oocytes were recovered (n=7), being classified as abnormal (14.3%), MI (57.1%), TI (14.3%) and MII (14.3%). The results suggest that the in vivo maturation system using the TIFOI method was adequate, since the oocytes exposed to this system presented kinetics of maturation similar to the in vitro, being even more homogeneous than in vitro with 8 h of maturation.