Animal Reproduction

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OPU-FIV

Intrafollicular transfer of immature oocytes (IFIOT) as an alternative for maturation of bovine oocytes

Otávio Augusto Costa de Faria[1], Nayara Ribeiro Kussano[2], Lucas Costa de Faria[1], Leticia Prates Martins[1], José Felipe Warmling Sprícigo[3], Margot Alves Nunes Dode[2]

[1]Universidade de Brasília (UnB)
[2]Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
[3]Universidade Federal de Goiás (*Departamento de Ciência Animal - UFG*)

E-mail: otavio.fariamv@gmail.com

Studies have demonstrated that oocytes matured in the pre-ovulatory follicle by intrafollicular transfer of immature oocytes (IFIOT) have nuclear maturation and lipid droplets accumulation similar to those matured *in vivo* (Faria *et al.*, Reproduction, Fertility and Development 33(5) 372-380, 2021). However, it is still unknown whether the ability to form an embryo and the amount of lipids remained similar to the *in vivo* matured after IVF and IVC. Therefore, the objective of this study was to assess whether IFIOT improves maturation and affects embryo production and quality. To do this, oocytes matured by IFIOT were compared with those matured *in vitro* (IVM) and with those matured *in vivo* after ovarian superstimulation (FSH). After selection, 25-30 COCs recovered from abattoir ovaries were allocated to the IVM (22 h) or were injected into a dominant follicle of a cow ovulator (diameter ≥10 mm) for IFIOT systems. For the FSH group donors were super stimulated with 100 mg of Folltropin (Folltropin-V; Vetoquinol). *In vivo* matured oocytes were recovered by OPU at 22 h after GnRH injection (i.m), which was performed at the moment of IFIOT and, in the other group, at 18 h after the last injection of FSH. *In vitro* and *in vivo* matured oocytes were then fertilized and cultured *in vitro.* On day 7 of culture, expanded blastocyst were either stained for analyses of mitochondrial activity, lipid quantification and total number of cells, using confocal microscopy, or cryopreserved by DT method to evaluate resistance to cryopreservation. After thawing, the embryos evaluated for expansion at 6 and 24 h of culture. Data were analyzed by Chi-square and ANOVA (GLIMMIX) tests. To date, nine replicates have been carried out. As expected FSH group showed a higher (P<0.05%) cleavage (n = 134/138, 97.10%) and blastocyst rate (n = 56/138, 40.58%) than the IVM (n = 377/454, 83.07% and n = 131/454, 28.85%) and IFIOT (n = 365/425, 85.88% and n = 127/425, 29.88%) groups, which, in turn, were similar between then (P>0.05). Embryos from the three groups were similar regarding the total cells number and mitochondrial activity. Conversely, the mean area occupied by lipids on embryo from IVM groups (n = 23, 12.9% ± 7.73) was higher (P<0.05%) than in embryos from FSH (n = 23, 5.81% ± 4.36) and IFIOT (n = 28, 5.32% ± 4.08) groups, which, by the way, were similar (P>0.05%). Even though embryos differ of intracellular lipids contend, they had similar response to cryopreservation evaluated by re-expansion, at 6 hours (IVM = 25/55, 45%; FSH = 15/31, 48%; IFIOT = 29/53, 55%; P>0.05%) and 24 hours (IVM =37/55, 67%; FSH = 19/31, 62%; IFIOT = 44/53, 83%; P = 0.0621) after thawing. Therefore, the results suggested that the effects of the maturation system can affect the IVF embryo production and their quality. Moreover, the IFIOT system provides embryos with similarities to those produced *in vivo*, regarding lipid content, and can be used as a tool for the maturation. Considering that lipids content may reflect embryo metabolism, maturation by IFIOT, brings new possibilities for reproductive technologies that require *in vitro* matured of oocytes. Acknowledgement: FAP-DF, Embrapa, CAPES.