Animal Reproduction

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SUPPORT BIOTECHNOLOGIES CRYOPRESERVATION AND CRYOBIOLOGY, DIAGNOSIS THROUGH IMAGING, MOLECULAR BIOLOGY, AND "OMICS"

Intrafollicular immature oocyte transfer (IFIOT) for *in vivo* maturation of vitrified bovine oocytes

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The cryopreservation of bovine oocytes has great potential for the expansion and use of animal reproduction biotechniques, as well as for genetic conservation. The aim of this study was to increase the embryo development and quality of fresh and cryopreserved COCs following *in vivo* maturation through IFIOT. *Cumulus*-oocyte complexes (COCs), obtained from abattoir ovaries, were randomly assigned to 4 treatment groups: 1) fresh COCs matured *in vitro* (MF; n = 210); 2) fresh COCs matured *in vivo* by IFIOT (TF; n = 236); 3) vitrified/warmed immature COCs matured *in vitro* (MV; n = 216); and 4) vitrified/warmed immature COCs matured *in vivo* by IFIOT (TV; n = 215). The vitrification and heating protocols followed the Cryotop® methodology, the IFIOT was carried out following the methodology described by IFIOT-Embrapa®. For IFIOT, 26 Nelore females were subjected to a synchronization protocol based on P4/E2 to ensure the presence of a pre-ovulatory follicle (≥10mm) on the day of the injection. After IFIOT, 50 mg IM of lecirelin (GnRH, Tec- Relin®, Agener União) was administered to induce the LH peak. For IVM, the oocytes remained in maturation medium for 20-22 h, just like the *in vivo* groups in the preovulatory follicle, until the matured COCs were retrieved by OPU. After maturation, the COCs from all groups were subjected to IVF and IVC. Cleavage rate was evaluated on D2 and blastocyst rates on D6 and D7. Moreover, the kinetics development was evaluated on D6 and D7. Finally, on D7, Bx embryos were evaluated for diameter (µm), total cell number, and percentage of apoptotic cells (TUNEL®). Statistical analyzes were performed using the Chi- square test, ANOVA and Tukey test (SAS; $P \leq 0.05$). Cleavage rate was lower for the vitrified groups (MV = 22.6 and TV = 21.8%) compared to the fresh groups (MF = 90.0 and TF = 86.0%). Blastocyst rate on D6 differed among all groups (MF = 22.3^{b}; TF = 31.3^{a}; MV = 0.9^{d}; and TV = 4.6%^{c}). However, on D7 differences in blastocyst rates were only observed between fresh and vitrified (MF = 44.2^{a}; TF = 47.0^{a}; MV = 2.3^{b}; and TV = 4.6%^{b}). Regarding blastocyst quality evaluations, both cryopreserved groups (MV and TV) did not achieve enough blastocysts for statistical analysis. On D6, TF embryos developed faster than MF embryos, since 24.3% (18/74) of the embryos reached the Bx stage compared to 8.5% (4/47), respectively. However, no difference in developmental stages were observed on D7 [TF =54 (60/111) and MF = 44.0% (41/93)]. Finally, TF blastocysts presented greater diameter [192 ± 16.4 (n =48) *vs.* 179 ± 16.3 µm (n = 26)], number of cells [165 ± 11.4 (n = 38) *vs.* 156 ± 11.3 (n = 24)] and fewer proportion of apoptotic cells [2.8 ± 0.01 (n = 38) *vs.* 4.2% ± 0.01 (n = 24)], compared to MF blastocysts. In summary, *in vivo* maturation using the IFIOT technique for immature vitrified COCs did not improve embryo development or quality. However, for fresh oocytes, IFIOT accelerated embryo development on D6 and increased the blastocyst quality on D7.