Animal Reproduction

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

# Can the time and conditions of incubation of *in vivo*- derived goat embryos compromise embryonic cryosurvival?

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Evidence suggests that the conditions after collection may compromise the survival of *in vivo*-derived (IVD) embryos after cryopreservation. To test this hypothesis, 150 IVD embryos (grade I and II) from 25 Alpine goats were divided into three groups after recovery on day 6 (D6; D0 = AI): P3 (n=50) – embryos maintained in a 35 mm cell culture dish with 2 mL of holding medium (HM; 301 mOsm/kg) at 37 °C for 3 h on a hotplate; P6 (n = 50) – embryos kept under the same conditions as the P3 group for 6-8 h; and T6 (n = 50) embryos maintained in cryovials containing 1 mL of HM at 37°C, for 6-8 h, in a transporter. After incubation, the embryos were reclassified and subjected to slow freezing and the media from each group was frozen for osmolarity analysis. After thawing, 45 blastocysts (n = 15 per group) were dry-frozen in pools of five embryos for analysis of differential expression of genes related to cellular stress (PRDX.1 and HSP70), embryonic quality (CDX2, NANOG, TGFB1 and NRF1) and apoptosis (BAX and BCL2), using RT-qPCR and $2^{-\Delta\Delta Ct}$ method, after normalization with ACTB and GAPDH genes. After thawing and before IVC, 10 blastocysts from each group were analyzed for mitochondrial activity (MitoTrackerRed), glutathione (GSH; Cell Tracker Blue) and reactive oxygen species (ROS; H2DCHFDA) levels. Results are presented as percentage (%) or mean ± SDM. Differences were considered significant when P≤0.05 in ANOVA followed by Tukey's test (parametric data), Kruskal-Wallis followed by Dunn's test or chi-square test (non-parametric). Increase in medium osmolarity was observed in all groups after the incubation period: 15% (347/301) in P3, 49% (301/447) in P6 and 1% (301/307) in T6. Regarding the stage of development after incubation, frequencies were assessed by Kappa test revealing a significative increase ($p<0.05$) of blastocysts and expanded blastocysts in the T6 group (27,5% and 42,5%, respectively), meanwhile P6 group had no difference ($p>0.05$) among these stages (14,1 and 34,62%, respectively). Despite this, no difference ($P>0.05$) was observed in the survival rate after cryopreservation (61, 70 and 78%). Levels of GSH ($44 \pm 4^{a, b}$, $48 \pm 6^{a}$ and $39 \pm 2^{b}$), ROS ($10 \pm 3^{a}$, $10 \pm 1^{a}$ and $18 \pm 2^{b}$) and mitochondrial activity ($38 \pm 20^{a}$, $32 \pm 3^{a}$ and $81 \pm 46^{b}$) differed between groups. Higher expression ($P<0.05$) of genes associated with cellular stress (HSP70 and PRDX1) and proapoptotic (BAX) were observed in the P6 group, compared to the T6 group. However, both groups were similar ($P>0.05$) to the P3 group. The abundance of other transcripts did not differ ($P>0.05$) between the groups. Despite previous incubation on the transporter did not modify the survival rate after cryopreservation, when compared to incubation on the hot plate, the analysis performed revealed that the experimental condition of the T6 group is able to better maintain molecular aspects of IVD goat embryos after thawing, such as apoptotic incidence and ATP production.

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