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CALF PRODUCTION BY ARTIFICIAL INSEMINATION WITH SPERMATOZOA OBTAINED FROM EPIDIDYMIDES REFRIGERATED AT 5°C FOR LONG PERIODS AFTER DEATH

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The viability study of spermatozoa recovered postmortem animal is important to determine the maximum period to extract viable cells of the epididymides. In this work bovine testicles were collected in abattoir, transported to the laboratory and stored at 5°C for different periods (0h, 24h, 48h e 72h). The spermatozoa were retrieved from each epidydimides, evaluated and diluted in tris-egg yolk-glycerol 7% medium and cryopreserved in liquid nitrogen. The morphological and functional characteristics of the spermatozoa were analyzed in vitro and in vivo. The data were submitted to analyze of variance One Way Anova and t test with 5% of significance. Pathologies of sperm immaturity, motility decreased after 72 h of epididymides refrigeration and after thaw sperm were observed. The membrane and acrosome integrity were only affected in G48 h and G72 h groups after cryopreservation. However, the sperm capacity of fertilization post-cryopreservation was sufficient to promote two pregnancies and birth of healthy calves from G24 h and G72 h groups. These results indicated that recovery and cryopreservation of chilled epididymal sperm from dead animals is a viable option to preserve male gametes to compose a germplasm bank.

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CRYOTOLERANCE OF MORULAE AND BLASTOCYSTS PRODUCED *IN VIVO* IN *BOS INDICUS*

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The aim of this study was to evaluate the cryotolerance of morulae and blastocysts produced *in vivo* in Sindhi and Nellore (*Bos indicus*) donors. In Experiment 1, 24 lactating and non-lactating Sindhi donors were superovulated with 100 mg porcine FSH with protocols in which the last two FSH treatments were replaced or not by 300 IU eCG. Embryos were collected 7 days after ovulation induction and embryo development and quality degree were accessed according to Ahmad *et al.* (1995; Biol Reprod, 52:1129-1135). Two thirds of the embryos were cryopreserved, by conventional freezing or vitrified using a new vitrification method (Vitri-ingá®, INGÁMED, Perobal, PR, Brasil), similar to the Cryotop method. After that, embryos were thawed/warmed and transferred to synchronized recipients, simultaneously to fresh embryos. In Experiment 2, 31 Nellore cows were superovulated with 133 mg porcine FSH and two thirds of the embryos were cryopreserved and transferred similarly to Experiment 1. Results were analyzed using generalized linear models and are presented as least squares means ± standard error. In Experiments 1 and 2, fresh embryos had a higher conception rate at Day 30 than those vitrified and frozen (54.8 ± 7.4^{a}, 17.7 ± 7.3^{b} and $19.5\pm6.6\%^{b}$, respectively; Pd"0.0013) in Sindhi donors (n=231 embryos) and (46.0 ± 6.1^{a}, 31.2 ± 5.4^{b} and $28.1\pm5.3\%^{b}$; respectively; Pd"0.04) in Nellore donors (n=297 embryos). There was no difference between the conception rates of morulae and blastocysts at 30 days (27.8±5.6 and 28.2±8.3%, respectively; P>0.90) and at 60 days (27.1±5.1 and 28.4±7.9%, respectively; P>0.90) in Sindhi donors. In Nellore donors, developmental stage also seemed to not have influenced conception rates at 30 days (39.1±4.8 and 30.5±4.9% for morulae and blastocysts, respectively; P>0.17) or at 60 days (30.6±4.6 and 23.9±4.3%; P>0.24). Finally, there was no effect of embryo quality (Grade 1 versus 2) in conception rates of fresh, vitrified and frozen embryos at 30 or 60 days. We concluded that fresh embryos had higher viability than the cryopreserved ones in Zebu breeds and different stages of embryonic development as well as the cryopreservation methods had similar cryotolerance in *Bos indicus* embryos.

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