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Comparison of Two Methods of Cryopreservation of Testicular Tissue from Prepubertal Lambs

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Abstract : The cryopreservation of testicular tissue emerges as an alternative for the preservation of the reproductive potential of individuals who still cannot produce sperm; however, they will undergo treatments that may affect their fertility (e.g., chemotherapy). Therefore, the present work aims to compare two cryopreservation methods (slow freezing and vitrification) in testicular tissue of prepubertal lambs. For that, to obtain the testicular tissue, the animals were castrated and the testicles were collected immediately in a physiological solution supplemented with antibiotics. In the laboratory, the testis was split into small pieces. The total size of the testicular fragments was 3×3x1 mm^3 and was placed in a dish contained in Minimum Essential Medium (MEM-HEPES). The fragments were distributed randomly into non-cryopreserved (fresh control), slow freezing (SF), and vitrified. To SF procedures, two fragments from a given male were then placed in a 2,0 mL cryogenic vial containing 1,0 mL MEM-HEPES supplemented with 20% fetal bovine serum (FBS) and 20% dimethylsulfoxide (DMSO). Tubes were placed into a Mr. Frosty™ Freezing container with isopropyl alcohol and transferred to a -80°C freezer for overnight storage. On the next day, each tube was plunged into liquid nitrogen (NL). For vitrification, the ovarian tissue cryosystem (OTC) device was used. Testicular fragments were placed in the OTC device and exposed to the first vitrification solution composed of MEM-HEPES supplemented with 10 mg/mL Bovine Serum Albumin (BSA), 0.25 M sucrose, 10% Ethylene glycol (EG), 10% DMSO and 150 µM alpha-lipoic acid for four min. The VS1 was discarded and then the fragments were submerged into a second vitrification solution (VS2) containing the same composition of VS1 but 20% EG and 20% DMSO. VS2 was then discarded and each OTC device containing up to four testicular fragments was closed and immersed in NL. After the storage period, the fragments were removed from the NL, kept at room temperature for one min and then immersed at 37 °C in a water bath for 30 s. Samples were warmed by sequentially immersing in solutions of MEM-HEPES supplemented with 3 mg/mL BSA and decreasing concentrations of sucrose. Hematoxylin-eosin staining to analyze the tissue architecture was used. The score scale used was from 0 to 3, classified with a score 0 representing normal morphologically, and 3 were considered a lot of alteration. The histomorphological evaluation of the testicular tissue shows that when evaluating the nuclear alteration (distinction of nucleoli and condensation of nuclei), there are no differences when using slow freezing with respect to the control. However, vitrification presents greater damage ($p < 0.05$). On the other hand, when evaluating the epithelial alteration, we observed that the freezing showed scores statistically equal to the control in variables such as retraction of the basement membrane, formation of gaps and organization of the peritubular cells. The results of the study demonstrated that cryopreservation using the slow freezing method is an excellent tool for the preservation of pubertal testicular tissue.

Keywords : cryopreservation, slow freezing, vitrification, testicular tissue, lambs
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