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Evaluation of different fragment sizes and cryoprotectants for cryopreservation of feline testicular tissues
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Cryopreservation of testicular tissue is considered an alternative and efficient method to maintain cell-to-cell contact, allowing their survival and future use in xenotransplantation. However, up to now there is no ideal protocol for cryopreservation of feline testicular tissue. For that reason, this study aimed to evaluate tissue damage of feline testicles sectioned in two different sizes (0.3 cm³ or 0.5 cm³) and submitted to different cryoprotectants (Propanediol 3% or Glycerol 3%) through histomorphology and, for the first time, Thiobarbituric Acid Reactive Substances test (TBARS). Testicles were obtained from 12 domestic cats (crossbreed, 1 to 5 y), transferred into a 50ml falcon tube containing NaCl 0.9% and transported in a isothermal box (18°C) to the laboratory within 2 h. Testicular tissue was separated from the vessels, tunica albuginea and epididymides, washed three times in saline solution supplemented with streptomycin + penicillin and sectioned in 0.3 cm³ and 0.5 cm³ sized pieces (measured with a digital pachymeter). One fragment of each size was immediately evaluated by TBARS and semi-quantitatively by histomorphology, scored as follow: (i) detachment of cells from the basement membrane was scored as 0 if absent, 1 <75% and 2 >75% of the circumference, (ii) gap formation and shrinkage were scored as 0 if absent, as 1 if slight and as 2 if more obvious; (iii) distinction between Sertoli cells and spermatogonia nuclei was scored as 0 if easy, 1 if difficult and 2 if impossible, (iv) observation of nucleoli was scored as 0 if easy (visible in 40% of cells) and scored as 1 if indistinguishable; (v) pyknotic nuclei was scored as 0 if absent, as 1 if <40% and as 2 if >40% were pyknotic. The remaining 0.3 and 0.5 cm³ fragments were placed in cryotubes with 1mL Egg yolk Tris Equex STM extender containing 3% of glycerol or 3% propanediol for 10 min at room temperature, then were maintained at 4°C for 30 min and finally were laid horizontally on a rack 4 cm above the liquid nitrogen vapor for 10 min, before being plunged into liquid nitrogen. For thawing, the samples were exposed to air for 10 sec and submerged into 37°C water bath for 30 sec. The testicular tissues were placed in extender medium at 37°C for 5 min. Frozen-thawed fragments were also evaluated by TBARS and histomorphology. Statistical analysis was performed by using One-way ANOVA with Student–Newmann–Keuls post hoc test to test the interaction of fresh and frozen-thawed testicular tissue, with P < 0.05. Fresh and cryopreserved tissues generally exhibited a similar morphology concerning detachment of cells from the basement membrane (i) and observation of nucleoli (iv), with a great proportion scored as 0. When present, alterations were slight and the morphology was considered to be good (most classified in scores 1). Pyknosis was the main anomaly observed as score 2 in 54.6% and 58.4% of 0.3 cm³ fragments cryopreserved in propanediol and glycerol, respectively (16.7% scored 2 in fresh tissue). In TBARS evaluation, 0.5 cm³ fragments cryopreserved in glycerol produced less free radical compared to the 0.3 cm³ cryopreserved in glycerol or propanediol. Our results showed that glycerol was more efficient than propanediol to cryopreserve 0.5 cm³ testicular fragments; this might be attributed to the fact that glycerol molecular weight is larger than propanediol and so its perfusion in the testicular tissue is slower. Moreover, in 30 min equilibration time, propanediol was more toxic for all sizes of testicular fragments.