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EFFECT OF TWO DIFFERENT REFRIGERATION PROCESSES ON THE QUALITY OF CHILLED AND FROZEN/THAWED EPIDIDYMAL SPERMATOZOA

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Purpose of the work. Cryopreservation of epididymal spermatozoa is a useful tool to preserve genetic material of valuable stallions after emergency castration or unexpected death. Often, testicles and epididymides are sent refrigerated to the laboratory. The collection of epididymal spermatozoa is a simple procedure1 that reduces the volume of the material to be shipped, and may improve the quality of the chilled epididymal sperm cells. In the present study we compared the characteristics of chilled and frozen/thawed epididymal spermatozoa (SPZ), using refrigeration of the epididymides vs. direct refrigeration of the epididymal sperm cells.

Materials and used methods

For that, epididymides of nine stallions subjected to routine castrations were utilized. After the orchiectomy, one testicle and corresponding epididymis were refrigerated at 5ºC (EPI R) for 24 h. The epididymal cauda of the contralateral testicle was immediately separated and spermatozoa were flushed with 50 ml Kenney medium and refrigerated for 24 h at 5ºC (ESPZ R). The same flushing method was applied to collect SPZ from the EPI R group, after the refrigeration period. Spermatozoa concentration was assessed with the Newbauer chamber immediately after flushing. After 24-h chilling, SPZ were frozen utilizing the Botucrito? extender in 0.5 mL straws2. Weight of testicles and tail of the epididymides were recorded. Viability (eosin-nigrosin staining) and sperm cell morphology (Diff-Quick, optical microscopy, 1000 x magnification) were evaluated after 24-h refrigeration and after freezing/thawing. Progressive motility (optical microscopy) was assessed after the 24 hr refrigeration following centrifugation (10 min, 900g), after addition of Botucrito? extender (MPE) and after thawing (MPT). Previously to motility assessment, SPZ were warmed to 37ºC. Thawing was done immersing the straws for 1 minute in a water bath at 37ºC. Total motility (TM), linear velocity (VSL), and percentage of rapid sperm of frozen/thawed SPZ (RAP%) was evaluated with a CASA system (ISAS?).

Outcomes. The weight of left (174.30±14.41) and right testicles (176.90±11.84) and left (13.86±0.89) and right cauda epididymides (12.35±1.03) did not differ (p>0.05). Total number of SPZ recovered was 8878±1112x106 for EPI R and 9014±1456x106 for ESPZ R (p>0.05). No difference (p>0.05) in percentage of viable cells or in abnormal sperm cells was found between EPI R and ESPZ R before and after cryopreservation. MPE and MPT (61.00±7.97 vs. 50.00±10.41; 29.38±4.58 vs. 24.29±5.05, for EPI R and ESPZ R, respectively) did not differ (p>0.05).

After thawing no differences (p>0.05) were observed between experimental groups regarding TM, VSL and RAP% (42.95±6.29 vs. 49.46±7.74; 16.63±1.83 vs. 19.24±2.24; 4.25±9.93 vs. 6.74±1.69 for EPI R and ESPZ R, respectively).

Conclusions. In conclusion, both methods of refrigeration were equally efficient for chilling and freezing epididymal SPZ. The method of choice to chill epididymal SPZ may be chosen based on operator preference and/or cost of shipping.

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**Bibliography**


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