Evaluation of frozen stallion spermatozoa using two different moments of glycerol addition

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The widespread use of frozen stallion spermatozoa has lagged behind that of bull spermatozoa. However, despite the difficulties found in the cryopreservation of stallion spermatozoa, many researches have been done to elucidate such differences between these two species. Although glycerol is the most common cryprotectant used to freeze stallion semen, some doubts still remain unclear about what happens to spermatozoa according to the chosen method. The objective of this study was to verify the differences between frozen-thawed stallion spermatozoa when the addition of glycerol is carried-out before or after equilibration time.

Materials and methods: Forty-three ejaculates from four stallions of different breeds and ages, with known fertility were used. Semen was collected using artificial vagina ("Botucatu" Model) and then analyzed as standard procedures for volume (mL), pH, osmolarity, concentration ($x10^6$/mL), hyposmolarity test (HO), eosin staining, membrane integrity analysis, subjective motility (%), and the parameters given by CASA (HTM-IVOS-10). Afterwards, semen samples was diluted (1:1) in Kenney medium for centrifugation at 600x g for 10 min at room temperature. Spermatozoal pellet was divided into two samples. One of them was resuspended in M9 extender (Papa et al, 1998) without glycerol (Sample A) and the other in the similar extender containing 5% glycerol (Sample B). The final volume of both resuspended samples was equal to that prior to centrifugation. Sample B was packaged in 0.5-mL straws containing 100 million motile spermatozoa each, whereas the total volume of Sample A was kept in a glass tube. Both samples were cooled to 5°C for 60 min in an appropriated chamber. Before packing, Sample A was diluted 1:1 with a solution 10% glycerol and then it was packaged just as Sample B. Both sample straws were frozen at 6 cm above liquid nitrogen for 20 min and then plunged into liquid nitrogen for few seconds until being stored. After that, one straw of each sample was thawed into 46°C water bath for 20 sec and the same parameters initially evaluated were verified again. The differences between the frozen-thawed spermatozoa were compared with respect to the glycerol addition time by analysis of variance, considering each animal as a block.

Results and Discussion: No significant differences ($P<0.5\%$) were observed in the semen parameters between the samples with glycerol addition before or after equilibration time. These results are in agreement with Martin et al. (1979), Loomis et al. (1983) and Cochran et al. (1983). However, the authors of this study suggest glycerol be added prior to the equilibration period due to being more practicable to packing the semen at room temperature.

References

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