



CUSHIONED CENTRIFUGATION BEFORE CRYOPRESERVATION OF EPIDIDYMAL STALLION SPERMATOZOA



F.C.C. Santos^{1*}, J. M. Morrell², C.E.W. Nogueira³, B.R. Curcio³, M.M. Nunes⁴, E. Malschitzky⁵

¹Departamento de Medicina Veterinária, Universidade Federal de Roraima (UFRR), Boa Vista, Roraima, Brazil;

²Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden;

³Departamento de Clínicas Veterinária, Faculdade de Veterinária, Universidade Federal de Pelotas (UFPel); Pelotas, Rio Grande do Sul, Brazil;

⁴Discente do Programa de Pós Graduação em Medicina Veterinária, Universidade Federal de Viçosa (UFV);

⁵Curso de Medicina Veterinária, Universidade Luterana (ULBRA)



Introduction

- Unexpected death, orchietomy or traumatic injuries can prematurely end the stallion's reproductive life, since sperm harvesting from the cauda epididymis is the last chance to preserve spermatozoa.
- For cryopreservation, the centrifugation step aims to increase sperm concentration, although this may induce detrimental effects on sperm quality and also lead to loss of spermatozoa.
- In order to protect the cell and reduce sperm loss during centrifugation, semen can be under laid with dense colloid, called cushioned centrifugation.

The objective of this study was:

- To determine the effect of cushioned centrifugation of frozen-thawed epididymal stallion sperm quality.

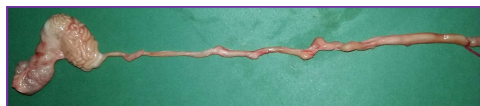
Material and Methods

Epididymal stallion sperm collection:

- Ten Crioulo stallions, aged 4 years-old, were submitted to elective bilateral orchietomy under general and local anesthesia.
- The cauda and the deferent duct were dissected and sperm were recovered by retrograde flush of the cauda of epididymies with Kenney (1975) extender.

Semen processing

- Sperm concentration, total and progressive motility were determined by computer-assisted sperm analyzer (CASA) AndroVision (Minitub®, Tiefenbach, Germany)
- Samples were extended to a final concentration of 100×10^6 spermatozoa/mL and submitted to different centrifugation protocols:



- The supernatant was removed, the pellet was re-suspended and evaluated regarding sperm quality.

Statistical analyses

- Descriptive statistics (expressed by mean \pm standard error mean, Kruskal-Wallis one-way test, analysis of variance (ANOVA) followed by comparison between means by Tuckey test.
- Significance was set as $p < 0.05$.

Results

- No significant difference was observed between treatments Conventional and Cushioned regarding total motility, progressive motility, sperm morphology, mitochondrial functionality, membrane integrity and DNA integrity

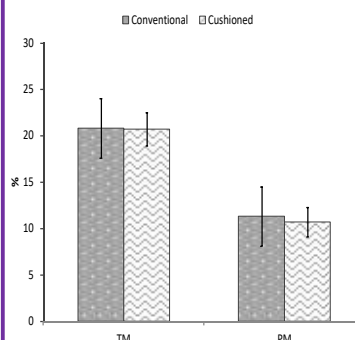


Figure 1. Total motility (TM) and progressive motility (PM) of frozen-thawed epididymal stallion sperm submitted to Conventional and Cushioned centrifugation prior cryopreservation.

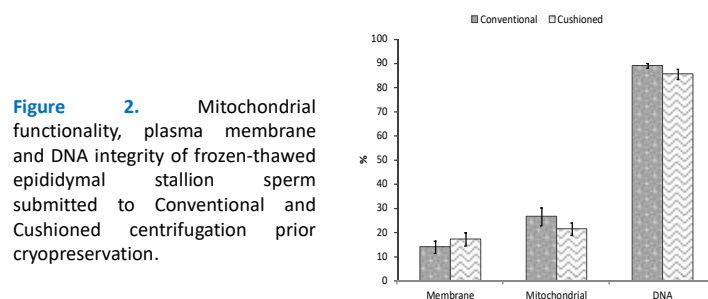


Figure 2. Mitochondrial functionality, plasma membrane and DNA integrity of frozen-thawed epididymal stallion sperm submitted to Conventional and Cushioned centrifugation prior cryopreservation.

Conclusion

- Cushioned centrifugation performed before cryopreservation had neither a beneficial nor a detrimental effect on thawed epididymal stallion sperm quality.

References

- Kenney, R.M.; Berman, R.V; Cooper, W.L et al. Minimal contamination techniques for breeding mares: technique and preliminary findings. In: Annual Convention of American Association Equine Practitioners, 21, 1975, Boston. Proceedings...Boston, AAEP, p.327-336, 1975.

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