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Comparison of different cryoprotectants for freezing donkey jack (*Equus asinus*) semen

Santiago L Bruno[1,*], Deborah M Neild[2], Jessica P Plaza[2], Alejandro A Ferrante[2], María I Carretero[2], Gustavo EA Olivieri[1], Carolina P Bianchi[3,4], Marcelo H Miragaya[2]

[1] *Haras Militar "General Lavalle", Dirección de Remonta y Veterinaria, Ejército Argentino, Tandil, Argentina*
[2] *Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, INITRA, Cátedra de Teriogenología, Buenos Aires, Argentina*
[3] *Universidad Nacional del Centro de la Provincia de Buenos Aires, Facultad de Ciencias Veterinarias, Laboratorio de Endocrinología, Tandil, Argentina*
[4] *Centro de Investigación Veterinaria de Tandil (CIVETAN), Tandil, Argentina*

The objective of this study was to extrapolate a rapid sperm freezing technique developed in horses to Remonta Argentino donkey semen, evaluating, *in vitro* and *in vivo*, the effect of freezing media that included two cryoprotectants, dimethylformamide (DMF) and methylformamide (MF) at two different concentrations (5 and 7%). Twenty-four ejaculates from 8 fertile jacks (n=8; r=3) were processed with 8 extenders: BotuSemen Gold with 5% or 7% MF and 5% or 7% DMF and EDTA-glucose with 5% or 7% MF and 5% or 7% DMF, all containing 11% lactose, 20% egg-yolk and Equex and frozen: to -15°C (10-12°C/min) then to -120°C (25-40°C/min.), then plunged in N2. Post-thaw evaluations included sperm motility (Computer Assisted Semen Analysis; AndroVision, Minitube, Tiefenbach, Germany), morphology (Diff Quick stain), membrane function and acrosome status (combined hypoosmotic test and Coomassie blue stain). Sperm data were analyzed using Kruskal Wallis and pregnancy data using Chi-Square test. Values are mean ± SD. Differences were observed in the post-thaw progressive sperm motility between individuals ($p<0.05$) resulting in three subgroups (low: 9.23 ± 0.16; intermediate: 22.92 ± 9.81 and high: 41.33 ± 5.41). Within each subgroup, there were no *in vitro* differences among the 8 media used for any of the sperm characteristics evaluated. Samples in BotuSemen Gold with 5% DMF, however, tended to show highest percentages ($P>0.05$) of sperm with acrosomes and functional membranes (DMF: 5%: 53.67 ± 22.01; 7%: 33.92 ± 23.4; MF: 5%: 44.5 ± 20.46; 7%: 38.75 ± 27.4). In vivo, 300×10^6 PM sperm were deeply inseminated post-ovulation in 30 mares: 15 with BotuSemen Gold 5% DMF and 15 with BotuSemen Gold 7% DMF. A 46% (7/15) pregnancy rate was obtained using the extender with 5% DMF and no pregnancies (0%; 0/15) were obtained using the extender with 7% DMF ($P=0.003$). Perhaps uterine inflammatory response post-insemination was too high when using 7% DMF or sperm chromatin was damaged, affecting *in vivo* results. In conclusion, the rapid semen freezing technique was successfully applied in jacks. Regarding the extenders, no significant *in vitro* differences were observed using either BotuSemen Gold or EDTA-glucose media, whether adding MF or DMF at either 5% or 7%. However, when inseminating mares, pregnancies were only obtained using 5% DMF. It would be interesting to analyze sperm DNA and peroxidation characteristics as well as the uterine response to insemination to determine possible reasons for the failure in fertility and the variability observed among individuals.