ELSEVIER

Animal Reproduction Science 94 (2006) 82–84

ANIMAL REPRODUCTION SCIENCE

www.elsevier.com/locate/anireprosci

Abstract

Effect of cryoprotectant on donkey semen freezability and fertility☆

J.V. Oliveira, M.A. Alvarenga, C.M. Melo, L.M. Macedo, J.A. Dell'Aqua Jr., F.O. Papa *

UNESP-FMVZ, Departmento de Reproducao Animal e Radiologia Veterinaria, Distrito de Rubiao Jr, s/n Botucatu, SP, CEP 18618-000, Brazil

Available online 6 April 2006

1. Introduction

As a consequence of indiscriminate slaughter, some donkey breeds are at risk of extinction. For example, the Poiton breed has 180 animals world wide, and the Brazilian donkey population is around 80 animals (Phillippe, 1994; Mariante and Cavalcante, 2000). The use of donkey frozen semen is an important tool for maintaining this species. However, few studies have evaluated donkey semen quality or fertility (Vieira et al., 1985; Trimeche et al., 1998; Papa et al., 1999; Vidament et al., 2005). The aim of the present study was to evaluate the effect of different cryoprotectants on donkey semen and fertility using mares and jennies.

2. Materials and methods

Thirteen ejaculates were collected from a Brazilian donkey, and each ejaculate was diluted at 1:1 (semen/extender) using Botu-Semen^{TM} extender and centrifuged at $600 \times g$ for 10 min. The supernatant was dispensed and the pellet re-suspended in a concentration of 200×10^6 sperm/ml using MP 50 extender (Papa et al., 2002) in association with different cryoprotectants comprising various combinations of dimethyl sulphoxide (DMSO), dimethyl formamide (DF), dimethylacetamide (DA) and glycerol (GLY). These were: M1 (2% DMSO + 2% DF), M2 (3% DF), M3 (2% DA + 2% GLY), M4 (3% DA), M5 (3% GLY + 2% DF) and M6 (3% DMSO % + 2% GLY).

☆ This paper is part of the special issue entitled Proceedings of the Ninth International Symposium on Equine Reproduction, Guest Edited by Margaret J. Evans.

* Corresponding author.
E-mail address: papa@fmvz.unesp.br (F.O. Papa).

0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2006.04.010

Table 1
Mean (±S.D.) values of total motility (TM), progressive motility (PM) membrane integrity (PMI) of sperm frozen using six different cryoprotectants

Cryoprotectant combination	TM	PM	PMI
M1	63.6 ± 8.847	36.5 ± 5.0	44.0 ± 10.2
M2	63.9 ± 8.9	37.7 ± 7.4	42.3 ± 7.5
M3	69.4 ± 7.0	43.5 ± 7.1	47.8 ± 10.4
M4	69.2 ± 8.7	42.2 ± 9.5	48.6 ± 10.8
M5	61.8 ± 11.7	40.0 ± 9.0	41.1 ± 11.6
M6	65.2 ± 12.5	39.2 ± 9.9	43.3 ± 11.1

The samples were packed into 0.5 ml straws, maintained at 5 °C for 1 h and then frozen at 6 cm above liquid nitrogen level for 20 min before being plunged into the liquid nitrogen. The straws were thawed at 46 °C for 20 s and evaluated for total and progressive motility by CASA and the membrane integrity using fluorescent probes (Harrison and Vickers, 1990). The fertility trial was conducted on jennies (53 artificial inseminations with 800×10^6 spermatozoa pre and post ovulation). Based on unsatisfactory pregnancy rates in all groups, a control fertility trial was performed using 10 mares inseminated with the same donkey semen and insemination protocol as for the jennies, with semen that was frozen with 3% glycerol and 2% dimethylformamide (M5).

3. Results

There were no differences in sperm parameters after use of the six cryoprotectant combinations (Table 1) and none of the 53 inseminations of jennies produced a pregnancy. When mares were inseminated with donkey semen, the pregnancy rate was 40%.

4. Discussion

The present study demonstrates that the cryoprotectants used were efficient in protecting the sperm during the cryopreservation process. However, despite the satisfactory in vitro results, no jennie became pregnant with frozen semen. Thus, further investigations are necessary to optimize pregnancy rates in jennies to the level obtained with mares.

Acknowledgement

This study was supported by grants from FAPESP and the animals were feed with dry food from Purina™.

References

Harrison, R.A., Vickers, S.E., 1990. Use of fluorescent probes to assess membrane integrity in mammalian spermatozoa. J. Reprod. Fert. 88, 343–352.

Mariante, A.S, Cavalcante, N., 2000. Animais do descobrimento. Raças domésticas da história do Brasil. Embrapa sede/Embrapa Recursos Genéticos e Biotecnologia, pp. 182–183.

Papa, F.O., Meira, C., Simon, J.J., Dell'Aqua Jr., J.A., Leme, D.P., 1999. Pregnancies in mares using donkey (*Equus asinus*) frozen semen. Arq. Fac. Vet. UFRGS, Porto Alegre 27, 262.

Papa, F.O., Zahn, F.S., Dell'Aqua Jr., J.A., Alvarenga, M.A., 2002. Utilização do diluente MP50 para a criopreservação do sêmen equino. Rev. Bras de Reprod. Anim. 26, 184–187.

Phillippe, M.A., 1994. Inventaire de la Population de la Race Baudet du Poiton. Parc Naturel Regional du Marais, La Ronde.

Trimeche, A., Renard, P., Tainturier, D.A., 1998. Procedure for poitou jackass sperm cryopreservation. Theriogenology 50, 793–806.

Vidament, M., Vincent, P., Yvon, J.M., Bruneau, B., Martin, F.X., 2005. Glycerol in semen extender is a limiting factor in the fertility in asine and equine species. Anim. Reprod. Sci. 89, 302–305.

Vieira, R.C., Arruda, R.P., Manzano, A., 1985. Inseminação intracornual de eqüídeos com sêmen congelado em palhetas de 0.5 ml. In: Anais da XXII Reunião Anual da SBZ, Balneário Camburiú, SC, 298 pp.