Animal Reproduction Science 121S (2010) S178–S179

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Abstract

Influence of sperm motility factors on spermatozoa obtained from different epididymal segments☆

C.M. Melo*, F.O. Papa, L.C.O. Magalhães, P.N. Guasti, G.A. Monteiro, I. Martin, B.R. Avanzi, F.C. Landim-Alvarenga, M.A. Alvarenga, J.A. Dell'Aqua Jr.

Frederico Ozanam Papa, Department of Animal Reproduction and Veterinary Radiology, FMVZ, UNESP, Distrito de Rubião Jr s/n, Botucatu - SP, Cep: 18618-670, Brazil

1. Introduction

Spermatozoa, formed within the seminiferous tubules of the testes by spermatogenesis, are immotile when released into luminal fluid and transported to the epididymis, where they gain the ability to move and fertilize the ovum. In the epididymis, which comprises the caput, corpus, and cauda, the segment where most spermatozoa attain their full fertilizing capacity appears to be the proximal cauda. The cauda epididymides are the regions where spermatozoa are stored before ejaculation. At ejaculation, the stored spermatozoa with the surrounding fluid are mixed with the alkaline secretions of the male accessory sex glands.

Epididymal sperm harvested from stallions have been frozen and used to obtain pregnancies; in fact the first equine pregnancy generated using frozen sperm was obtained using frozen-thawed epididymal sperm (Barker and Gandier, 1957). However the *in vivo* fertility of cauda epididymal spermatozoa tends to be low (Morris et al., 2002). Pasquini et al. (2008) compared the influence of motility-enhancing media on the freezability of epididymal sperm in horses and concluded that incubation in Talp + progesterone, Fert-Talp or Sperm-Talp media, commonly used in IVF procedures, improved equine epididymal sperm motility after freezing. Melo et al. (2009) evaluated the influence of those substances on epididymal sperm from sub-fertile stallions after freezing, and verified a pre- and post-thaw motility improvement over frozen ejaculated semen when samples were incubated with Sperm-Talp and with Fert-Talp. These results were probably related to the deleterious effect of seminal plasma in stallions with poor quality semen. Based on these previous experiments, the aim of the present study was to verify the influence of motility-enhancing media on the freezability of sperm obtained from various epididymal segments.

2. Materials and methods

Ten stallions from different breeds were castrated and sperm were immediately harvested from the epididymis. Spermatozoa from the cauda were obtained using retrograde flushing with Botu-Semen™ medium. Spermatozoa from the corpus were recovered using the float-up method, in which each corpus was incised in 12–15 locations, suspended in Botu-Semen™, and sperm were allowed to float into the extender for 10 min (Bruemmer, 2006) before filtering the samples through fine nylon mesh to remove epididymal tissue. The samples from the corpus and cauda were split into three parts and diluted 1:1 with the following extenders: Botu-Semen™ (BS); Talp + caffeine (Sperm-Talp) and Talp + heparin + penicillamine, hypotaurine and epinephrine (PHE) (Fert-Talp). After incubation at room temperature (25 °C) for 15 min, samples were centrifuged at 600 × *g* for 10 min, the supernatant discarded, and the pellet resuspended using Botu-Crio™. For freezing, the semen was packed into 0.5 mL straws and maintained at 5 °C for 20 min, followed by 20 min at 6 cm above liquid nitrogen before immersion. After thawing at 46 °C for 20 s, the samples were analyzed by CASA (HTM – IVOS 12). Plasma membrane integrity was evaluated using fluorescent probes (CFDA and PI). Data were analyzed by ANOVA

☆ This paper is part of the supplement entitled "Proceedings of the Tenth International Symposium on Equine Reproduction", Guest Edited by Margaret J. Evans.

* Corresponding author. Tel.: +55 14 38116249; fax: +55 14 38116249. *E-mail address:* cely_marini@yahoo.com (C.M. Melo).

0378-4320/$ – see front matter
doi:10.1016/j.anireprosci.2010.04.114

Table 1
Mean values (±SD) for total motility (TM), progressive motility (PM), path velocity (VAP), progressive velocity (VSL), track speed (VCL) and plasma membrane integrity (PMI) of frozen-thawed samples from (a) the equine corpus epididymis and (b) the cauda epididymis in various extenders.

Extender	TM	PM	VAP	VSL	VCL	PMI
(a) Corpus epididymis						
BS	8.5 ± 9.1^{a}	3.0 ± 3.1	73.0 ± 4.8	60.5 ± 13.1	141.5 ± 23.8	20.0 ± 23.7
Fert-Talp	24.0 ± 16.0^{ab}	7.0 ± 7.4	82.5 ± 2.1	64.5 ± 6.5	157.0 ± 13.3	29.0 ± 9.8
Sperm-Talp	31.5 ± 11.6^{b}	10.0 ± 4.7	84.0 ± 2.8	64.5 ± 8.6	160.5 ± 16.3	27.0 ± 12.3
(b) Cauda epididymis						
BS	43.0 ± 20.7^{a}	14.0 ± 11.9^{a}	90.5 ± 13.6	72.0 ± 29.2	161.0 ± 20.8	32.5 ± 9.9
Fert-Talp	69.0 ± 14.2^{b}	31.0 ± 8.9^{ab}	91.5 ± 10.2	73.0 ± 6.9	170.5 ± 16.8	42.0 ± 10.6
Sperm-Talp	70.5 ± 12.8^{b}	36.0 ± 10.1^{b}	92.0 ± 8.4	78.5 ± 6.7	168.0 ± 16.2	36.0 ± 7.4

[a,b] Different lower case letters within the same column differ ($P < .05$).

(GraphPad Instat, Version 3.00, 1997) followed by a Tukey test.

3. Results

Both Sperm-Talp and Fert-Talp increased sperm motility for samples from the epididymal corpus and cauda. In addition, post-thaw progressive motility was higher in samples from the cauda that had been incubated with Sperm-Talp and Fert-Talp before freezing (Table 1).

4. Discussion

We conclude that the use of Fert-Talp and Sperm-Talp improved sperm motility parameters of equine epididymal sperm after freezing. These benefits were also observed in previous studies with cauda epididymal sperm (Pasquini et al., 2008) and with epididymal sperm from sub-fertile stallions (Melo et al., 2009). Previous studies from our laboratory also revealed an improvement of equine epididymal sperm after incubation with Sperm-Talp and Fert-Talp before freezing (Pasquini et al., 2008).

The benefit obtained from the use of Fert-Talp and Sperm-Talp is probably connected to the presence of sperm motility factors that may increase intracellular cAMP, elevating sperm motility. Although seminal plasma also contains sperm motility factors, an increase in sperm motility was not achieved when seminal plasma alone was used. Similar results were obtained by Morris et al. (2001) who did not observe any influence of either seminal plasma or density gradients on motility of epididymal semen.

Sperm-Talp is a medium commonly used in bovine IVF and contains caffeine and heparin, among other components such as BSA. Fert-Talp is made by adding PHE (penicillamine, hypotaurine and epinephrine) to the Sperm-Talp. The incubation of bovine frozen-thawed semen with PHE improved progressive motility and all the velocity parameters, especially for very poor semen quality (Person et al., 2007). Papa et al. (unpublished data) incubated bull epididymal semen with Sperm-Talp before freezing and also observed improved sperm parameters.

The results of the present experiment reinforce the contention that incubation of equine epididymal semen with Sperm-Talp and Fert-Talp media has a beneficial effect on sperm parameters without being deleterious to sperm viability. Further studies are necessary to evaluate the influence of these media on *in vivo* fertility.

Acknowledgement

This study was supported by grants from FAPESP Proc. Number 2008/57504-2.

Conflict of interest

None.

References

Barker, C.A.V., Gandier, J.C.C., 1957. Pregnancy in a mare resulting from frozen epididymal spermatozoa. Can. J. Comp. Vet. Med. Vet. Sci. 21, 45–51.

Bruemmer, J.E., 2006. Collection and freezing of epididymal stallion sperm. Vet. Clin. North Am. Equine Pract. 22, 677–682.

Melo, C.M., Papa, F.O., Monteiro, G.A., Guasti, P.N., Avanzi, B.R., Landim-Alvarenga, F.C., Alvarenga, M.A., Dell'Aqua Jr., J.A., 2009. Improvement of frozen epididymal semen from subfertile stallions using sperm motility factors. In: Proc. 11th Int. Cong. World Equine Vet. Assn, 2009, Guarujá, SP, Brazil, http://www.ivis.org/proceedings/weva/2009/310.pdf?LA=1.

Morris, L.H.A., Hunter, R.H.F., Tiplady, C.A., Allen, W.R., 2001. Low numbers of spermatozoa in the equine oviduct are compatible with fertilisation. In: Proc. From Epididymis to Embryo, Monograph Series No. 6, Havemeyer Foundation, New Orleans, USA, pp. 30–31.

Morris, L.H.A., Tiplady, C., Allen, W.R., 2002. The in vivo fertility of cauda epididymal spermatozoa in the horse. Theriogenology 58, 643–646.

Pasquini, D.F., Melo, C.M., Papa, F.O., Fioratti, E.G., Landim-Alvarenga, F.C., Alvarenga, M.A., Zahn, F.S., De Vita, B., Dell'Aqua Jr., 2008. Effects of seminal plasma and sperm motility factors on viability of epididymal sperm of stallions. Anim. Reprod. Sci. 107, 338–339.

Person, C.N., Lester, T.D., Person, M.D., Rorie, R.W., 2007. Effects of penicillamine, hypotaurine and epinephrine (PHE) on post-thaw bovine sperm parameters, as measured by computer-assisted sperm analysis. Arkansas Animal Science Department Report, vol. 553. pp. 47–50. http://arkansasagnews.uark.edu/553-11.pdf (accessed July 19).