Journal of Equine Veterinary Science 33 (2013) 40-43

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Effect of Removing Seminal Plasma Using a Sperm Filter on the Viability of Refrigerated Stallion Semen

Carlos Ramires Neto [a], Grabriel Augusto Monteiro MSc [b], Rafaela Fatima Soares [a], Cesar Pedrazzi DVM [c], Jose Antonio Dell'aqua Jr. PhD [b], Frederico Ozanan Papa PhD [b], Marco Antonio Alvarenga PhD [b]

[a] School of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, São Paulo, Brazil
[b] Department of Animal Reproduction and Veterinary Radiology, School of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, São Paulo, Brazil
[c] CEAFEPE Tecnologia Veterinária Ind., Sorocaba, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 11 March 2012
Received in revised form
20 April 2012
Accepted 27 April 2012
Available online 7 June 2012

Keywords:
Cooled semen
Seminal plasma
Stallion
Sperm filter
Centrifugation

ABSTRACT

Cooling of equine semen obtained from some stallions results in lower seminal quality and viability when the seminal plasma (SP) is present. The objective of this study was to evaluate the effect of the removal of SP using a Sperm Filter on the viability of cooled stallion semen. For this purpose, 31 stallions were used. Their ejaculates were divided into three groups: CN, semen was diluted with an extender; FLT, SP was removed by filtration; and CT, SP was removed by centrifugation and cooled to 15°C for 24 hours. Sperm kinetics and plasma membrane integrity were evaluated immediately after collection (T0) and after 24 hours of refrigeration (T1). No difference ($P > .05$) was noted at T1 for total sperm motility (TM), progressive sperm motility, or plasma membrane integrity when semen samples from all the stallions were analyzed. However, when samples from stallions termed "bad coolers" were analyzed (TM = <30% at T1), a difference was observed in TM and progressive sperm motility for CN compared with FLT and CT at T1. Sperm recovery was greater when SP was removed using the filter (FLT) to that when the SP was removed by centrifugation (CN) (89% vs. 81%). Thus, we concluded that filtering with a Sperm Filter is an efficient and practical method for removal of SP from stallion ejaculates, with lower sperm loss than centrifugation. We also found that the presence of SP reduces the quality and viability of cooled semen from stallions whose semen is sensitive to the process of refrigeration.

1. Introduction

Cooling of stallion semen reduces the metabolic activity of the sperm and thereby increases the time that the sperm remains fertile [1]. Cooled semen can be stored and transported [1], and the rates of conception obtained using cooled semen are similar to those obtained with fresh semen [2]. This semen processing approach, together with artificial insemination, minimizes the spread of diseases and allows for the breeding of geographically distant animals [3,4].

Although it provides benefits in terms of reducing postbreeding endometritis [5], seminal plasma (SP) has been reported as being deleterious to the in vitro preservation of refrigerated semen [6-9].

SP is a fluid produced by the rete testis, epididymis, and accessory glands, and is expelled in fractions during ejaculation. Its function involves the transport and supply of metabolic substrates to the sperm and also participates in

Corresponding author at: Marco Antonio Alvarenga, PhD, Department of Animal Reproduction and Veterinary Radiology, School of Veterinary Medicine and Animal Science, São Paulo State University, Botucatu, São Paulo 18610-970, Brazil.
E-mail address: malvarenga@fmvz.unesp.br (M.A. Alvarenga).

0737-0806/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
doi:10.1016/j.jevs.2012.04.008

the process of sperm maturation [10]. It also contains substances that protect and stimulate the cells [1]; however, owing to its variable composition among individuals, SP can be beneficial or deleterious to sperm quality [2]. Thus, it is sometimes necessary to remove SP from seminal samples to increase the time that the cooled semen is viable [7,11].

Centrifugation is the technique normally used to remove SP and to concentrate sperm from the ejaculate; however, the intensity and time of centrifugation can interfere negatively with the motility, plasma membrane integrity (PMI), and quantity of sperm recovered. Sieme et al. [12] observed that in some stallions, the process of centrifugation damages the cells. Dell'aqua et al. [13] showed that centrifugation at 600× *g* for 10 minutes provided the best quality and the least sperm loss during the centrifugation process.

Recently, a new method for removal of SP from stallion ejaculate using a filter made of a synthetic hydrophilic membrane (Sperm Filter, CEAFEPE Tecnologia Veterinária Ind., Sorocaba, São Paulo, Brazil) was proposed [14]. This filter was shown to be efficient for the retention and preservation of the viability of stallion sperm, allowing only the passage of SP. Afterward, the retained spermatozoa are resuspended with an extender until the desired sperm concentration per milliliter of semen is reached.

The aim of this study was to evaluate the effect of removing SP using the Sperm Filter on cooled stallion semen.

2. Material and Methods

2.1. Collection of Semen and Division into Groups

Thirty-one stallions, ranging in age from 5 to 20 years, of the Quarter Horse, Mangalarga Marchador, and Thoroughbred breeds were used.

After collection with an artificial vagina, semen from each ejaculate was diluted 1:1 with a skim milk–based extender (Botu-Sêmen; Botupharma, SP, Brazil), and the ejaculates were divided into three groups: CN, FLT, and CT. For the CN samples, the semen was further diluted with Botu-Sêmen until reaching a concentration of 50×10^6 sperms/mL. For the FLT samples, the semen was filtered using the Sperm Filter (Pat req. US2010/0099075), and the sperms retained in the filter were resuspended with Botu-Sêmen to a final concentration of 50×10^6 sperms/mL. For the CT samples, the semen was centrifuged to 600× *g* for 10 minutes, and the pellet was resuspended in Botu-Sêmen at a final concentration of 50×10^6 sperms/mL.

2.2. Cooling Semen

Immediately after collection and processing, the semen samples from the three groups were conditioned in the same commercial Botu-Flex (Botupharma, Brazil) refrigeration system for 24 hours at 15°C.

2.3. Analysis of the Semen

The semen samples were analyzed immediately after collection and processing (T0) and 24 hours after refrigeration (T1) at 15°C (Botu-Flex).

The following motility parameters were analyzed: total sperm motility (TM; %), progressive sperm motility (PM; %), rapid sperm (%), velocity of trajectory (μm/s), linear progressive velocity (μm/s), and curvilinear velocity (μm/s), using Computer-Assisted Semen Analysis (Hamilton-Thorne, Beverly, MA). In addition, the PMI (%) was analyzed using epifluorescence microscopy (Leica Microsystems, DMLB, Germany) with the fluorescent probes 6-carboxyfluorescein diacetate and iodine propidium [15].

The semen, after filtration and centrifugation, was diluted to the same initial volume to calculate sperm loss, and a Neubauer chamber was used to determine concentration of the rediluted aliquots.

2.4. Classification of the Stallions

First, all stallions (n = 31) were analyzed in the same group (experiment 1). Then, these same animals were subdivided into two categories, based on observed sperm motilities after cooling (experiment 2) (24 hr/15°C). The stallions were classified as sensitive to the seminal refrigeration process ("Bad coolers") if they showed less than 30% TM in CN after cooling (T1) and were classified as "Good coolers" if they showed more than 30% TM in CN at T1.

2.5. Statistical Analysis

All the sperm parameters were compared with analysis of variance using the program GraphPad InStat 3 (GraphPad Software, La Jolla, CA). Differences were considered significant at a probability level of $P < .05$ [16].

3. Results

In experiment I, when values from all stallions were included in the analysis, there were no differences ($P > .05$) for any parameter measured (Table 1).

Table 1
Mean values and standard deviations determined by Computer-Assisted Semen Analysis (Hamilton-Thorne, HTM-IVOS) for TM, PM, VAP, VSL, VCL, RAP, and PMI for all stallions (experiment 1) by groups CN, FLT, and CT

Group	TM (%)	PM (%)	VAP (μm/s)	VSL (μm/s)	VCL (μm/s)	RAP (%)	PMI (%)
CN T0 (n = 31)	71.7 ± 12.4	33.8 ± 11.4	114.1 ± 15.8	88.1 ± 9.6	61.0 ± 14.4	54.7 ± 14.7	71.7 ± 12.4
FLT T0 (n = 31)	72.3 ± 13.1	34.6 ± 12.5	113.1 ± 17.3	87.0 ± 11.6	61.2 ± 16.8	57.6 ± 12.1	72.3 ± 13.1
CT T0 (n = 31)	68.2 ± 13.8	31.2 ± 11.2	113.9 ± 16.8	90.2 ± 16.8	58.9 ± 15.8	35.6 ± 13.3	68.2 ± 13.8
CN T1 (n = 31)	37.1 ± 23.3	14.2 ± 13.2	93.8 ± 20.1	70.6 ± 16.8	28.8 ± 24.6	35.3 ± 15.3	37.1 ± 23.3
FLT T1 (n = 31)	51.0 ± 19.3	21.8 ± 12.4	96.8 ± 19.6	73.3 ± 14.7	41.0 ± 21.6	45.5 ± 13.4	51.0 ± 19.3
CT T1 (n = 31)	47.9 ± 20.3	17.1 ± 10.7	87.9 ± 17.9	66.7 ± 13.3	35.1 ± 18.9	47.2 ± 13.8	47.9 ± 20.3

TM, total motility; PM, progressive motility; VAP, velocity of trajectory; VSL, linear progressive velocity; VCL, curvilinear velocity; RAP, rapid sperm; PMI, plasma membrane integrity; CN, semen containing seminal plasma; FLT, semen filtered to remove seminal plasma; CT, semen centrifuged to remove seminal plasma; T0, before refrigeration; T1, after refrigeration.