Theriogenology xxx (2014) 1–8

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of heterologous and homologous seminal plasma on stallion sperm quality

J.M. Morrell [a,*], A. Georgakas [a,b], N. Lundeheim [c], D. Nash [b], M.C.G. Davies Morel [b], A. Johannisson [d]

[a] Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden
[b] Department of Biological, Environmental and Rural Sciences, Aberystwyth University, Wales, UK
[c] Department of Animal Breeding and Genetics, SLU, Uppsala, Sweden
[d] Department of Anatomy, Physiology and Biochemistry, SLU, Uppsala, Sweden

ARTICLE INFO

Article history:
Received 27 November 2013
Received in revised form 17 March 2014
Accepted 21 March 2014

Keywords:
Seminal plasma
Single layer centrifugation
Sperm kinematics
Sperm chromatin structure assay

ABSTRACT

Removing most of the seminal plasma (SP) from stallion semen has been shown to improve survival during cooled storage, yet adding small quantities of SP may improve pregnancy rates or cryosurvival. Furthermore, there is considerable controversy about whether the stallion's own SP or heterologous SP produces the best effect, possibly because of the variation between stallions in SP proteins or because some homologous SP remained in the sperm preparation. The SP is removed completely from stallion spermatozoa prepared by colloid centrifugation. Thus, the aim of the present study was (1) to investigate the effect of adding back SP to colloid centrifuged spermatozoa to determine its effect on spermatozoa; and (2) to investigate whether the stallion's own SP had a greater or lesser effect than heterologous SP. Conventional semen doses were sent from a stud overnight to the laboratory using standard transport conditions. Once at the laboratory, the semen samples were used for single layer centrifugation with Androcoll-E, and the resulting sperm preparations were treated with heterologous SP. Adding SP had a small but significant effect on sperm motility but no effect on the proportion of spermatozoa that had acrosome reacted. There were significant increases in hydrogen peroxide production and chromatin damage ($P < 0.001$). When homologous and heterologous SP were compared, considerable variation was observed between stallions, so that it was not possible to predict whether homologous or heterologous SP, or no SP, will produce the best motility for spermatozoa from any given stallion. Therefore, it is necessary to test different combinations of spermatozoa and SP to find the optimal effect on motility. The SP from most stallions increased reactive oxygen species and chromatin damage. In conclusion, the interaction between SP and spermatozoa depends on the origin of both SP and spermatozoa. If it is desirable to add SP to stallion sperm samples, it should be done directly before insemination rather than before storage, because of increased hydrogen peroxide production and sperm chromatin damage.

1. Introduction

There are conflicting reports about the benefits or otherwise of seminal plasma (SP) in many species [1], no more so than in the horse. Seminal plasma is the fluid part of semen, which functions to activate epididymal spermatozoa and transport them to the site of semen deposition, but it is known to exert an effect on the female reproductive tract as well and may regulate ovarian function [2]. Some components of SP are responsible for preventing sperm capacitation (decapacitation factors). Proteins found in SP are thought to modulate fertility [3,4] such as horse SP

* Corresponding author. Tel.: +4618671152; fax: +461867113545.
E-mail address: jane.morrell@slu.se (J.M. Morrell).

0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.03.020

proteins and other members of the family of cysteine-rich secretory proteins—CRISP proteins [5,6]. However, sperm samples that have had all SP removed by colloid centrifugation are fertile [7–9]. Semen plasma also has beneficial effects on spermatozoa, affecting sperm motility [10,11] in fresh sperm samples and after cryopreservation [12]. In mares susceptible to persistent breeding-induced endometritis, the presence of some SP in the artificial insemination (AI) dose may be necessary for optimum fertility [13], possibly acting via immune-regulatory molecules to activate and modulate immune responses [14].

In contrast to the benefits of SP, removal of most of the SP from stallion semen increases sperm survival during cold storage and reduces chromatin damage [15,16]. Cryopreservation protocols for stallion semen suggest removing more than 90% SP before adding the cryoextender [17] although Moore, et al. [18] did not observe a protective effect when various proportions of SP were included in cryopreserved semen. Mari, et al. [19] were unable to influence fertility in an AI trial by adding 5% SP to sperm doses immediately before deep intrauterine insemination of a small number of spermatozoa. Thus, the whole issue of the effects of SP is confusing.

The main problem with adding SP to ejaculated sperm samples to study its effects is that the spermatozoa may have already been activated by components of SP on ejaculation. Colloid centrifugation, such as single layer centrifugation (SLC), separates spermatozoa from SP and even removes SP proteins from the sperm surface [20]. Thus, known quantities of SP can be added to SLC-selected spermatozoa to enable the effects of SP to be measured. In previous studies, homologous SP was found to increase the motility and velocity of SLC-selected spermatozoa [10,21]. In a preliminary study comparing homologous and heterologous SP with a limited number of ejaculates, there was considerable variation between stallions as to whether homologous or heterologous SP produced the most effect. The aim of the present study, therefore, was (1) to investigate the effect of adding back SP to colloid centrifuged spermatozoa to determine its effect on spermatozoa; and (2) to investigate whether the stallion's own SP had a greater or lesser effect than heterologous SP.

2. Materials and methods

Semen collection was carried out according to standard veterinary and husbandry procedures. The experimental protocol was reviewed and approved by the Ethical Committee for Experimentation with Animals, Uppsala, Sweden (C345/9) before the start of the experiment.

2.1. Preparation of SP

Semen was collected from 10 warmblood stallions of known fertility, 4- to 15-years old, kept at a commercial stud in Sweden (Flyinge AB) during the nonbreeding season. Semen was collected up to three times per week for freezing, by allowing the stallions to mount a phantom and ejaculate into an artificial vagina (Colorado or Missouri, depending on the stallion). Gel was removed with an inline filter. Aliquots (10 mL) of semen were immediately centrifuged at 500× *g* for 10 minutes to pellet the spermatozoa and collect the SP supernatant. After checking the supernatant microscopically for the presence of spermatozoa, sperm-free SP aliquots were frozen individually until required. The seasonal pregnancy rates of these donor stallions after AI with cooled semen during the subsequent breeding season varied from 67% to 86%.

2.2. Semen collection

Semen was collected from 17 warmblood stallions of known fertility, 4- to 15-years old, kept at a commercial stud in Sweden (Flyinge AB) during the breeding season. Semen was collected as described previously. Sperm concentration was measured using a SpermaCue photometer (Minitűb GmbH, Tiefenbach, Germany), and motility was assessed subjectively. The ejaculate was extended in INRA96 (IMV Technologies, L'Aigle, France) to provide approximately 1 billion motile spermatozoa per dose (a standard cooled semen dose in Sweden). The doses were placed in insulated boxes with a cold pack for overnight transport, according to standard practice. The temperature inside the insulated box was approximately 6 °C.

2.3. Single layer centrifugation

On arrival at the laboratory at the Swedish University for Agricultural Sciences (approximately 24 hours after semen collection), the samples were allowed to equilibrate to room temperature before performing SLC. Aliquots (15 mL) were used for SLC [22]. Briefly, after equilibrating the semen and colloid to room temperature (approximately 22 °C) for 30 minutes, the extended semen from the commercial AI dose was carefully layered on top of Androcoll-E-Large (15 mL) in 50-mL Falcon tubes. After centrifugation at 300× *g* for 20 minutes, the resulting sperm pellet was aspirated into a clean tube containing 2 mL INRA96.

2.4. Sperm concentration

Sperm concentration in the SLC-selected samples was measured using a Nucleocounter SP-100 (ChemoMetic, Allerød, Denmark) as previously described [23]. INRA96 was added to a fixed number of spermatozoa (30×10^6) to provide a final volume of 0.5 mL after adding the SP (see experimental design).

2.5. Sperm evaluation

2.5.1. Computer-assisted sperm analysis

Motility assessment was made on both control and SLC-selected samples using a Sperm Vision (Minitüb, Tiefenbach, Germany), connected to an Olympus BX51 microscope (Olympus, Tokyo, Japan) with a heated stage (38 °C). Aliquots (6 μL) of the sperm samples were pipetted onto a warm glass slide, and an 18 × 18 mm cover slip was placed on top. Sperm motility of approximately 1000 spermatozoa in eight fields of view was analyzed using the Sperm Vision software program version 3.5 with previously established settings [24]. The cell identification area was set at 14 to 80 μm^2; spermatozoa having an average orientation change of head of less than 17° were defined as immotile, those