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Immediate and delayed (after cooling) effects of centrifugation on equine sperm

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Abstract

The objectives of this study were to determine the effects of centrifugation on equine sperm total and progressive motility, viability, and acrosomal integrity. We hypothesized that although high centrifugation forces would be detrimental to equine Equus caballus sperm, recovery rates would increase. Ejaculates from six stallions were collected, extended to a concentration of 25×10^6 cells/mL, and subjected for 10 min to (1) no centrifugation (NC) or (2) centrifugation at $400 \times g$, (3) $900 \times g$, or (4) $4500 \times g$. Before and after centrifugation (Day 0), and after 24 h of cooling (Day 1), sperm motility was assessed by computer-assisted semen analysis, and samples were stained with SYBR-14/propidium iodide (PI) for viability and with PI/fluorescein isothiocyanate (FITC)–Peanut aglutinin (PNA) (*Arachis hypogaea*) for acrosomal integrity. The effect of treatment and day on motility, viability, and acrosomal integrity was determined using a mixed linear model. Compared with the other treatments, centrifugation at $4500 \times g$ reduced all end points measured ($P < 0.05$). Both $400 \times g$ and $900 \times g$ yielded lower recovery rates than that of $4500 \times g$ (NC = $100.0 \pm 0.0\%$; $400 \times g = 54.4 \pm 8.6\%$; $900 \times g = 75.0 \pm 7.1\%$; $4500 \times g = 97.9 \pm 2.8\%$; $P < 0.05$). Centrifugation at $400 \times g$ or $900 \times g$ did not damage equine sperm. Based on these findings, further studies of centrifugal forces between $900 \times g$ and $4500 \times g$ are warranted to determine the optimal force that maximizes recovery rate, minimizes sperm damage, and does not affect fertility.
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Keywords: Acrosome; Centrifugation; Motility; Plasma membrane; Recovery rate; Sperm

1. Introduction

Large volumes of seminal plasma are not an ideal medium for storing equine extended sperm [1–3]; therefore, one of the main objectives in processing fresh cooled or frozen equine semen is to reduce the amount of seminal plasma in the ejaculate. Dilution of seminal plasma is commonly achieved by the addition of a semen extender or by centrifugation of extended semen, partial removal of seminal plasma, and resuspension of the sperm pellet with fresh semen extender.

Reports on the effects of centrifugation on equine sperm have been conflicting; however, evaluation has often been based solely on sperm motility postcentrifugation. Although centrifugation decreased sperm motility in several studies [3–6], other studies have shown no detrimental effect on sperm motility [7–9]. Furthermore, centrifugation was beneficial to sperm motility of fresh cooled semen [10]. In addition to motility, other sperm function aspects can be evaluated

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using fluorescent probes, for example, SYBR-14 and propidium iodide (PI), to assess damage to the plasma membrane integrity (viability) [11] and plant lectins to assess the acrosomal reactive status.

Because the effects of centrifugation on equine sperm have not been fully elucidated, recommended centrifugal forces used for processing equine semen are conservatively low ($400 \times g$ to $600 \times g$) [3,5,8,10]. Unfortunately, application of low centrifugal forces for processing fresh cooled semen can result in a loss of 20% to 30% of sperm in the supernatant [5,7,12].

The objectives of this study were to evaluate the effects of various centrifugal forces on equine sperm motility (total and progressive), integrity of the plasma membrane (viability), and acrosomal reactive status. Additionally, sperm recovery rates were determined.

2. Materials and methods

Semen was collected from six stallions Equus caballus, two Arabs and four Quarter Horses (age range, 7 to 25 yr). All belonged to Louisiana State University: five were from the Department of Animal Sciences, and one was from the School of Veterinary Medicine's teaching herd. These stallions were maintained on pasture during the entire study and were fed a 12% protein commercial ration and free choice Alicia Bermuda hay.

2.1. Semen collection

Before semen collection, each stallion was teased with a mare in estrus, the stallion's penis was washed with cotton soaked in warm water, and semen was collected into a Hanover or Missouri artificial vagina during mount of a phantom mare. The artificial vagina was equipped with an in-line nylon filter to exclude gel from the semen sample. Immediately after collection, the filter containing the gel from the ejaculate was removed, and semen was transported to the laboratory within 15 min.

2.2. Semen processing

Upon arrival at the laboratory, semen volume was measured and sperm motility was determined using a computer-assisted semen analysis system (CASA; Sperm Vision; Minitube, Verona, WI, USA), and sperm concentration was evaluated using a hemocytometer, as described [13]. Semen was then diluted with a prewarmed semen extender (INRA96; IMV Technologies, Maple Grove, MN, USA) to a concentration of 25×10^6/mL in a plastic bag (Whirl-Pak; Nasco, Fort Atkinson, WI, USA). After extension, motility and concentration were reevaluated. A sample of extended semen was obtained to assess the integrity of the plasma membrane and acrosome. Four aliquots (40 mL) of the extended semen were placed into four 50-mL conical centrifuge tubes (Corning, Corning, NY, USA). Each tube was centrifuged (Eppendorf 5804; Hamburg, Germany) at one of four centrifugal forces for 10 min. The treatment groups were noncentrifuged (NC) or centrifuged at $400 \times g$, $900 \times g$, or $4500 \times g$. The $4500 \times g$ treatment group was included to create damage to sperm, thereby serving as a negative control. After centrifugation, the supernatant was partially removed, leaving a residual volume of ~3 mL, and extender was added to resuspend the sperm and restore the volume to 40 mL. Concentration and motility of the resuspended, postcentrifugation semen was assessed. A sample of the resuspended, postcentrifugation semen was then obtained to assess integrity of the plasma membrane and acrosome. Each resuspended semen aliquot was packaged in a plastic bag, the air removed, and the bag placed in a passive cooling device (Equitainer; Hamilton Thorn Research, Danver, MA, USA). After cooling for 24 h, sperm motility, and integrity of the plasma membrane and acrosome were assessed for the last time.

2.3. Motility

Sperm motility was analyzed by placing 2 μL semen in a 20-μm high, four-chamber slide (Leja Products B.V., Nieuw-Vennep, The Netherlands) over a slide warmer at 37.5 °C. The slide was then placed on an optical microscope warmed stage at 37.5 °C (Olympus BX41; Olympus America Inc., Center Valley, PA, USA). Using the ×20 phase-contrast objective, sperm motility was analyzed with CASA. Mean percentages of total and progressive motility were assessed from seven fields with at least 100 sperm in each field. After 24 h of cooling, a 25-μL sample of cooled semen from each treatment group was placed in a microcentrifuge tube. The cooled semen in the microcentrifuge was warmed for 5 min at 37.5 °C prior to motility analysis.

2.4. Plasma membrane integrity (viability)

Fluorescent dyes were purchased from Molecular Probes Inc. (Eugene, OR, USA) unless otherwise indicated. The SYBR-14 and PI (Live/Dead Sperm Viability Kit) were used to assess integrity of the plasma membrane. In this method, SYBR-14 is a permeable nucleic acid fluorescent dye that stains sperm with an intact plasma membrane green (viable), whereas PI is