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Original Research

Relationship of Sperm Quality to Fertility after 4 Days of Cooled Storage of Equine Semen

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ABSTRACT

This study evaluated measures of sperm quality in relation to fertility achieved with fresh semen or semen cooled and stored. Semen from 1 stallion was collected and processed to provide 3 treatments: group 1 received fresh semen; group 2 received cooled semen containing 50% seminal plasma (SP) stored for 4 days; and group 3 received cooled semen containing 50% SP stored for 1 day, then centrifuged and resuspended in fresh extender containing 10% SP on days 1 to 3. Inseminates were evaluated for sperm motion characteristics and the percentage of sperm with intact membranes (SMI). Mares (n = 34) in estrus were treated with an ovulation-inducing drug and inseminated with 100 million membrane-intact sperm on the following day. Pregnancy status was determined via transrectal ultrasonography 2 weeks after ovulation. The mean percentage of SMI was higher in group 1 (81%, initial) than in group 2 (74%, day 4) or group 3 (74%, day 4) ($P < .05$). The median percentages of total sperm motility differed among the groups (77%, 5%, 59% for groups 1, 2, and 3 respectively; $P < .05$). Median values for the percentages of progressively motile sperm and curvilinear velocity for group 1 (55%, 216 μm/s) and 3 (37%, 186 μm/s) were higher than for group 2 (1%, 73 μm/s) ($P < .05$). Pregnancy rates did not differ among groups (5 of 11, 45% in group 1; 5 of 11, 45% in group 2; and 7 of 12, 58%, in group 3; $P = .77$). These data suggest that, at least for this stallion, sperm membrane integrity may be a more valuable means of assessing potential fertility of cooled-stored semen than sperm motion characteristics.

1. Introduction

Cooled, transported stallion semen is commonly used to breed horses in North America, Europe, and Australia, but fertility achieved from breeding with transported semen is often reduced compared to that from breeding mares with fresh semen [1,2]. Factors contributing to the lowered fertility achieved with cool-transported semen include reduction in the intensity of mare management, quality of mares bred (age, beginning reproductive status), and sperm quality after cooling and storage [3]. In a recent study, mares bred on the farm with fresh semen had a 2.5-fold higher probability of becoming pregnant (18% higher, first cycle pregnancy rate) than mares bred with cooled-transported semen from the same stallions [2]. It is generally assumed that sperm quality declines over time after collection, extension, and cooled storage [4]. However, it is unclear what contributes to that decline. Factors such as concentration or source of seminal plasma (SP) in stored semen [5,6] have been shown to contribute to the demise

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of sperm quality over time. Additionally, a reduction in metabolizable substrates or an increase in metabolic waste products in the stored semen might also contribute to a decline in sperm quality. It has recently been shown that sperm quality (motion characteristics and DNA quality) can be prolonged (4 days) by centrifuging stored semen and replacing the supernatant with fresh extender daily [7]. However, whether the resulting improvement in sperm quality using this technique would improve fertility is unknown. The purpose of this experiment was to evaluate whether daily centrifugation and resuspension of cooled semen to maintain sperm quality throughout 4 days of storage would improve fertility compared to that with semen that was merely extended and stored for 4 days. Our hypothesis was that simple (1:1) dilution of semen in extender would limit the longevity of sperm quality and fertility after storage, which could be overcome by centrifugation and resuspension in fresh extender at 24-hour intervals and yield fertility similar to that achieved with fresh semen.

2. Materials and Methods

2.1. Stallion and Semen Collection

One fertile Quarter Horse stallion, aged 9 years, was selected for its known fertility and was used for this trial. A mare in behavioral estrus was used for sexual stimulation, and the stallion was mounted on a breeding phantom for semen collection. Immediately before collection, the penis was cleansed with water and dried while erect. Semen was collected using an artificial vagina (Missouri model; Nasco, Ft. Atkinson, WI) lubricated with a small volume (3-5 mL) of sterile nonspermicidal lubricant (Priority Care; First Priority, Inc., Elgin, IL) and fitted with a nylon micromesh filter (Animal Reproduction Systems, Chino, CA) to separate the gel-free portion of the ejaculate.

2.2. Semen Processing

The volume of gel-free semen was measured. Sperm concentration and percentage of sperm with intact membranes were determined using an automated cell counter (NucleoCounter SP-100; ChemoMetec A/S, Allerød, Denmark) [8]. Semen was diluted in extender (INRA 96, IMV, Maple Grove, MN) to provide the following treatment groups. Group 1 received fresh semen diluted (by direct extension) in extender to 30×10^6 sperm/mL and used for breeding within 1 hour. Group 2 received semen diluted 1:1 in extender and subjected to cushioned centrifugation, with resuspension to 30×10^6 sperm/mL in extender containing 50% autogenous SP and stored in 15-mL plastic conical bottom tubes (14.5 mL per tube) for 4 days at 8°C, as described by Love et al [7], prior to insemination. Group 3 received semen diluted 1:1 in extender and subjected to cushioned centrifugation, followed by dilution to 30×10^6 sperm/mL in extender containing 50% autogenous SP and stored in 15-mL plastic conical bottom tubes (14.5 mL per tube) for 24 hours at 8°C but subsequently subjected to cushioned centrifugation and resuspension and further cooled storage in fresh extender containing 10% autogenous SP at days 1 (24 hours), 2 (48 hours), and 3 (72 hours), as described by Love et al [7], prior to insemination. A total of 18 ejaculates were required to complete the project.

Prior to insemination, aliquots of semen were analyzed for sperm motion characteristics by using a computerized semen analysis system (IVOS 2; Hamilton Research, Inc., South Hamilton, MA) and for assessment of the percentage of sperm with intact plasma membranes (SMI) as previously described [7,8]. Analyses of sperm motility and SMI were carried out immediately after collection and extension for fresh semen (group 1) as well as on day 4 (at 96 hours) of cooled storage for groups 2 and 3.

2.3. Mares and Insemination

Mares ($n = 34$) in estrus were examined by transrectal ultrasonography at approximately 2-day intervals to monitor follicle size. Seven mares (group 1 had 3 mares; group 2 had 2 mares; and group 3 had 2 mares) that developed a small amount of anechoic intrauterine fluid during estrus were treated once with ecbolic administration (20 units of oxytocin, intramuscularly [IM] or 250 μg of cloprostenol, IM). Fluid was not detected in any ecbolically-treated mare on the following day. Once a follicle of ≥30-mm diameter and uterine edema were detected, 0.5 mg of a gonadotropin-releasing hormone (GnRH) analog (BioRelease Histrelin; BET Pharm) was administered IM. Mares were bred the following day with inseminates adjusted to contain 100 million membrane-intact sperm delivered transcervically into the uterine body, and were examined daily thereafter to detect ovulation. Mares were examined 2 weeks postovulation via transrectal ultrasonography to determine pregnancy status.

2.4. Statistical Analyses

Group differences in percent of total sperm motility, percent of progressive sperm motility, curvilinear velocity (VCL; μm/s), and percent of SMI were evaluated by analysis of variance (ANOVA) procedures. When data for dependent variables failed the Shapiro-Wilk normality test, the Kruskal-Wallis ANOVA on ranks statistic was used to test for differences in median values among the groups; and when significant, Dunn's method of pairwise multiple comparison procedures was used to identify the group or groups that differed from the others. The Holm-Sidak method was used for pairwise comparisons of normally distributed data. Group differences among pregnancy rates were evaluated by Fisher's exact test.

2.5. Animal Use

All experimental procedures were performed according to the US National Institutes of Health Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training (http://history.nih.gov/research/downloads/US_Principles.pdf), and were approved by the Laboratory Animal Care Committee at Texas A&M University.