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The relationship between sperm quality in cool-shipped semen and embryo recovery rate in horses

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ABSTRACT

The relationship between the quality of cool-shipped stallion semen and fertility has not been adequately described. This study evaluated sperm quality of cool-shipped semen from 459 ejaculates (N = 130 stallions) that were used for insemination of 196 embryo donor mares (n = 496 estrous cycles). Embryo recovery rate (ERR; %) increased, as all sperm measures (e.g., motility, viability, DNA quality, morphology, concentration, and total number) increased. Threshold values are reported for each sperm quality measure (e.g., total sperm motility ≥ 65%) that separate two ERR groups (e.g., average: ~50% ERR; high: ~65% ERR).

1. Introduction

The use of cool-shipped stallion semen is a common and accepted method for short-term sperm preservation in the horse industry. Nevertheless, there are concerns about the fertility of this breeding method [1,2]. As with fresh semen, reduced fertility occurs with cool-shipped semen from a combination of factors (e.g., mare, management, and stallion) including sperm quality. Typically, a semen evaluation is performed immediately after its collection or after a period of cooled storage. Although the tests used to evaluate the quality of fresh and cool-shipped semen before shipment are often similar, various sperm features may change after cooled storage. Diagnostically, the clinician must determine and interpret the results of a semen evaluation to render an opinion as to whether the semen contributes to reduced fertility. Information is sparse regarding the relationship between the quality of cool-shipped sperm and fertility outcome. The objectives of this study were to (1) describe the relationships between embryo recovery rate (ERR) and measures of sperm quality after cooled storage and (2) describe and identify a threshold where sperm quality measures are associated with a change from lesser to higher fertility.

2. Materials and methods

2.1. Animals and breeding

During the 2009 breeding season in the Northern Hemisphere, ejaculates (N = 459; mean age [range]: 3.5 ± 4.8 years [1–35]) from American Quarter Horse or American Paint Horse stallions (N = 130; mean age [range]: 11 ± 5 years [3–25]) were collected and shipped to a single embryo transfer facility in Central Oklahoma. Mares (N = 196; mean age [range] = 11.6 ± 6.0 years [2–26];

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insemination during 459 total estrous cycles; each mare was bred for a mean of 2.3 ± 1.5 estrous cycles) were examined per rectum by ultrasonography, and when an ovarian follicle reached 35 mm in diameter with evidence of moderate-to-heavy uterine edema, semen was ordered for insemination, and an ovulation synchronization agent was administered. Breeding occurred either the same day as semen collection (same day; n = 330 inseminations) or the day after semen collection (T24; n = 129 inseminations). Ovulation was confirmed, and embryo recovery attempt was performed on the donor mare uterus 7 to 8 days after ovulation. Recovered embryos were transferred to synchronized recipient mares.

2.2. Semen processing and evaluation

The semen was collected at locations remote from where mares were inseminated. The conditions under which the stallions were maintained and methods of semen collection and processing were unknown. On arrival at the breeding farm, cool-shipped semen samples were evaluated for total volume, total and progressive sperm motility, sperm concentration, and viability. Sperm motility was subjectively evaluated by a single observer, using phase-contrast microscopy (Olympus CH-2; Olympus America Inc., Center Valley, PA, USA) at × 200 magnification. Sperm concentration and viability were determined using a fluorescence-based sperm counter (NucleoCounter SP-100; ChemoMetec A/S, Allerød, Denmark) [3,4]. In addition, small aliquots (~0.5 mL; n = 289) of semen were immediately snap frozen in liquid nitrogen for subsequent evaluation of DNA quality using the sperm chromatin structure assay [5] or fixed (n = 366) in buffered formol saline for sperm morphologic evaluation [6] using differential interference contrast microscopy under × 1250 magnification (Olympus BX-60; Olympus America Inc.).

2.3. Statistics

Fertility outcome (embryo recovery) was determined and matched with each sperm quality measure (i.e., total and progressive sperm motility, viability, DNA quality, morphology, and sperm concentration) of each sample. The relationship between sperm quality and ERR was described and measured using a modification of the cumulative sum (CUSUM) technique [7,8]. This technique is commonly used to measure a binary change in outcome (e.g., surgical success) as a result of a sequential change (e.g., time). In this study, we measured a binary outcome (i.e., embryo recovery) as a result of a sequential change (i.e., increase in sperm quality). Using an Excel spreadsheet, each sperm quality measure (e.g., total sperm motility, progressive sperm motility, sperm concentration, total sperm number, viable sperm, morphologically normal sperm, mean$_{\alpha t}$, standard deviation$_{\alpha t}$, COMP$_{\alpha t,}$ mode$_{\alpha t}$, semen volume, total motile sperm, total progressively motile sperm, total viable sperm, total progressively motile viable sperm, total morphologically normal, total motile morphologically normal) for the study was sorted sequentially in ascending order (e.g., from low to high motility). Data regarding embryo recovery outcome (i.e., embryo recovered = +1; no embryo = −1) were also recorded. Embryo recovery outcomes (-1 or +1) were summed, starting from the lowest to the highest sperm quality value to give a CUSUM for each sperm quality measure. The results were described in graphic form with sperm quality values plotted in ascending order on the x-axis and cumulative embryo recovery values on the y-axis. The intent of this approach was to identify changes in fertility as the sequential variable (i.e., measure of sperm quality) increased. An example of this approach is represented in Supplemental Material. The point at which the slope of the graph changed was considered a threshold value for that measure. For example, a slope of zero (horizontal line) would represent an ERR of 50% per cycle, with no change occurring because of a sequential increase in the measure plotted. A positive slope would represent an increase in ERR as the measure increased, whereas a negative slope would indicate a decrease in the ERR as the measure increased.

A general linear model procedure was used to examine differences between mean sperm quality values (SAS Institute Inc., Cary, NC, USA) in the ERR groups previously identified in the CUSUM procedures. The least significant difference statistic was used for mean separation when treatment *F*-ratios were significant. Chi-square analysis was used to compare ERR between fertility groups. A significance level of P = 0.05 was used.

3. Results

Overall, the ERR of mares bred once per estrus was similar to mares bred more than once per estrus (Table 1).

The mares inseminated with same-day semen had a higher ERR (65%) than the mares bred with T24 semen (51%; Table 2; P < 0.05). Total and progressive sperm motility were higher (P < 0.05) for same-day semen than those for T24 semen; however, the percent morphologically normal sperm was lower (P < 0.05), and the percentage of proximal droplets was higher (P < 0.05) in the same-day semen than that for T24 semen. All other sperm qualities and composite sperm variables (i.e., total motile, total progressively motile, total viable, total morphologically normal, total motile morphologically normal) were similar between the two groups (Table 2); therefore, the same-day and T24 samples were combined for further analysis. Mare age was lower (P < 0.05) in the same-day sample than in the T24 samples

Table 1
Comparison of the embryo recovery rate of mares that had only one breeding (1×) with mares that had two or more breedings (>1×) during their estrous period.

Cycle number	Bred 1×/estrus[a]	Bred >1×/estrus	Total
1	97/150 (65)	22/40 (55)	119/190 (63)
2	59/93 (63)	16/22 (72)	75/115 (65)
3	31/57 (54)	9/14 (64)	40/71 (56)
4	18/36 (50)	8/10 (80)	26/46 (57)
5–8	14/29 (48)	5/8 (63)	19/37 (51)
Total	219/365 (60)	60/94 (64)	279/459 (61)

Columns represent the embryos recovered/total cycles bred (% embryo recovery rate).
[a] Within rows, there is no difference (P > 0.05) between mares bred 1× or >1×/estrus.