Fertility of Detomidine HCl–Induced Ex Copula–Ejaculated Stallion Semen After Storage at 5°C

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Detomidine HCl–induced ex copula–ejaculated stallion semen is well suited for storage and insemination. When extended, slow-cooled, and stored at 5°C, this semen can be used in a transported or stored semen breeding program without detrimental affect on fertility. Authors’ address: College of Veterinary Medicine, University of Illinois, 1008 W. Hazelwood Drive, Urbana, IL 61802. © 1999 AAEP.

1. Introduction
To date, a variety of methods for inducing ex copula ejaculation in stallions have been described. All methods apply a variety of agents that affect genital smooth muscle activity, and most have a range of overall reliability of success (20–70%), with considerable variability among individual stallions. There are several characteristics of pharmacologically induced ejaculates that make them well suited for storage and insemination. A higher total number of spermatozoa per ejaculate is commonly reported, allowing for more inseminations per collection. Additionally, a characteristically lower volume of seminal plasma results in increased longevity of the extended ejaculate. Previous reports have demonstrated pregnancies from imipramine- and xylazine-induced ex copula ejaculations. The purpose of the following fertility study was to demonstrate in vivo and in vitro qualities of detomidine HCl–induced ex copula–ejaculated stallion semen that was extended, slow-cooled, and stored at 5°C over time.

2. Materials and Methods
The in vivo portion of this study was performed from July through September 1998. Nine pony mares, ranging in age from 3 to 11 years and weighing between 258 and 441 kg, and six Warmblood mares, ranging in age from 17 to 23 years and weighing from 473 to 670 kg, were used. Before inclusion in the study, mares were determined to have normal reproductive tracts based on rectal palpation and transrectal ultrasonography; otherwise, their reproductive potential was not evaluated. Estrous cycles of the mares were synchronized by 2 treatments with prostaglandin F2α (PGF2α) given 15 d apart. After the second luteolytic injection, mares were teased daily with a teaser stallion to indicate the onset of behavioral estrus. Once exhibiting behavioral estrus, or 3 days after the second luteolytic injection, the reproductive tracts of the mares were examined via rectal palpation and transrectal ultrasonography. Evaluation of the mares continued with daily teasing and every-other-day ultrasonographic examination of ovarian activity until a follicle >30 mm in diameter was detected. Once a >30-mm follicle was detected, the mares were examined daily until ovulation. Once a follicle had obtained a maximum diameter of at least 33 mm, mares were given 2500 IU human chorionic gonadotropin IV and insemi-
nated every other day according to their assigned treatment.

One pony stallion 4 years of age provided all ejaculates used for insemination purposes. Semen was collected, the gel portion removed, and the gel-free ejaculate extended to a concentration of 34 million spermatozoa/ml. The aliquots were cooled to 5°C and stored in an Equitainer® for 6–78 h before insemination. Mares were assigned in random pairs to be inseminated with ex copula– or in copula–induced ejaculated semen, every other day until ovulation. Pregnancy was detected by means of transrectal ultrasonography on days 12, 13, and 14 after ovulation. On day 14, the mares were administered a luteolytic dose of PGF2α, and on the subsequent estrus, inseminated with the alternate semen treatment. After pregnancy determination of the second assigned insemination treatment, the mares were given a luteolytic dose of PGF2α. Mares were then reassigned to treatment group and the study repeated so as to follow each mare through a total of 4 ovulatory cycles.

A. In Copula Ejaculates
Semen was collected with a Missouri model artificial vagina and the use of a live-jump mare. Immediately after collection, the ejaculate was filtered and the gel fraction of the sample removed.

B. Ex Copula Ejaculates
Before induction, the stallion was teased (<10 min) with a mare exhibiting behavioral estrus. After the teasing episode, the stallion was returned to his stall and allowed up to 10 min of undisturbed rest. At the conclusion of the rest period, the stallion was given 0.02 mg/kg detomidine HCl (IM) (t = 0 min). Five minutes after the initial injection (t = 5 minutes), a semen-collection device was placed on the stallion. A second injection, 0.01 mg/kg detomidine HCl (IM), was administered (t = 15 minutes), and the stallion was monitored from a distance to detect ejaculation.

C. Statistics
Differences in pregnancy rates (mares with vesicles observed by ultrasonography/mares inseminated) between groups were analyzed using McNemar’s test for two related samples. Measures relating to follicular activity of the mares and seminal characteristics of the ejaculates were analyzed using Kruskal-Wallis nonparametric analysis of variance and the Tukey t-test.

3. Results
A. Pregnancy Rates
The pregnancy rates of mares were not found to be different \( p = 0.7266 \) for mares inseminated with the first artificial vagina (AV1) versus the first chemical (CH1), the second artificial vagina (AV2) versus the second chemical (CH2) \( p = 0.4531 \), AV1 versus AV2 \( p = 0.7055 \), or CH1 versus CH2 \( p = 0.6875 \) collections. Conceptions per estrous cycle were higher when using chemically obtained semen and averaged 0.80 (CH1), 0.53 (CH2), and 0.40 (AV1 and AV2) among treatment groups.

B. Preovulatory Follicles
The day before ovulation, follicle diameters averaged 41.9 mm (CH1), 40.0 mm (AV1), 42.9 mm (CH2), and 43.2 mm (AV2) with no significant difference between \( p = 0.2118 \) or within \( p = 0.4716 \) treatment groups.

C. Insemination to Ovulation Interval
Mares received 1.0 to 1.3, for an average of 1.1 inseminations per ovulation. All inseminations occurred prior to ovulation, with a range of 12–36 h from last insemination to ovulation. The average time from last insemination to ovulation was 19 hours for in copula–ejaculated semen and 22 hours for chemically collected ejaculates.

D. Ovulation Interval and Administration of PGF2α
The interval from day 14 (PGF2α) to the subsequent ovulation ranged from 4 to 11 d with an average of 7 d in the mares inseminated with ex copula–ejaculated semen, ranged from 5 to 12 d, with an average of 7 d in the mares receiving AV collected semen, with little difference \( p = 0.0902 \) between collection methods.

E. Frequency of Collection and Interval to Ejaculation
The stallion was ejaculated a total of 23 times, 16 of the 23 were in copula ejaculates and 7 of the 23 were ex copula in nature. The collections were obtained over a period of 10 wk for an average of 2.3 collections per week, with a range of 1–4 collections per 7-d period. The stallion was allowed a minimum of 24 h between ejaculations. In copula collections were consistent with less than 1 min from mount to ejaculation required per collection. The time required for ex copula ejaculations ranged from 17 to 47 min (CH1), with an average of 27.5 min per ex copula ejaculation, and 17 to 44 min (CH2), with an average of 26.0 min per ex copula ejaculation. Differences in time to ejaculation were significant \( p = 0.000 \) between methods of collection.

F. Ejaculate Volume, Concentration, and Total Sperm Output
Gel-free ejaculate volumes ranged from 10 to 31 ml with an average of 23 ml per ejaculate for in copula–collected semen. Volumes ranged from 13 to 29 ml with an average of 22 ml per ejaculate from chemically collected semen. The gel free volume was not different between \( p = 0.929 \) or within \( (0.874) \) treatment groups. Raw semen concentrations averaged 115 million/ml with in copula ejaculates, averaged 205 million/ml for the chemically induced ejaculates, and differed significantly \( p = 0.003 \) between collection methods. Total sperm
ejaculated ranged from 1.1 to 4.1 billion, with an average of 2.4 billion for in copula ejaculates. Chemically induced ejaculates consistently produced larger number of sperm with a range of 2.5 to 5.6 billion, an average of 4.5 billion per ejaculate, and were significantly higher \((p < 0.0001)\) in number of total sperm output compared with in copula ejaculates.

G. Total and Progressive Sperm Motility (0 h)
Subjective total and progressive motility estimates of raw and extended semen were obtained immediately following collection of the ejaculate and removal of the gel fraction. Estimates of total motility ranged from 60% to 90% with an average of 77% over all collections. *Ex copula* ejaculates showed higher total \((p = 0.018)\) and progressive motility \((p = 0.010)\) when evaluated raw at 0 h postcollection. Estimates of total motility ranged from 85% to 99% and were significantly \((p < 0.00001)\) higher in chemically induced ejaculates extended and evaluated at 0 h post/collection. Progressive motility of extended semen ranged from 80% to 95% over all ejaculates and again demonstrated superior motility \((p = 0.0003)\) at 0 h postcollection, when obtained through chemically induced ejaculation.

H. Sperm Longevity
Subjective estimates of total and progressive motility were obtained over time until insemination. At 6 h, total motility \((p = 0.0001)\) and progressive motility \((p < 0.000001)\) differed between collection methods. At 24 h postejaculation, total motility \((p = 0.005)\) and progressive motility \((p < 0.00001)\) estimates differed between collection methods. Estimates of total and progressive motility were additionally obtained at time of insemination and differed \((p = 0.0029\) and \(p < 0.00001)\) respectively according to method of semen collection. The average age of semen stored before insemination was significantly different \((p = 0.005)\) between treatment groups, ranging from 6 to 29 h (CH1), 6 to 7 h (AV1), 8 to 78 h (CH2), and 6 to 12 h (AV2) with an average of 27 h for chemically collected ejaculates and 7 hours for ejaculates collected with an artificial vagina.

4. Discussion
In this study, pharmacologically induced *ex copula*–ejaculated stallion semen showed higher concentrations and total sperm numbers per ejaculate. These results support findings of previous research\(^3\) regarding these objective characteristics. Estimates of total and progressive motility at time of collection, over time and at time of insemination were superior in *ex copula*–ejaculated semen as compared to artificial vagina–obtained ejaculates. Partial removal of seminal plasma has been shown\(^4\) to improve motility of stored semen in some stallions. A decreased total volume of seminal plasma in relation to increased concentration of sperm cells, as is the nature of these ejaculates, may explain the improved motility of the extended semen at collection and over time in storage.

Pregnancies have previously been reported\(^3,4\) in mares inseminated with imipramine and xylazine induced *ex copula*–ejaculated semen. This study is the first to compare fertility of detomidine HCl–induced *ex copula*–ejaculated semen, stored over time to AV-collected semen. Procedures for handling, storing, and evaluating semen, were consistent between methods, as were quality, quantity, and timing of insemination doses in relation to ovulation. Pregnancy rates were similar and average conceptions per cycle higher in mares inseminated with chemically induced ejaculates.

Various methods are described for chemically obtaining semen from stallions. A wide range of reliability exists among these methods and among individual stallions. Stallions for whom these methods are indicated are those who suffer physically, psychologically, neurologically, or behaviorally. As a result of their deficit, semen cannot be collected from these stallions, or it cannot be collected without posing danger to the stallion, the jump mare, or the handler.

Therapeutic approaches to managing and aiding emission and ejaculation are described\(^5\) and should first be implemented when managing a difficult stallion. When these methods are not successful, methods for pharmacologically inducing ejaculation become available. The interval times from induction to collection (10–90 min) as well as the variations in reliability of success (20–70%) of these methods should be considered before their implementation.

Semen obtained by use of these methods is known to undergo freezing and thawing procedures without a negative effect on postthaw motility.\(^1\) In addition to application in frozen semen programs, this study has shown the favorable qualities of detomidine HCl–induced *ex copula* ejaculates for use with transported semen. When slow-cooled and stored at 5°C, these ejaculates can be used to inseminate mares without concern of detrimental effect on fertility.

References and Footnotes

\(^{*}\)Equitainer, Hamilton-Thorn Research, Beverly, MA.