A comparison of electroejaculation and epididymal sperm collection techniques in stallions

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Abstract — The purpose of this study was to evaluate 2 methods of semen collection that could be used as terminal procedures in stallions with irreparable conditions, such as fractures or colic. Electroejaculation was attempted under general anesthesia. Forty-eight hours later, the ponies were castrated and 2 different epididymal sperm collection techniques were attempted by using a flushing or floating method. Additionally, the effect of supplemental seminal plasma was evaluated. Experimentally, electroejaculation was found to be a safe but ineffective method of terminal semen collection. Viable sperm cells were successfully recovered with both types of epididymal collection. The flotation method was least cumbersome and showed a tendency to be superior to flushing in terms of sperm motility and percentage of cells passing through glass wool/sephadex filtration, although differences did not reach significance. The addition of seminal plasma to epididymal spermatozoa prior to cryopreservation was of no value. In conclusion, either method of epididymal sperm collection is an acceptable method of terminal semen collection.

Résumé — Comparaison entre l’électroéjaculation et les techniques de récolte de sperme épididymaire chez les étalons. Le but de cette étude était d’évaluer 2 méthodes de récolte de semence qui pourraient être utilisées comme techniques terminales chez des étalons atteints d’affections irrécupérables telles que fractures ou coliques. L’électroéjaculation a été tentée sous anesthésie générale. Quarante-huit heures plus tard, les poneys ont été castrés et 2 différentes techniques de récolte de sperme épididymaire ont été essayées, soit une méthode de rinçage et une méthode de flottaison. De plus, l’effet d’une supplémentation en plasma séminal a été évalué. Expérimentalement, l’électroéjaculation s’est révélée une méthode sécuritaire mais inefficace de récolte terminale de sperme. Des spermatozoïdes viables ont été récupérés avec les 2 méthodes de prélèvement épididymaire. La méthode de flottaison était plus simple et avait tendance à être supérieure à celle du rinçage en terme de motilité et de pourcentage de cellules passant au travers d’un filtre de laine de verre/séphadex. Cependant, les différences n’étaient pas significatives. L’addition de plasma séminal aux spermatozoïdes épididymaires avant la cryoconservation n’avait pas d’effet. En conclusion, les deux méthodes de récolte de sperme épididymal constituent une façon valide de prélèvement terminal de semence.

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Introduction

Traumatic injuries or colic conditions can prematurely end the breeding careers of many stallions. In such circumstances, owners may request a final semen collection to allow propagation of valuable genetics. Little documented research exists on appropriate collection techniques for use in terminal cases. Our goal was to develop a model for terminal sperm cell collection in stallions.

Historically, little value has been placed on the use of electroejaculation in horses. The use of electroejaculation in standing or sedated horses is discouraged due to their temperament and the likelihood of trauma to the horse, the operator, or the handlers. The technique has been used with some success in chemically immobilized Przewalski horses (1). However, death following electroejaculation of a sedated, terminally ill domestic stallion has been reported (2). The potential use of electroejaculation under general anesthesia has been discussed, but its use has not been reported in a clinical setting (3,4). Electroejaculation under general anesthesia would simplify semen collection from stallions with acute irreparable conditions.

Successful epididymal sperm collections with resulting pregnancies have been reported in a number of species,
including goats, red deer, dogs, and humans (5–9). Similarly, frozen equine epididymal spermatozoa have been proven to be fertile (10,11); however, recent attempts at achieving pregnancies have been challenging (11). While individual stallion variation exists, overall single cycle pregnancy rates following the use of frozen ejaculated semen range from 30% to 50.6% (12,13). The pregnancy rates following the use of frozen epididymal sperm are anecdotally extremely low using standard insemination techniques. However, a recent study achieved rates of 17% to 30% by depositing the semen onto the papillae of the uterine tubal junction by using hysteroscopy (11). Possibly, the poor functional quality of frozen epididymal sperm is related to the absence of seminal plasma, a normal component of ejaculated semen. Seminal plasma may have important roles in sperm motility, membrane integrity, transport through the uterus, and the avoidance of phagocytosis (14,15).

In this study, electroejaculation and 2 epididymal collection techniques were evaluated, as well as the effect of adding low levels of seminal plasma to semen samples for storage in liquid nitrogen.

We considered that electroejaculation under general anesthesia could be a viable option for semen collection, although there was concern about retrograde ejaculation and urine contamination, which could hinder the fertility following freezing. By comparing electroejaculation under general anesthesia with epididymal collection techniques our goal was to identify a procedure that would allow efficient and efficacious recovery of semen for use in cases of acute terminal injury or illness. We utilized 2 postcastration techniques in an attempt to develop an efficient method of semen collection from the distal epididymis and vas deferens. Furthermore, we wanted to investigate the use of seminal plasma and its effect on post-thaw motility and acrosomal membrane integrity when added to epididymal spermatozoa.

Materials and methods

Seven intact sexually mature male ponies between 2 and 4 y of age and weighing between 160 and 250 kg were used. All ponies had palpably normal descended testicles. The project was approved by the University of Minnesota Institutional Animal Care and Use Committee.

Anesthetic protocol

Each pony was anesthetized on 2 occasions, 48 h apart. Ponies were fasted for 12 h prior to each anesthetic episode. Ponies were premedicated with xylazine (Phoenix Pharmaceutical, Saint Joseph, Missouri, USA), 0.5 mg/kg bodyweight (BW), IV, induced with 5% guaifenesin (Phoenix Pharmaceutical) 50 to 70 mg/kg BW, IV, followed by 5% thiopental (Abbot Laboratories, Chicago, Illinois, USA) 5 mg/kg, intubated, and then maintained on 1.5% to 2.5% halothane (Abbot Laboratories) in oxygen (1 to 2 L/min), delivered via a large animal semi-open circle system. Each pony was positioned in lateral recumbency for electroejaculation and in dorsal recumbency for castration.

Before the electroejaculation procedure was performed, the manure was evacuated from the rectum.

Electroejaculation

Electroejaculation was performed during the first period of anesthesia. Due to the size of the animals, commercial electroejaculation probes designed for use in rams were deemed most suitable. Two models of electroejaculators (Ram Ejaculator [RE] and Pulsator II [PII]; Lane Manufacturing, Denver, Colorado, USA) were used on each pony. Both models were equipped with 35-mm diameter probes that have 3 ventrally located, longitudinally oriented, electrodes. A similarly patterned 60-mm diameter yearling bull probe, available for the PII, could not be placed in the rectum of these ponies. Following the unsuccessful ejaculation attempts with the 35-mm ram probes and the inability to place the 60-mm bull probe, a 48 cm long, 40-mm diameter probe with 6 ring electrodes was used on 2 of the larger ponies in an attempt to increase stimulation of pelvic nerves.

The self-contained RE uses a sine wave pattern at a frequency of 25 to 30 Hz, with a maximum output of 6 volts and 0.2 amps. Stimulations with this probe were commenced in a pulsatile pattern with approximately 2 s of stimulation per burst and 1 s rest between bursts. As ponies failed to respond, the length of each burst was gradually increased to a maximum of 10 s.

The PII also uses a sine wave pattern and has a maximum output at 25 Ω resistance of 45 volts and 1 amp. Stimulation was commenced at the lowest setting and increased in a stepwise fashion until ejaculation was achieved, or all 8 power levels had been used. At each power setting, the stimulus was started at zero and then increased to maximum where it was maintained for 2 to 3 s before returning to zero. Approximately 4 stimuli were given at each power setting. If the pony was actively ejaculating, the stimulus was continued at that power setting until the ejaculation was complete. If an attempt was unsuccessful, it was repeated after a short break. The maximum total time for electrostimulation attempts in any animal was 15 min.

During the procedure, all 7 ponies had their heart rate, respiratory rate, and depth of anesthesia monitored continuously; 6 of the ponies were monitored by electrocardiogram (ECG) (ECG-103; Brentwood, Danbury, Connecticut, USA) in a base apex lead, and 5 by direct arterial pressure. Evidence of elevated heart rate, respiratory rate, nystagmus, or change in anesthetic depth was noted. The anesthetic record was later evaluated along with electrocardiogram strips obtained before, during, and after electrical stimulation. The ponies were maintained at a surgical plane of anesthesia.

The ejaculate was collected into a sterile test tube and analyzed for volume, sperm concentration, progressive motility, and morphology. The level of urine contamination was evaluated by gross examination and pH value, with 8.0 or above indicating the presence of urine in the sample.

Following both successful and unsuccessful electroejaculation attempts, the penis was cleansed and a sterile urinary catheter was passed into the pony’s bladder, which was then lavaged with 500 mL of lactated Ringer’s solution. The lavage fluid was collected and evaluated for the presence of semen by allowing it to sediment over 4 h, after which the top half of the fluid was decanted. The remaining fluid was centrifuged at 400 × g and the
resultant pellet was analyzed under a light microscope at 400 × for the presence of sperm.

Epididymal collection
Two days after electroejaculation, the ponies were reanesthetized and castrated by a modified open technique (16). The vas deferens was clamped to avoid loss of semen during the 5-minute transit to the laboratory. The individual testicles were placed in sterile plastic containers with tight sealing lids and were transported in Styrofoam coolers warmed by water bottles heated to 37°C. In the laboratory, an epididymodeferentectomy was performed. The cauda epididymis and vas deferens were isolated from each testicle, and the left and right epididymes were randomly assigned to be processed by 1 of 2 methods: Method 1, the standard flushing technique, consisted of catheterizing the lumen of the vas deferens with an 18-gauge tubing adaptor (Becton-Dickinson, Rutherford, New Jersey, USA) and retrograde flushing of the vas deferens and cauda epididymis with 5 mL of warmed (37°C) commercial milk-based semen extender (E-Z Mixin with amikacin and penicillin; Applied Reproduction Systems, Chino, California, USA). The resultant mixture was collected in a warmed sterile test tube. In method 2, the flotation technique, between 10 and 15 slashes were made horizontally in the distal epididymis and vas deferens with a #10 scalpel blade. The epididymis was placed in a 50 mL conical tube and covered with approximately 5 mL of the same commercial semen extender. The samples were then agitated and incubated at room temperature for 10 min. Both procedures were performed by the same investigator (SM), with the flush procedure occurring during the time that the float was incubating.

Seminal plasma
Four hours prior to the epididymal collection, semen was collected from 2 separate fertile horse stallions. The gel-free semen was centrifuged and the seminal plasma removed immediately by using a pipette. Seminal plasma from both horses was pooled prior to use. During final preparation for freezing, seminal plasma (5% by volume) was added to half of each of the samples from the traditional (flush) collection method and the experimental (float) collection method.

Freezing
The semen was processed and frozen by one author (LD) to minimize variation. Semen was centrifuged at 400 × g for 10 min. Following centrifugations, sufficient supernatant was aspirated such that when the lactose-EDTA-egg yolk-based freezing extender (17) was added, a sperm/commercial semen extender : freezing extender ratio of 30:70 and a sperm concentration of 400 × 10⁶/mL would be achieved. Following aspiration of the supernatant but prior to addition of the freezing extender, the sperm pellet was resuspended by gentle pipetting. After a 30-min equilibration period in freezing extender at room temperature, semen was packed in 0.5 mL straws (Minitube, Verona, Wisconsin, USA) and placed horizontally on a copper rack 2 to 3 cm above liquid nitrogen for 20 min before being plunged into the liquid nitrogen. The semen was frozen with 200 × 10⁶ sperm per straw (except for 1 with 9 × 10⁶ due to low numbers). The frozen semen was stored in liquid nitrogen until it was thawed and evaluated 2 to 4 wk later.

Semen evaluation
Semen was evaluated immediately following collection and after cryopreservation. Frozen semen was thawed in a 37°C water bath for 30 s. Each collection was manually counted following dilution in a 1:100 commercial white blood cell and platelet dilution chamber (Unopette; Becton-Dickinson). Diluted samples were loaded into a hemocytometer (Hauser Scientific, Horsham, Pennsylvania, USA) and counted at 400 × with a light microscope. A computer assisted semen analysis system (CAS:HTM-200; Hamilton-Thorne Research, Danvers, Massachusetts, USA) was used to evaluate total and progressive motility. Prior to analysis, semen samples were diluted to a concentration of 50 × 10⁶ in commercial milk-based semen extender (E-Z Mixin extender) at 37°C. Aliquots (7 µL) were loaded into a 10-µm deep counting chamber (Makler chamber; Sefi-Medical Instruments, Haifa, Israel) for analysis with the following settings: temperature 37°C, 3 fields per sample, low size gate 0.3, high size gate 2.5, low intensity gate 0.4, and high intensity gate 2.5. Morphology was analyzed by light microscopy under oil immersion at 1000 × by using slides stained with nigrosin and eosin. One hundred sperm cells were analyzed and classified according to the system recommended by the Society for Theriogenology (18). In addition, duplicate aliquots of post-thaw samples were evaluated by using glass wool sephadex (GWS) filtration (15) to calculate the percentages of sperm with intact plasma/acrosomal membranes. Briefly, the plunger from a plastic 3-mL syringe was removed and glass wool (Johns-Manville code 112, type 475; Denver, Colorado, USA) was loosely packed to the 0.3-mL mark. A 20% slurry of cross-linked dextran beads (Sephadex G15; Pharmacia Fine Chemicals, Uppsala, Sweden) was prepared in a 3% sodium citrate solution and allowed to swell for at least 4 h at room temperature. A 0.7-mL volume of the slurry was layered over each glass-wool filter and packed by adding 3 mL of 3% sodium citrate solution 3 times to each syringe, each addition being allowed to drain by gravity. Immediately after the sodium citrate had finished draining, the prepared filters were hung in dry10-mL test tubes and 50 µL of thawed semen was layered on the filter. Once the semen had drained into the filter a further 3 mL of sodium citrate was added. Duplicate control samples were prepared by mixing 50 µL of semen with 3 mL of 3% sodium citrate. The number of sperm in the effluent from filter samples and in the control samples was determined by counting in a hemocytometer and these results were used to calculate the percentage of cells passing through the filter for each treatment.

Statistical evaluation
Data were tested for normal distribution prior to analysis. Paired t-tests were used to compare the effects of epididymal recovery method on total sperm number, motility, and morphology prior to freezing. Two-way analysis of variance (ANOVA) was performed to compare the effects of recovery technique and addition of seminal
Table 1. Effect of recovery method and seminal plasma addition on post-thaw parameters of cryopreserved epididymal spermatozoa (mean, sₓ)

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<thead>
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<th>Seminal Plasma (%)</th>
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<tr>
<td></td>
<td>Float</td>
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<tr>
<td></td>
<td>0</td>
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<tr>
<td>Total motility (%)</td>
<td>32.6, 7.5</td>
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<tr>
<td>Progressive motility (%)</td>
<td>18.0, 4.1</td>
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<tr>
<td>GWS (%)</td>
<td>26.6, 10.0</td>
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GWS — glass wool Sephadex

Results

Electroejaculation

Ponies monitored with electrocardiographs were found to have normal, unchanged rhythms aside from intermittent electrical interference associated with the activated ejaculation probe. Evaluation of anesthetic records following the procedure failed to demonstrate any change in anesthetic monitoring parameters (heart rate, respiratory rate, mean arterial pressure, and depth of anesthesia) during the electrical stimulation. There was no evidence of rectal trauma. All ponies achieved an erection. During electrostimulation, musculoskeletal movement consisted of hindlimb extension and pelvic thrusting, typical for that seen in other species.

Only 3 of the 7 ponies had any evidence of semen during attempted electroejaculation. Two of these samples were achieved at the highest setting of the probe and resulted in small volumes with high levels (grossly visible) of urine contamination. The semen from these 2 ponies had no discernible motility when evaluated within 5 mins of collection. The 3rd pony ejaculated a very small volume of semen without gross urine contamination, but the motility was poor with no progressively motile sperm. Two ponies passed a small amount of urine during the procedure with no evidence of semen. Despite achieving erections, the remaining 2 ponies did not show any evidence of ejaculation as a result of electrical stimulation. The larger probe with 6 ring electrodes did not result in an ejaculation.

Fluid collected from the urinary bladder revealed the presence of semen in the bladders of 4 of the 7 ponies, including the 2 ponies that produced urine contaminated semen during electroejaculation. The number of sperm in the urine collected from the bladder was not determined due to the large volume of flush used, but the quantity was subjectively graded as moderate to large in comparison with urine debris and crystals.

Effect of recovery technique on prefreeze semen parameters

The epididymal flush procedure took approximately 15 min to accomplish when performed by an experienced person. The flotation technique required only 5 min of operator time and an additional 10 min of incubation in extender for the semen to “float out.” There was no difference between the techniques in the number of sperm recovered (flush: 4485 × 10⁶, sₓ = 1551 × 10⁶; float: 4886 × 10⁶, sₓ = 2422 × 10⁶; P = 0.78). There was a tendency toward a higher percentage of total motility in sperm recovered by flotation compared with flushing (74%, sₓ = 10% versus 61%, sₓ = 8%; P = 0.06) but no difference in the percentage of progressive motility (float 35%, sₓ = 5%, flush 31%, sₓ = 7%; P = 0.27). Similarly, there was no difference between the techniques with regard to percentage of sperm with normal morphology (float 58.4%, sₓ = 9.5%, flush 57.7%, sₓ = 10.9%; P = 0.82).

Table 2. Relationship between prefreeze and post-thaw progressive motility of epididymal sperm recovered by flushing or floatation and cryopreserved with or without the addition of seminal plasma (mean, sₓ)

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<td>0</td>
<td>5</td>
<td>0</td>
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<tr>
<td>Prefreeze Post thaw</td>
<td>18.4</td>
<td>16.3</td>
<td>17.6</td>
</tr>
<tr>
<td>P (paired t-test)</td>
<td>0.08</td>
<td>0.02</td>
<td>0.03</td>
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<tr>
<td>Spearman’s r</td>
<td>-0.71</td>
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plasma on sperm motility following thawing of cryopreserved samples. Data for sperm passage through GWS filtration showed significant deviation from the normal distribution and was analyzed by a nonparametric Friedman test. Within recovery technique, the change in progressive motility between prefreeze and post-thaw samples was assessed by 1-way paired t-tests with Bonferroni correction, and possible correlations were examined by Spearman’s correlation coefficient. Results are presented as mean and standard error of the mean (sₓ) and significance was set at P < 0.05. Analyses were performed by using a commercial statistical package (SPSS version 11.0; SPSS, Chicago, Illinois, USA).
Effect of recovery technique and seminal plasma on post-thaw semen parameters

The interaction term between recovery method (flush or float) and freezing technique (0% versus 5% seminal plasma) was not significant, nor did it increase the model fit for post-thaw sperm motility; therefore, it was not included in the final analysis.

There were no significant effects of recovery method or seminal plasma addition on any of the post-thaw parameters examined (Table 1).

The relationship between progressive motility before and after cryopreservation is shown in Table 2. Within recovery technique, there were significant decreases in progressive motility ($P < 0.05$) following cryopreservation, except for sperm recovered by the floatation technique and frozen without additional seminal plasma, where significance was approached. The only correlation that approached significance was for the floatation recovery technique without the addition of seminal plasma ($r = -0.71; P = 0.07$).

Discussion

Our results suggest that preservation of genetics from valuable stallions by using terminal collection techniques is possible. However, since our subjects were healthy, the effects of severe disease processes on the success of these procedures remains to be determined and could certainly alter the recovery rates of semen.

Electroejaculation, while proven to be safe for the operator and patient, was not an effective method of obtaining viable semen under the conditions of this experiment. When ejaculation was achieved, the level of urine contamination was significant, reducing the potential of obtaining viable semen. The one grossly uncontaminated sample did not have sufficient motility, volume, or concentration to be used as an acceptable dose of semen. In one case report, in which the stallion underwent cardiac arrest following successful collection by using the electroejaculation probe, the motility was 10% compared with 65% motility following castration and flushing the ductus deferens (2). Urine contamination may have contributed to the low motility with ejaculation compared with epididymal collection. Urine contamination has been cited as one of the reasons that electroejaculation in horses may not be an acceptable method of collection (4). The use of ring type electrodes worn on the gloved hand of the operator may reduce this contamination and provide improved sperm recovery by allowing more targeted stimulation of the reproductive tract. This technique may be worthy of investigation in suitably sized subjects.

The presence of sperm in the urinary bladder in 4 of 7 ponies would suggest that retrograde ejaculation might be common in ponies when electroejaculation is attempted under general anesthesia. Retrograde ejaculation in conscious horses is considered rare but has been documented (19), and it is commonly associated with electroejaculation in other species (20–22). The presence of sperm in the urethra during catheterization can yield small numbers of sperm in the urinary bladder (19); however, the apparent large quantity of sperm isolated from the bladders of ponies in this study suggests that retrograde ejaculation was occurring during electrical stimulation. In dogs, an increase in the occurrence of retrograde ejaculation has been linked to the administration of xylazine (23). In contrast, in stallions, xylazine is used as part of the protocol to obtain ex-copula ejaculations (24). To our knowledge, there is no documentation on the frequency of retrograde ejaculation in these stallions. It is possible that the administration of xylazine for premedication in the anesthetic protocol or the combination of anesthetic drugs contributed to the occurrence of retrograde ejaculation. Tricyclic antidepressants, such as imipramine, have been used as therapy for urospermia in stallions in an effort to increase tone of the bladder sphincter (25). Brinsko (19) hypothesized that these drugs could be used to decrease the frequency of retrograde ejaculation. Such therapy might have improved the recovery of sperm during electroejaculation in this experiment. However, such treatment has been found to be ineffective for cases of urospermia in a controlled trial (26).

Options for removal of semen from the distal epididymis include aspiration (9), flushing (7), and flotation (8,27) or any combination of techniques (3,5,28). In this study epididymal sperm were successfully harvested by both the flushing and flotation techniques. The collection of spermatozoa was more rapidly achieved by the flotation technique than by the flushing technique, as the lumen of the vas deferens or epididymis did not have to be catheterized. The ease of the flotation technique accelerated the active collection process while preserving the viability of resultant sperm. The flotation technique produced slightly higher prefreezing total and progressive motility and number of sperm collected over the flushing technique, although the counts were not statistically significant. Due to repeated incisions and subsequent incubation of the cauda epididymis and vas deferens, there was more blood contamination with this procedure; however, this did not appear to be detrimental to the sperm under the conditions of our experiment. The flotation collection method may be selective for the more functional spermatozoa that are able to swim out of the epididymis. Alternatively, flushing the spermatozoa from the epididymis may be more traumatic to individual sperm cells. However, there was essentially no difference in the post-thaw motility between the 2 recovery methods. Individual stallion variation existed within groups, although there was no discernible pattern to assist in predicting this trend in a clinical setting. In the absence of significant differences between the recovery techniques, either method would appear to be acceptable.

Progressive motility before freezing in this study ranged from 4% to 62% with a mean of 33%, somewhat lower than the results of Braun et al (29), who obtained prefreezing motility rates of 25% to 75% following collection after normal stallion castrations. However, the stallions in that study were on a regular collection schedule prior to castration, whereas ours were sexually rested. Progressive motility following thaw of cryopreserved epididymal sperm was 1% to 43% (mean 16.3%), comparable with 4% to 49% obtained by Braun et al (29).

Compared with sperm obtained at ejaculation, epididymal sperm are more resistant to cold shock, making
them potentially more successful candidates for freezing and thawing than sperm mixed with accessory sex gland fluids during ejaculation (28). In a direct comparison with ejaculated sperm, epididymal sperm were found to have superior post-thaw motility (30). Volkmann et al (31) reported superior post-thaw progressive motility for epididymal sperm frozen without seminal plasma compared with both epididymal sperm with seminal plasma added prior to cryopreservation and frozen ejaculated sperm from the same stallions. However, similar to our results, other workers have found no significant effect of seminal plasma on post-thaw motility of epididymal sperm (32). The same large variations between stallions in post-thaw motility of ejaculated sperm have been shown to exist in epididymal sperm and the two are highly correlated (29,33). This lead Braun et al (29) to suggest that the freezability of equine spermatozoa is determined when it leaves the epididymus, prior to mixing with the seminal plasma.

A puzzling result of our study is the apparently negative correlation between prefreeze and post-thaw motilities for semen recovered by flotation and frozen without additional seminal plasma (r = -0.71, P = 0.07). This was driven by the change in stallion ranking between prefreeze and post-thaw motility. The stallions with the 3 highest prefreeze progressive motility rankings dropped to be in the bottom 4 post-thaw rankings, while the 3 with the lowest prefreeze motilities became the highest ranked on post-thaw motility. While this may be a reflection of the individual stallion variation in response to freezing, we do not currently have an explanation as to why this should have occurred in this treatment group and not the others. Further investigations are required to determine whether this is a sampling effect for this group of stallions or whether a true trend exists.

Plasma membrane and acrosomal integrity is critical to fertility, and the negative impact of cooling and cryopreservation on the fertility of sperm is mediated largely through damage to these structures (34). With GWS filtration, we found no significant effect of either recovery method or the addition of seminal plasma on membrane integrity following cryopreservation. The treatment rankings were highest for the flotation method of recovery, irrespective of seminal plasma addition, and lowest for the flush method of recovery without the addition of seminal plasma. Most other tendencies within this study also favored the flotation method of recovery.

Semen quality is dependent on many factors, including season of the year, previous sexual activity, and individual stallion variability, especially when pertaining to cryopreservation (35). Therefore, success in terminal collections with limited amounts of semen may be challenging. To our knowledge, terminal collection and cryopreservation of epididymal sperm in horses has resulted in few documented pregnancies and poor pregnancy rates (10,11). Further advances in technology are required to improve the utilization of terminally collected sperm.

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