Equine semen quality following sperm exposure to seminal plasma stored under different conditions

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Abstract

This study addressed equine sperm quality following exposure to seminal plasma stored under different conditions. Objectives were to compare fresh versus snap-frozen homologous seminal plasma; to compare homologous versus allologous frozen seminal plasma; and to determine the optimal processing/freezing method for long-term preservation of seminal plasma. For the latter objective, seminal plasma was subjected to the following storage conditions: immediate storage of seminal plasma at -20, -80, or -196°C (Groups 20, 80 and 196, respectively); storage of seminal plasma at 4°C for 24 h prior to freezing at -20, -80, or -196°C (Groups 4-20, 4-80, and 4-196, respectively); or storage of raw semen at 4°C for 24 h prior to isolating seminal plasma and freezing at -20, -80, or -196°C (Groups RW-20, RW-80, and RW-196, respectively). Seven ejaculates were collected from each of three fertile stallions that served as sperm donors and seminal-plasma donors. Seminal plasma was also obtained from seven other stallions by centrifugation and filtration of raw semen. Following exposure of sperm to seminal plasma treatments and cooled storage of extended semen for 24 h, sperm motion characteristics (total motility [TMOT, %], progressive motility [PMOT, %], curvilinear velocity [VCL, μm/s]), plasma membrane intactness (PMI, %), acrosomal membrane intactness (AI, %), and sperm DNA quality (COMP, %) were evaluated. Comparison of fresh versus frozen homologous seminal plasma revealed no effect of treatment on any experimental endpoint ($P > 0.05$). Progressive motility was higher in semen exposed to frozen allologous seminal plasma, as compared to frozen homologous seminal plasma, for one of three stallions ($P < 0.05$). Storage method for seminal plasma did not impact PMI or COMP; however, Group RW-20 yielded lower TMOT than Group 4-80 ($P < 0.05$). Group RW-20 yielded lower VCL than all other treatments. Highest VCL was detected in Groups 80, 196, 4-80, and 4-196. A treatment x stallion interaction was detected for PMOT. No difference was observed for two stallions ($P > 0.05$); however, Group 20 yielded higher PMOT than Groups 80 or 196 for the remaining stallion ($P < 0.05$). Our findings suggest that: 1) seminal plasma can be frozen for later use, obviating the need to process fresh semen to supply seminal plasma, 2) allologous seminal plasma may be beneficial for selected stallions, and 3) semen should not be stored in the raw form for an extended period prior to processing seminal plasma for frozen storage.

Keywords: Equine, seminal plasma, sperm, semen quality, storage

Introduction

The effects of seminal plasma on sperm function are an active area of investigation in the horse, in areas as broad as the effects of seminal plasma on post-breeding endometritis;[1] effects on quality and fertility of frozen semen;[2,3] and effects on sperm quality in cool-stored semen.[4-14] Both inhibitory and stimulating effects of seminal plasma on sperm capacitation have also been investigated in other species,[15-17] and applications to stallion sperm have been proposed.[18] Raw semen can be subjected a centrifugation/filtration process as a means of harvesting sperm-free seminal plasma for future use; however, information is also unavailable regarding optimal methods for processing and storage of the seminal plasma. Another area that has not been well-researched is individual stallion variation in seminal plasma effects on sperm. Clinically, there are reports of stallions whose seminal plasma exerts a depressing effect on sperm quality; yet, when seminal plasma from another stallion is substituted, sperm quality improves when the semen is subjected to cooling[4] or cryopreservation.[2] Despite the increasingly

widespread use of seminal plasma addition/replacement clinically, data are sparse regarding the effect of adding allologous seminal plasma to semen when incorporated soon after ejaculation. The objectives of this study were to: compare fresh versus snap-frozen homologous seminal plasma and homologous versus allologous frozen seminal plasma on semen quality following cooled storage; and determine the optimal freezing method for long-term preservation of seminal plasma.

Materials and methods

Animals

Sexually-active mature light-breed stallions (n=10) with good sperm quality and no known fertility problems were used in this study. All animals were fed a pelleted diet with access to fresh water and roughage and all were in good body condition. Stallions were kept in stalls with occasional turn out in paddocks. Two to three daily ejaculates were collected from each stallion to reduce extragonadal sperm reserves prior to collection of semen and/or seminal plasma for the experimental procedures. Three stallions (11 to 24 years of age) were used as donors of both seminal plasma and sperm. Seven different stallions (9 to 20 years of age) were used as donors only of seminal plasma.

Semen collection

Semen was collected using a lubricated Missouri-model artificial vagina (Nasco, Ft. Atkinson, WI) fitted with a semen receptacle (Animal Reproduction Systems, Chino, CA) containing a nylon mesh in-line filter (Animal Reproduction Systems) to separate gel-free and gel-containing fractions of the ejaculate. Stallions were exposed to an ovariectomized mare to stimulate penile erection. The erect penis was rinsed with warm water and then patted dry with clean, disposable towels. After penile cleaning, stallions were again sexually stimulated and allowed to mount a breeding dummy for semen collection.

Semen processing

The gel-free semen volume was measured by weight (Model TP600S Precision Plus top-loading balance, Ohaus Corporation, Florham Park, NJ) and recorded in mL (1 g ≈ 1 mL), and sperm concentration was determined using a fluorescence-based instrument (NucleoCounter® SP-100™, Chemometec, Allerød, Denmark). The gel-free semen was diluted with extender (INRA 96; IMV, Maple Grove, MN) and then subjected to cushioned centrifugation, using 40-mL capacity glass nipple-bottom centrifuge tubes.[18] To prepare glass nipple tubes for centrifugation, 30 µL of cushion fluid (Minitube of America, Inc., Verona, WI) was pipetted into the bottom of the nipple underneath 1 mL of INRA 96 extender. Following extension of semen to a concentration of 30 x 10^6sperm/mL in INRA 96, approximately 1 x 10^9 sperm were carefully layered on top of the INRA-96 in the nipple tubes. Loaded nipple tubes were centrifuged at 400 x g for 20 min at room temperature. The supernatant was aspirated and the resulting sperm pellet was resuspended in INRA 96 extender, then transferred to a 50-mL conical-bottom tube and further diluted with extender and seminal plasma (30-32 mL) to obtain a final concentration of approximately 30 x 10^6 sperm/mL. Extended semen was mixed with seminal plasma (20%, v/v) based on seminal-plasma treatments dictated by experimental protocols. Prepared vials of extended semen were then packaged in a commercial semen-transport container (Equitainer® II; Hamilton Research, Inc., South Hamilton, MA) for 24 h of cooled storage. Following cooled storage, aliquots of extended semen were packaged in capped 0.6-mL polypropylene tubes (Fisherbrand™ snap-cap flat-top graduated microcentrifuge tubes, Fisher Scientific, Pittsburgh, PA) and then immediately frozen (-80°C) until analyzed for sperm DNA integrity, using the sperm chromatin structure assay (SCSA). The remaining contents were warmed for 15 min at 37°C and then evaluated for sperm motion characteristics, as well as plasma-membrane and acrosomal-membrane intactness.

Seminal plasma processing and storage techniques

Gel-free raw semen was centrifuged at 2000 x g for 10 min at room temperature using 15-mL plastic conical-bottom tubes (VWR International, LLC, Radnor, PA). The seminal plasma was decanted

and filtered through tandem nylon syringe filters (5.0- and 1.2-µm pore diameters, Spectrum Chemical Manufacturing Corp., New Brunswick, NJ) to remove any residual sperm. Aliquots (1.0- to 1.8-mL) of filtered seminal plasma were packaged in capped polypropylene tubes (Cryogenic vials [2.0-mL]; Corning Life Sciences, Lowell, MA) and stored according to experimental specifications.

Experimental treatments

Fresh versus frozen-thawed seminal plasma. Seven ejaculates from each of three stallions were subjected to centrifugation and sperm resuspension in extender, as described above. Semen was diluted to a final concentration 30 x 10^6 sperm/mL in extender containing 20% (v/v) freshly prepared (unfrozen) seminal plasma or seminal plasma that was flash-frozen (-196°C) immediately prior to thawing and use. Volume of extended semen ranged from 30-32 mL.

Homologous versus allologous seminal plasma. Seven gel-free ejaculates from each of three stallions were subjected to centrifugation and sperm resuspension in extender, as described above. Semen was diluted to a final concentration 30 x 10^6 sperm/mL in extender containing 20% (v/v) frozen (-196°C)/thawed seminal plasma from the same stallion (homologous) or from each of seven other seminal-plasma donor stallions (allologous).

Seminal plasma frozen-storage conditions. Seven gel-free ejaculates from each of three stallions were subjected to centrifugation and sperm resuspension in extender, as described above. Semen was diluted to a final concentration 30 x 10^6 sperm/mL in extender containing 20% (v/v) allologous seminal plasma (from seven different stallions) that had been processed and stored in various manners. Treatment groups consisted of the following: Group 20 (seminal plasma processed immediately following collection and frozen at -20°C); Group 80 (seminal plasma processed immediately following collection and frozen at -80°C); Group 196 (seminal plasma processed immediately following collection and frozen at -196°C); Group 4-20 (seminal plasma processed immediately following collection, stored at 4°C for 24 h and then frozen at -20°C); Group 4-80 (seminal plasma processed immediately following collection, stored at 4°C for 24 h and then frozen at -80°C); Group 4-196 (seminal plasma processed immediately following collection, stored at 4°C for 24 h and then frozen at -196°C); Group RW-20 (raw semen stored at 4°C for 24 h, then processed for seminal plasma and frozen at -20°C); Group RW-80 (raw semen stored at 4°C for 24 h, then processed for seminal plasma and frozen at -80°C); and Group RW-196 (raw semen stored at 4°C for 24 h, then processed for seminal plasma and frozen at -196°C).

Computer-assisted sperm motion analysis (CASMA). Sperm motion characteristics were analyzed in a manner similar to that previously described.[19] Warmed (37°C) analysis chambers (fixed height of 20 µm) affixed to microscope slides (Leja Standard Count 2 Chamber slides; Leja Products, B.V., Nieuw-Vennep, The Netherlands) were slowly loaded with a 6-µL volume of extended semen. The slides were then placed on a stage (37°C) and inserted into the CASMA instrument (IVOS Version 12.0, Hamilton-Thorne Research) for evaluation. A total of 10 microscopic fields and a minimum of 500 sperm were examined per sample. Preset values for the IVOS system consisted of the following: frames acquired–45; frame rate–60 Hz; minimum contrast–70; minimum cell size–4 pixels; minimum static contrast–30; straightness (STR) threshold for progressive motility–50; average-path velocity (VAP) threshold for progressive motility–30; VAP threshold for static cells–15; cell intensity–106; static head size–0.60 to 2.00; static head intensity–0.20 to 2.01; static elongation–40 to 85; LED illumination intensity–2200. Experimental endpoints included: 1) percentage of motile sperm (TMOT); percentage of progressively motile sperm (PMOT); and curvilinear velocity (VCL; µm/s).

Sperm acrosomal-membrane and plasma-membrane integrity. The intactness (integrity) of sperm acrosomal and plasma membranes was evaluated using a procedure described previously.[20] Fifty µL of extended semen were added to 133 µL of Dulbecco's phosphate buffered saline (PBS; Invitrogen Gibco,® Carlsbad, CA). Three µL of propidium iodide (Invitrogen Molecular Probes, Eugene, OR; 2.4 mM working solution) and 10 µL *Pisum sativum* agglutinin (PSA)-FITC conjugate (Sigma-Aldrich, St. Louis, MO; 0.05 mg/mL working solution) were added to the semen-buffer solution. The samples were incubated at room temperature (approximately 25°C) in the dark for 10 min. Fifty µL of semen were then mixed with 1 mL PBS and processed immediately on a flow cytometer (FACScan; Becton Dickinson,

Mountain View, CA). The sample was allowed to pass through the tubing for 30 sec before evaluation of cells. A cell flow rate of approximately 300 cells/s was used and a total of 5000 events were evaluated per sample. The voltage settings on the flow cytometer were as follows: SSC 240, FL1 798, FL2 657, and FL3 150. The compensation was set at FL1 1.9% of FL2, and FL2 18.8% of FL1. Data were acquired using a log scale and analyzed by WinList™ software (Verity Software House, Topsham, ME), with scatterplots divided into quadrants: minimal green and red fluorescence (representing sperm with intact plasma membranes and intact acrosomal membranes); minimal green and enhanced red fluorescence (representing spermatozoa with damaged plasma membranes and intact acrosomal membranes; minimal red and enhanced green fluorescence (representing spermatozoa with intact plasma membranes and damaged acrosomal membranes); and enhanced red and green fluorescence (representing spermatozoa with damaged plasma membranes and damaged acrosomal membranes). Data were sorted by sperm with intact plasma membranes, regardless of acrosomal status (PMI; %) and sperm with intact acrosomal membranes, regardless of plasma membrane status (AI; %).

Sperm chromatin structure assay (SCSA). The SCSA protocol was conducted as previously described.[21] All stock solutions (buffer solution [TNE; pH 7.4; 0.19 g disodium EDTA, 0.79 g Tris-HCl, 4.380 g NaCl in 500 mL deionized water], Triton-X [2.19 g NaCl, 1.0 mL of 2N HCI solution, 0.25-mL Triton-X, qs. 250 mL with deionized water], and acridine orange [pH 6.0; 3.8869 g citric acid monohydrate, 8.9429 g Na_2HPO_4, 4.3850 g NaCl, 0.17 g disodium EDTA, 4.0 µg/mL acridine orange stock solution (1.0 mg/mL), qs. 500 mL water]) were kept on ice throughout the duration of the procedure. Immediately prior to analysis, semen samples were thawed in a 37°C water bath. Nine-µL of frozen-thawed semen were aliquoted into a 5-mL Falcon tube and diluted to 200 µL with the TNE solution, then 400 µL Triton-X solution was added and the mixture was placed on ice for 30 s. A 1.2- mL aliquot of acridine orange stain was then pipetted into the tube. The mixture was analyzed via flow cytometry using the following settings: mean green fluorescence at 500 channels (FL-1: 500) and mean red fluorescence at 150 channels (FL-3: 150). All samples underwent an equilibrium period of 30 seconds prior to analysis. Five-thousand (5000) events were recorded at a rate of at least 200 events/s. Quantification of DNA denaturation in each cell was determined by the term alpha-t (αt), which is defined as the ratio of red/(red + green fluorescence). The alpha-t (αt) designation is used to describe the relationship between the amounts of green (double-stranded DNA) and red (single-stranded DNA) fluorescence. Data were acquired in a list-mode and values were calculated using WinList™ software (Verity Software House). Cells outside the main population (COMP) was the endpoint measured and represented the percentage of sperm outside the main population.

Statistical analysis

Percentage data were arc sine-root transformed for normalization prior to statistical analysis using SAS® (SAS Institute Inc., Cary, NC). Statistical tests were conducted on transformed data. Analysis-of-variance (ANOVA) procedures were used for data analysis, with the Tukey test used for mean separation when treatment F ratios were significant ($P < 0.05$). Level of significance was set at $P < 0.05$. Untransformed data are presented in the results section for clarity of interpretation.

Results

Comparison of fresh versus frozen homologous seminal plasma

No significant treatment differences were detected for experimental endpoints ($P > 0.05$; Table 1), and stallion-by-treatment interactions were not significant ($P > 0.05$).

Table 1. Main effect of fresh versus frozen/thawed homologous seminal plasma on measures of sperm quality (mean ± SEM) for three stallions following 24 h of cooled storage (n = 21 ejaculates).

	Treatment	
Laboratory parameter*	Fresh seminal plasma	Frozen/thawed seminal plasma
TMOT (%)	80 ± 1.8	79 ± 2.0
PMOT (%)	49 ± 1.7	48 ± 1.5
VCL (μm/s)	183 ± 6.8	187 ± 6.9
PMI (%)	85 ± 0.6	84 ± 0.7
AI (%)	88 ± 1.0	88 ± 1.1
COMP (%)	10 ± 0.8	9 ± 0.9

*TMOT = total sperm motility (%); PMOT = progressive sperm motility (%); VCL = curvilinear velocity (μm/s); PMI = sperm with intact plasma membrane (%); AI = sperm with intact acrosomal membrane (%); COMP = percentage of sperm with αt value outside the main population (%). Percentage data (TMOT, PMOT, PMI, AI, and COMP) were arc sine-root transformed for normalization prior to statistical analysis. Untransformed values for mean and SEM are presented in table to ease interpretation but statistical tests were conducted on transformed data. For each dependent variable, treatment differences were not detected ($P > 0.05$).

Comparison of frozen-thawed homologous versus allologous seminal plasma

Table 2 illustrates the main effects of frozen homologous versus allologous seminal plasma on experimental endpoints. No significant differences were detected between homologous and allologous treatment groups for variables TMOT, PMI, AI and COMP ($P > 0.05$). Allologous seminal plasma yielded a lower VCL when compared to homologous seminal plasma ($P < 0.05$). For variable PMOT, allologous seminal plasma yielded significantly higher values than did homologous seminal plasma ($P < 0.05$). Stallion-by-treatment interactions were detected for PMOT, where mean PMOT was lower for homologous seminal plasma than allologous seminal plasma for one of three stallions ($P < 0.05$). Treatment differences were not detected for the remaining two stallions ($P > 0.05$). The source of the allologous seminal plasma which yielded the highest PMOT differed with sperm from each of the three donor stallions.

Table 2. Main effect of frozen/thawed homologous versus allologous seminal plasma on measures of sperm quality (mean ± SEM) for three stallions following 24 h of cooled storage (n = 21 ejaculates).

	Treatment	
Laboratory parameter*	Homologous	Allologous
TMOT (%)	79 ± 2.0[a]	82 ± 1.7[a]
PMOT (%)	48 ± 1.5[b]	54 ± 1.9[a]
VCL (μm/s)	187 ± 6.9[a]	176 ± 7.8[b]
PMI (%)	84 ± 0.7[a]	85 ± 1.2[a]
AI (%)	88 ± 1.1 [a]	88 ± 1.1[a]
COMP (%)	9 ± 0.9[a]	8 ± 0.5[a]

*TMOT = total sperm motility (%); PMOT = progressive sperm motility (%); VCL = curvilinear velocity (μm/s); PMI = sperm with intact plasma membrane (%); AI = sperm with intact acrosomal membrane (%); COMP = percentage of sperm with αt value outside the main population (%). Percentage data (TMOT, PMOT, PMI, AI, and COMP) were arc sine-root transformed for normalization prior to statistical analysis. Untransformed values for mean and SEM are presented in table to ease interpretation but statistical tests were conducted on transformed data

[a,b] Within row, means with different superscripts differ ($P < 0.05$).

Storage methods for frozen preservation of allologous seminal plasma

Data regarding the effect frozen storage methods for allologous seminal plasma on semen quality are provided in Table 3. A main effect of treatment was detected ($P < 0.05$) for all sperm motion

variables. Variable TMOT was higher for Group 4-80 as compared to Group RW-20 ($P < 0.05$), but TMOT for both treatment groups was similar to that of the remaining treatments groups ($P>0.05$). Group 20 exhibited higher PMOT than RW-80 and RW-196 ($P < 0.05$), but PMOT for these two treatment groups was similar to the remaining treatment groups ($P > 0.05$). Mean VCL was higher in Groups 80 and 4-80 than in Groups 20, 4-20, RW80 and RW-196 ($P < 0.05$), and was lower for Group RW-20 than that of all other treatment groups ($P < 0.05$). Main effects of treatment were not detected for variables PMI, AI, and COMP ($P > 0.05$).

A treatment-by-stallion interaction was detected for PMOT ($P < 0.05$). Mean PMOT was similar among treatment groups for two of three stallions ($P > 0.05$). For the remaining stallion, PMOT was higher in Group 20 than in Groups 4-20, 80, 4-80, 196, RW-196 and RW-196.

Table 3. Main effect of frozen-thawed seminal plasma that had previously been processed in various manners and stored for nine months at various freezing temperatures on measures of sperm quality (mean ± SEM) in three stallions following 24 h of cooled storage with allologous sperm (n = 21 ejaculates).

	Treatment‡								
Laboratory Parameter*	20	80	196	4-20	4-80	4-196	RW-20	RW-80	RW-196
TMOT (%)	81 ± 2^{ab}	82 ± 2^{ab}	82 ± 2^{ab}	82 ± 2^{ab}	83 ± 2^{a}	83 ± 2^{ab}	80 ± 2^{b}	82 ± 2^{ab}	80 ± 2^{ab}
PMOT (%)	58 ± 2.3^{a}	55 ± 2.1^{ab}	54 ± 1.9^{ab}	57 ± 1.8^{ab}	55 ± 1.9^{ab}	57 ± 2.3^{ab}	54 ± 2.1^{ab}	53 ± 2.4^{b}	53 ± 2.1^{b}
VCL (μm/s)	166 ± 7.4^{c}	177 ± 7.5^{a}	176 ± 7.8^{ab}	166 ± 7.6^{c}	178 ± 8.3^{a}	177 ± 7.7^{ab}	155 ± 7.4^{d}	168 ± 8.5^{bc}	164 ± 7.6^{c}
PMI (%)	85 ± 0.7^{a}	85 ± 0.8^{a}	85 ± 1.2^{a}	84 ± 1.4^{a}	86 ± 0.7^{a}	86 ± 0.8^{a}	86 ± 0.9^{a}	87 ± 0.7^{a}	86 ± 0.6^{a}
AI (%)	89 ± 0.9^{a}	89 ± 0.8^{a}	88 ± 1.1^{a}	89 ± 0.9^{a}	89 ± 0.8^{a}	89 ± 0.8^{a}	89 ± 0.9^{a}	89 ± 0.8^{a}	89 ± 0.8^{a}
COMP (%)	10 ± 0.8^{a}	9 ± 0.7^{a}	8 ± 0.5^{a}	9 ± 0.5^{a}	11 ± 0.7^{a}	10 ± 0.6^{a}	10 ± 0.6^{a}	10 ± 0.6^{a}	9 ± 0.5^{a}

*TMOT = total sperm motility (%); PMOT = progressive sperm motility (%); VCL = curvilinear velocity (μm/s); PMI = sperm with intact plasma membrane (%); AI = sperm with intact acrosomal membrane (%); COMP = percentage of sperm with αt value outside the main population (%). Percentage data (TMOT, PMOT, PMI, AI, and COMP) were arc sine-root transformed for normalization prior to statistical analysis. Untransformed values for mean and SEM are presented in table to ease interpretation but statistical tests were conducted on transformed data.

†20 = seminal plasma stored only at -20 °C; 80 = seminal plasma stored only at -80°C; 196 = seminal plasma stored only at -196°C; 4-20 = seminal plasma held at 4°C for 24 h and then stored at -20°C; 4-80 = seminal plasma held at 4°C for 24 h and then stored at -80°C; 4-196 = seminal plasma held at 4°C for 24h and then stored at -196°C; RW-20 = raw semen sample stored for 24 h at 4°C and then processed for seminal plasma which was then stored at -20°C; RW-80 = raw semen sample stored for 24 h at 4°C and then processed for seminal plasma which was then stored at -80°C; RW-196 = raw semen sample stored for 24 h at 4°C and then processed for seminal plasma which was then stored at -196°C.

[a-d] Within row, means with different superscripts differ ($P < 0.05$).

Discussion

This study evaluated the effect of seminal plasma processing and storage methods on resulting sperm quality following cooled storage. Our results indicate that seminal plasma can be processed and stored under different conditions without adversely affecting sperm quality when using clinically normal stallions.

There was no difference between fresh and snap-frozen/thawed seminal plasma on sperm quality, which suggests that seminal plasma can be processed for immediate use, or it can be stored frozen for later use. This permits the clinician more flexibility in the processing of seminal plasma for use with fresh, cooled, or cryopreserved semen. Data from a previous study suggested that freezing of seminal plasma prior to mixing with semen could negatively impact sperm motility.[22] The current findings are not supportive of this notion. The source of the frozen-thawed seminal plasma could have impacted

experimental outcomes in these two studies. We evaluated the effects of homologous versus allologous seminal plasma on sperm semen quality to determine if the origin of seminal plasma would affect sperm quality. Clinically, there are reports of stallions whose seminal plasma exerts a suppressive effect on sperm quality; yet when seminal plasma from another stallion is substituted, sperm quality improves when the semen is subjected to cooling[4] or cryopreservation.[2] In the present study, allologous seminal plasma was generally similar to homologous seminal plasma for maintaining sperm motion characteristics, membrane integrity and DNA integrity after 24 h of cooled storage; however, we did note a stallion-by-treatment interaction, whereby sperm from one stallion exhibited improved progressive motility following cooled storage when mixed with allologous seminal plasma, as compared to homologous seminal plasma. This finding supports the report of Varner et al. where incorporation of allologous seminal plasma improved the sperm velocity in a subfertile stallion.[4] It also supports the findings of Aurich et al. where the seminal plasma from stallions with good post-thaw sperm quality improved sperm quality of stallions with poor post-thaw sperm quality.[2] The fact that sperm from one stallion in our study did show a preference for allologous seminal plasma for PMOT suggests that it may be appropriate to test sperm from problematic stallions in the clinical setting to determine whether allologous seminal plasma may be more appropriate than homologous seminal plasma when processing semen for cooled storage. Our data suggest that it may be important to test various sources of allologous seminal plasma when performing this procedure, as no single source of allologous seminal plasma yielded consistently high values for sperm quality when added to the semen of the three sperm donors. Others have reported inconsistencies in use of homologous versus heterologous (implying allologous) seminal plasma among stallions, but that study involved overnight storage of extended semen containing homologous seminal plasma for transport to the laboratory prior to conducting the experiments.[23] This may have confounded experimental results, as components of homologous seminal plasma likely incorporated into sperm membranes during this time.[23] The term, heterologous, was inappropriately used by Morrell and coworkers, as the term would indicate that the seminal plasma was derived from a different species from the recipient.

We evaluated various processing methods and frozen-storage temperatures for seminal plasma in an effort to determine the technique(s) for accomplishing this task that might optimize resulting semen quality following cooled storage. The findings generally supported our hypothesis that sperm quality would not be affected by seminal plasma storage temperature (-20 °C, -80°C or -196°C). We also hypothesized that cooled storage of raw semen for 24 h prior to processing of seminal plasma would be detrimental. While this treatment condition was not dramatically different than the other methods used for processing seminal plasma, the data certainly suggest that some resulting sperm-motility values could be suppressed when this method is used. The seminal-plasma samples in this experiment were stored for nine months prior to use. Based on the findings of this study, we consider it feasible for a veterinary practice to store seminal plasma using a conventional freezer (-20°C), especially if liquid nitrogen, dry ice, or a -80°C-freezer is not readily accessible; however, we did not test freezers with automatic defrost (frost-free) systems. It is possible that defrost cycles of such freezers would be deleterious to some components of seminal plasma.

In summary, fresh and frozen seminal plasma yielded similar results for sperm quality, regardless of freezing temperature for storage. As such, we contend that seminal plasma can be banked for future use, eliminating the need for processing a fresh sample when seminal plasma is needed. Semen from certain stallions may benefit from using allologous seminal plasma, as compared to homologous seminal plasma. Differences detected among processing/freezing methods for seminal plasma were slight, suggesting that considerable flexibility may be permitted in techniques for storage.

Acknowledgment

Funding for this project was provided by the Legends Premier Stallion Season Auction, Texas A&M University.

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