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Use of a Silane-Coated Silica Particle Solution to Enhance Semen Quality of Stallions (21-Nov-2003)

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Abstract
Use of a proprietary silanated silica particle density gradient improved spermatozoal motility and morphology of stallions. Post-centrifugation resuspension in a milk-based extender improved spermatozoal motility more than pre-load dilution media. Sperm morphologic defects were separated differentially along the discontinuous gradient, yielding a higher percentage of morphologically normal sperm in the pellet.

1. Introduction
Discontinuous density gradients have been used to select populations of higher quality sperm for assisted reproductive techniques in humans [1]. Density gradients separate cells (sperm) with differing specific gravities into different layers after centrifugation. The most commonly used discontinuous density gradient contained silica particles coated with polyvinylpyrrolidone [a]. This product has been removed from commercial use. An alternative discontinuous gradient [b] that is used in humans, employs silane-coated silica particles in an isotonic solution for separation of sperm [1,2]. The objective of this study was to determine the optimal formulation and processing procedures necessary to use a similar product offered by the same company (labeled as EquiPure) [c] with equine sperm, based on in vitro sperm characteristics.

2. Materials and Methods
Six ejaculates were collected from five light-breed horses using a Missouri-model artificial vagina. Aliquots of gel-free semen were preserved in buffered formal saline or diluted to 25 x 10^6 sperm/ml in extender [d] to obtain initial (pre-treatment) values for sperm morphologic and motility endpoints.

Table 1. Treatment Applications for Discontinuous Density Centrifugation Gradients

Treatment	Diluent	Density Gradient (80:40)	Wash Media	Resuspension Media
1	Buffer A	Gradient A	Wash A	Wash A
2	Buffer A	Gradient A	Wash A	CST
3	CST	Gradient A	Wash A	Wash A
4	CST	Gradient A	Wash A	CST
5	Buffer B	Gradient B	Wash B	Wash B
6	Buffer B	Gradient B	Wash B	CST
7	CST	Gradient B	Wash B	Wash B
8	CST	Gradient B	Wash B	CST

Buffer A - Proprietary media (Stallion TALP, NidaCon International AB, Göteborg, Sweden) adjusted to pH 6.9.

CST - EZ - Mixin CSTT extender (Animal Reproduction Systems, Chino, California, USA).
Buffer B - Proprietary media (Stallion TALP, NidaCon International AB, Göteborg, Sweden) adjusted to pH 7.2.
Gradient A - Proprietary centrifugation gradient (EquiPure[tm] 100, NidaCon International AB, Göteborg, Sweden; pH 7.3) adjusted to 80 and 40% concentrations with Buffer A.
Gradient B - Proprietary centrifugation gradient (EquiPure[tm] 100, NidaCon International AB, Göteborg, Sweden; pH 7.6) adjusted to 80 and 40% concentrations with Buffer B.
Wash A - Buffer A containing bovine serum albumin (fraction V; 5 mg/ml).
Wash B - Buffer B containing bovine serum albumin (fraction V; 5 mg/ml).

Semen was also subjected to one of eight density-gradient treatments to evaluate the effects of dilution medium, density gradient pH, and resuspension media on experimental endpoints (Table 1). One- milliliter aliquots of diluted (1:1) semen were layered over a discontinuous gradient [80% layer (2 ml) and 40% layer (2 ml)] in 15-ml centrifugation tubes. Loaded tubes were centrifuged at 200 g for 30 min. The supernatant was removed by aspiration, and sperm at the semen-40% layer interface and 40% layer-80% layer interface were preserved in buffered formol saline for morphologic analysis. The aspirated sperm pellets were washed in 3-ml media at 400 g for 15 min, and the pellets were placed in resuspension media to obtain a final sperm concentration of approximately 25 x 10^6 sperm/ml. Aliquots of resuspended pellets were preserved in BFS for morphologic analysis. The percentages of morphologically normal sperm and specific morphologic defects were determined using phase contrast microscopy (1250x magnification) [e]. Spermatozoal motion characteristics were evaluated using a computerized spermatozoal-motility analyzer equipped with a heated stage [f]. Six motility variables were evaluated: percentage motile sperm (MOT); percentage progressively motile sperm (PMOT); mean curvilinear velocity (VCL; µm/s), mean average-path velocity (VAP; µm/s), mean straight-line velocity (VSL; µm/s), and straightness (STR; %). The difference between initial (control) values and processed values were analyzed by analysis of variance (ANOVA) procedures for paired data.

3. Results

Type of dilution media (Buffer A, Buffer B, CST) had no effect on sperm morphologic or motility endpoints ($P > 0.4$). Gradient media had no effect on motility endpoints ($P > 0.4$); however, Gradient A yielded a higher difference in percentage of morphologically normal sperm (mean, 24%) after treatment than did Gradient B (mean, 15%; $P = 0.01$). In addition, Gradient A yielded a lower difference in percentage of bent tails after treatment than did Gradient B ($P = 0.01$). Type of resuspension media (Wash A, Wash B, CST) had no effect ($P > 0.1$) on morphologic endpoints, but CST yielded a higher difference in PMOT, VCL, VAP, and VSL after treatment than did Wash A or Wash B ($P < 0.01$). Mean change in STR was similar ($P > 0.1$) between Wash A and CST and was higher ($P < 0.05$) in both of these media than in Wash B. Overall, Treatments 4 and 6 yielded a higher difference in VAP after treatment than did Treatments 1, 3, 5, and 7 ($P < 0.05$).
The mean percentage of morphologically normal sperm tended to be higher ($P = 0.06$) in the processed sperm pellets than in the raw (unprocessed) semen. The mean percentages of abnormally shaped midpieces and distal droplets were lower ($P < 0.05$) in processed sperm pellets than in raw semen, and the mean percentage of premature germ cells tended to be lower ($P = 0.1$) in the processed sperm pellets. Comparing Treatment 6 with unprocessed semen, the mean MOT and PMOT were higher ($P < 0.05$) in processed sperm pellets than in unprocessed semen, and the mean STR also tended to be higher ($P = 0.06$) in processed sperm pellets. No effect was detected in sperm velocity endpoints ($P > 0.1$).
Differences were detected in most morphologic endpoints for sperm recovered from the 40% gradient, 80% gradient, and sperm pellets. A higher percentage of morphologically normal sperm was recovered from the pellet than in the 40% or 80% gradients ($P < 0.01$). Higher percentages of detached heads ($P < 0.01$), proximal droplets ($P < 0.05$), and premature (round) germ cells ($P < 0.01$) were detected in the 40% gradient compared with the 80% gradient or processed sperm pellets. Higher percentages of abnormally shaped midpieces were detected in the 40% and 80% gradients compared with the sperm pellets ($P < 0.01$). Higher percentages of abnormally shaped heads, bent tails, and coiled tails were also detected in the 40% gradient compared with the sperm pellets ($P < 0.05$).

4. Discussion

This study demonstrated that use of a proprietary silanated silica particle density gradient [c] can improve semen quality in stallions, as determined by measures of both spermatozoal motility and spermatozoal morphology. Density gradient pH did not effect spermatozoal parameters. Post-centrifugation resuspension media had a more profound effect on spermatozoal motility values than did pre-load dilution media. Use of a milk-based media for resuspension of processed spermatozoa enhanced several motility endpoints compared with the proprietary media. Several types of sperm morphologic defects were separated differentially along the discontinuous gradient, yielding a higher percentage of morphologically normal sperm in the pellet. Premature germ cells, detached heads, and proximal droplets were trapped more frequently in the 40% gradient, whereas abnormally shaped heads, abnormally shaped midpieces, bent tails, and coiled tails were trapped at similar frequencies in the 40% and 80% gradients. This centrifugation protocol may be useful in preparing sperm of subfertile stallions for low-dose insemination programs. Discontinuous centrifugation gradients may also be useful for separating sperm to study the effects of various morphologic defects on fertility in vivo and in vitro.

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Footnotes

[a] Percoll, Sigma Aldrich, St. Louis, MO.
[b] Pure Sperm, T NidaCon International AB, Göteborg, Sweden.
[c] Proprietary centrifugation gradients, EquiPure™ 100; NidaCon International AB, Göteborg, Sweden.
[d] EZ-Mixin CSTT extender, Animal Reproduction Systems, Chino, CA 91710.
[e] Olympus BX60, Olympus America, Inc., Melville, NY 11747.
[f] HTM IVOS; Version 10.8; Hamilton Thorne Research, Beverly, MA 01915.

References

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