

Improvement of sperm recovery rates after centrifugation of stallion semen

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Maximum sperm recovery with minimal sperm damage is one of the goals when centrifuging stallion semen. While gravitational force and time are parameters of concern, the height of the fluid column may be an important aspect that is commonly overlooked. Reducing the volume (column height) contained in a tube should increase sedimentation, thereby minimizing sperm loss in the supernatant. Our objectives were to compare sperm yield and viability after centrifugation and after 24 hours of storage at 4°C. We hypothesized that an increase in gravitational force would increase sperm recovery rate without inducing further immediate or long-term damage to the cells and a reduction in the height of the column would increase recovery rate.

Ejaculates were collected from 8 stallions. Each ejaculate was extended to 25 X 10⁶ cells/mL in a skim-milk extender and divided into 9 aliquots. Aliquots were centrifuged in 50-mL conical tubes under the conditions in Table 1. After centrifugation, sperm concentration in the supernatant was determined and sperm recovery rate was calculated. Each aliquot was resuspended to 25 X 10⁶ cells/mL and stored at 4°C for 24 h. Motility patterns were analyzed with a computer assisted semen analyzer (Minitube of America, Inc. Verona, WI) and sperm viability was evaluated with SYBR14/Propidium Iodide (Garner et al., *Biol. Reprod.* 1995; 53: 276-284). These parameters were assessed before and after centrifugation, and after 24 h of cooled storage. Means among treatments were compared using analysis of variance and paired comparisons were made between treatments using Tukey's test.

Table 1: Centrifugation conditions for the different treatments

Treatment	1	2	3	4	5	6	7	8	9
Force (x g)	0	400	400	400	400	900	900	900	900
Time (min)	0	10	10	5	5	10	10	5	5
Volume (mL)	40	40	20	40	20	40	20	40	20

There were no differences among treatments in total motility, progressive motility and percentage of live cells immediately after centrifugation and after 24 h of storage ($p>0.05$). Sperm recovery rate was higher for treatments 1 (100%), 3 (95.3 ± 5.6), 6 (91 ± 4.6), 7 (99.1 ± 0.8) and 9 (93.5 ± 10) compared to treatments 2 (74.3 ± 9.2), 5 (72 ± 11.3) and 8 (72.4 ± 11) (mean $\pm$ SD, $p<0.05$). Treatment 4 provided the lowest recovery rate (44.1 ± 8) ($p<0.05$).

The results supported our hypotheses. When using 400 x g for 10 min or 900 x g for 5 min, decreasing the height of the column of fluid increased the recovery rate. When dealing with a large volume of semen, recovery rate can be improved by centrifuging at 900 x g for 10 minutes without inducing immediate or long-term damage to spermatozoa.

Key words: equine, semen, centrifugation, cooled storage