Stallion sperm recovery rate after centrifugation and removal of the supernatant using different methods

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A reduction in sperm loss during centrifugation would yield an increase in viable sperm for cryopreservation or insemination. It was hypothesized that the method used to remove the supernatant after centrifugation would have an effect on sperm recovery rate. Some methods may reduce processing time and, therefore, sperm loss in the supernatant due to swim up of viable sperm from the pellet. Two experiments were conducted to test this hypothesis. In each experiment, two ejaculates were collected from each of three stallions, totaling six ejaculates per experiment. Semen was extended to 25×10^6 sperm/mL with a milk based semen extender, divided into three 40 mL aliquots, and centrifuged for 10 min at 900 × g in 50 mL conical centrifugation tubes. Then, 37 mL of supernatant was removed using one of three methods. In Exp. 1, the supernatant was removed with a 1.5 mL glass Pasteur pipette attached to a latex bulb (GPP), a 3 mL plastic transfer pipette (PTP), or vacuum suction (VS). Since the GPP yielded the best recovery rate in the first experiment, this method was subsequently compared with others in the second experiment. In Exp. 2, the supernatant was removed with the GPP, a 10 mL pipette attached to a manual pipettor (10P), or a combination of the 10P up to the 10 mL mark of the centrifugation tube and GPP thereafter (10PGPP). Concentration was determined with a hemacytometer in both the pre-centrifuged samples and the removed supernatants, and the sperm recovery rate was calculated. The means were compared among groups using ANOVA for repeated measurements. In Exp. 1, mean sperm recovery rate was higher in the GPP (83.58 ± 10.61%) than in PTP (66.61 ± 11.68%) or VS (61.62 ± 15.79%) groups (P < 0.05) (Mean ± SD). In Exp. 2, there were no differences in sperm recovery rates among groups GPP (92.15 ± 9.78%), 10P (91.81 ± 11.20%), and 10PGPP (75.62 ± 25.04%) (P > 0.05). Thus, the method used to remove the supernatant had an effect on sperm recovery rate, and the 1.5 mL glass Pasteur pipette yielded one of the highest rates, even with a longer processing time and the need of repeated pipetting. Therefore, the difference in sperm recovery rate may be due to changes in negative pressure or rate of fluid flow with the various methods rather than by processing time and potential for swim up. In conclusion, the glass Pasteur pipette, the 10 mL pipette, and the combination of both provided the highest sperm recovery rates after centrifugation and removal of the supernatant. The high recovery rate associated with the practicality in removing the supernatant with a 10 mL pipette makes this method recommended.

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