Single-layer colloid centrifugation as a method to process urine contaminated stallion semen after cryopreservation

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Urospermia is an important ejaculatory dysfunction that can be challenging to manage in some stallions. Currently, cryopreservation of urine contaminated semen is not recommended due to poor outcomes. However, some stallions continuously ejaculate urospermic samples and thus alternative cryopreservation techniques to overcome urine contamination are needed. We hypothesized that semen with low amounts of urine contamination can be cryopreserved without detrimental effects on semen parameters and that single-layer gradient centrifugation pre- and post-cryopreservation can be used enhance post-thaw semen parameters. The objective of this study is to evaluate the effect of single layer density gradient centrifugation on the selection of sperm with optimal motility parameters, viability, and morphology in post-thaw samples from ejaculates contaminated with low or high concentrations of urine. Forty-nine ejaculates from eight mature, healthy stallions were split into three portions one with no urine, and two portions with a low (20%) or high (50%) amount of urine added to the ejaculate. Prefreeze evaluation of semen included assessments with an automated sperm analyzer (motility parameters), flow cytometry (viability, acrosome integrity, mitochondrial potential, and chromatin stability) and morphology. Semen was extended with a commercial cooling extender (Equi-Pro Cool Guard Timentin, Minitube of America, Vernon, WI), cushion centrifuged (1000xg, 20 min) and resuspended to 200 million/mL in a commercial semen freezing extender (Botucrio, Botupharma Scottsdale, AZ). Resuspended semen was loaded in 0.5 mL straws and cryopreserved in liquid nitrogen. After six months, samples were thawed at 37°C for 30 s and processed through a single-layer density gradient centrifugation (1700 rpm, 30 min) (Equipure, Nidacon, Sweden). Total motility (TM) and progressive motility (PM) were assessed with Computer-Assisted Sperm Analysis. Sperm viability and yield were assessed with a Nucleocounter SP100 before and after centrifugation. Data were analyzed with ANOVA (one-way or repeated measures) and post-hoc analyses with Tukey's. Significance was set at $p<0.05$. As expected, sperm motility parameters were reduced with increasing urine contamination ($p<0.05$) (Table). It is worth noting that low amounts of urine contamination appears to only marginally reduce sperm motility and sperm viability of stallion cryopreserved sperm ($p<0.05$).

Table. Post-thaw sperm parameters before and after single-layer gradient centrifugation.

	Pre-gradient			Post-gradient		
	TM	PM	Viability%	TM	PM	Viability%
Control	35 ± 2^{aA}	24 ± 1.8^{aA}	45 ± 0.7^{aA}	51 ± 3.6^{aB}	40.3 ± 3.2^{aB}	54 ± 0.5^{aA}
Low	33 ± 0.7^{aA}	21 ± 1.14^{aA}	27 ± 0.2^{bA}	42 ± 2.2^{aB}	31 ± 3.9^{aB}	49 ± 0.7^{aB}
High	22 ± 1.8^{bA}	12 ± 1.5^{bA}	27 ± 0.3^{bA}	25 ± 2.8^{bA}	14 ± 2^{bA}	38 ± 0.6^{bB}

Different superscripts within rows $^{(abc)}$ and columns $^{(ABC)}$ denote differences ($p<0.05$).
Recovery rates (Control 32 ± 3.6, Low 28 ± 3, and High 27 ± 2.7) were not significantly different between groups.

In conclusion, urine contamination affects sperm motility parameters in a dose-dependent manner. Post-thaw gradient centrifugation improved motility and viability in Control and Low groups but only improved viability in the High group.

Keywords: Horse, urospermia, centrifugation, semen freezing ability