Cryosurvival of cooled transported stallion semen frozen in three commercial extenders

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Semen freezing is a common practice in equine reproduction, but requires special equipment and skills. Shipping cooled semen to a referral facility can enable more stallions to have semen frozen. Egg yolk (e.g. Botucrio, Lactose EDTA) and milk based (e.g. modified French) extenders are commonly used, but there is apparently no information comparing diluents for transported stallion semen. Objectives were to determine which extender is more suitable to freeze transported stallion semen and to determine if cryosurvival can be predicted. It was hypothesized that formamide containing egg yolk based extenders are more suitable for freezing transported stallion semen than milk based extender and that thawed semen quality is predictable based on prefreezing parameters. Semen was collected from stallions ($n = 20$) and extended in INRA 96 to 40 x 10^6 sperm/ml. Semen was shipped overnight to the laboratory in a passive cooling device (Equitainer). After arrival, aliquots were warmed and CASA used to assess total (TM) and progressive (PM) sperm motility and amplitude of lateral head displacement (AHL). Percentage of sperm with intact plasma and acrosomal membranes (IMIA; FITC PNA/PI) and percentage of apoptotic and necrotic sperm (Annexin V/PI) were evaluated with flow cytometry. Each ejaculate was divided into 3 aliquots, centrifuged, resuspended in Botucrio (BC; Botupharma, Scottsdale, AZ), EZ Mixin Cryomax Modified French (MFR; ARS, Chino, CA) or EZ Mixin Cryomax Lactose-EDTA (LE; ARS) and processed following manufacturer's instructions. Sperm parameters were reassessed after thawing and compared with ANOVA. Thawed samples with $PM \geq 30\%$ were considered acceptable. There was no difference in distribution of acceptable samples (BC = 55%, MFR = 35%, LE = 60%; Chi square). There was a reduction in TM, PM and early apoptosis ($p < 0.0001$), regardless of extender used (Table). Percentage of IMIA was higher ($p = 0.0008$) and late apoptotic sperm lower ($p < 0.0001$) prefreezing than after freezing in MFR or LE, but not BC. Percentage of necrotic sperm did not differ. A shipping test designed to predict poor thawed semen motility was considered positive if $TM < 70.79\%$ and $ALH < 4.19$ (below the 90% confidence interval for stallions with good freezing ability) prior to freezing. Sensitivity, specificity, positive and negative predictive values of the test were 62, 100, 100, and 80%, respectively. Whereas thawed semen motility of cooled transported sperm did not differ among extenders, BC had a better protective action on plasma and acrosomal membranes and prevented late apoptosis. Freezing ability of transported semen was predictable based on selected prefreezing parameters.

Table. Sperm parameters (mean ± SEM) before and after freezing.

Treatment	TMOT	PMOT	IMIA	Early apoptotic	Late apoptotic
Prefreeze	72.7 ± 2.7^{a}	65.6 ± 2.8^{a}	67.6 ± 5.9^{a}	7.7 ± 1.1^{a}	2.9 ± 0.5^{a}
BC	44.4 ± 3.1^{b}	35.1 ± 2.6^{b}	$52.1 \pm 4.9^{a,b}$	38.0 ± 4.5^{b}	$14.4 \pm 3.2^{a,b}$
MFR	37.8 ± 2.4^{b}	27.9 ± 2.1^{b}	40.4 ± 5.3^{b}	45.2 ± 3^{b}	16.2 ± 2.6^{b}
LE	43.0 ± 2.5^{b}	32.5 ± 2.4^{b}	48.6 ± 4.9^{b}	41.5 ± 4.3^{b}	15.9 ± 2.5^{b}

[a-c] Within a column, means without a common superscript differed ($p < 0.05$).

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