Length of Cyropreservation Has No Effect on Fertilizing Ability of Boar Spermatozoa

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Preserving genetic diversity in swine is important for the maintenance of healthy production populations, as well as the conservation of rare breeds that have genetic traits of merit. One method by which to accomplish this goal is the cryopreservation and long term banking of semen. However, semen cryopreservation in swine is not as efficient as that in other domestic species. The effect of long term storage of cryopreserved boar semen on the fertilizing ability of the sperm after thawing is relatively unknown, and may potentially decrease motility and forward progression. The objective of this research was to evaluate boar semen after varying cyropreservation lengths for its ability to successfully penetrate oocytes and support early embryonic development in vitro.

Oocytes derived from abattoir ovaries were matured in TCM199-based defined maturation medium for 40-42 h. Before fertilization, oocytes were denuded by vortexing with hyaluronidase and placed into mTBM fertilization medium. Straws were thawed at 50°C for 45 seconds and 2.5 mL of semen was layered onto a 45%/90% Percoll (Sigma, St. Louis, MO) gradient and centrifuged for 20 min at 1000 x g. The remaining semen was used for post-thaw analyses. After Percoll separation, the sperm pellet was washed with 4 ml D-PBS (Gibco, Grand Island, NY) with 1 mg/ml BSA (Sigma). For each boar (n=47), the percentage of motile sperm (0 to 100%), the rate of forward progression (0, no motility to 5, rapid forward movement) and initial concentration were evaluated post-thaw. Oocytes were coincubated with sperm (5.0×10^5/ml) for 5 h. A sample of oocytes was randomly selected 12 h post-insemination for assessment of pronuclear formation. The remaining oocytes were cultured in NCSU23 for 144 h, when blastocyst development was recorded. Each boar was examined in 2 replicates. The following parameters were analyzed: % motility, progression score, % cleavage (CLVG), % morula and blastocyst (MOR/BLST), % blastocyst (BLST), % normal penetration (2PN), % polyspermic penetration (PPN) and % total penetration (PN). Data collected for each boar were averaged. Results were analyzed using simple linear regression to determine correlation of measured parameters with length of cyropreservation. Length of storage time of cyropreserved samples varied from 10 to 231 months.

Of the 47 boars, 83 % had greater than 20 % motility post-thaw. Only one boar did not result in oocyte penetration after IVF, and 87 % of the boars penetrated greater than 15 % of inseminated oocytes. In vitro fertilization with 91 % of boars resulted in embryonic cleavage above 50 %, and 77 % of boars resulted in blastocyst development greater than 10 %. None of the parameters examined were significantly correlated with the length of cyropreservation (Table 1). This study demonstrates that boar semen can be successfully cryopreserved and stored for at least 20 years without affecting the ability of the sperm to be used successfully in an in vitro embryo production system. Thus, successful germplasm preservation can be achieved by cryopreservation of boar semen.

Table 1. Correlation between length of cyropreservation of boar semen and fertilization and developmental parameters in vitro

	%Motility	Progression Score	%CLVG	% MOR/BLST	% BLST	% 2PN	% PPN	% PN
Means	30.55±1.48	2.65±0.08	64.62±1.64	22.40±1.61	16.30±1.16	31±1.80	16.68±1.74	46±3.13
Correlation Coefficient [a]	0.03	-0.19	0.26	0.15	0.10	-0.15	-0.38	-0.27
R^2 [b]	0.001	0.04	0.07	0.02	0.01	0.02	0.14	0.07

[a]Pearson's correlation coefficient. Values are between -1 and 1; values near zero indicate no linear relationship. [b]Coefficient of determination. Values are between 0 and 1; values near zero indicate no linear relationship.