Reduced glutathione and field fertility with frozen-thawed boar semen

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The use of frozen-thawed boar sperm in artificial insemination is marginal (only 2% of swine inseminations). This is explained with different reasons, including the lower fertility rates and litter sizes when compared to fresh/extended semen.

In fact, sperm cryopreservation in pigs, as in other mammalian species, is known to damage sperm plasma membrane and nucleus and reduces sperm survival.

However, supplementing cryopreservation extenders with 2mM glutathione (GSH) is known to increase the resistance of nucleoprotein structure against freeze-thawing as well as sperm motility and survival at post-thawing. In a very recent study, we demonstrated that adding 2mM GSH to LEY and LEYGO extenders significantly improves fertility and prolificacy of boar frozen-thawed semen. Using that as a guide, we have cryopreserved boar semen in the presence (0 mM) or absence of 2mM GSH, prior to performing field fertility trials.

Materials and methods

Overall, 20 good freezability ejaculates coming from 10 different boars from the Pietrain breed were obtained from Swine Genetic

Services (Gepork SA). Ten ejaculates were used as farm controls for artificial insemination, whereas the other 10 were cryopreserved following the Westendorf method (1975), as modified by Casas et al. (2009).

All the ejaculates included in this study presented acceptable sperm quality (total sperm motility >80%, progressive sperm motility >60%; morphologically normal spermatozoa >85%; sperm viability >85%).

Fertility trials were performed with a total of 150 multiparous sows in a local breeding farm – Servicios Genéticos Porcinos SA, Sant Sadurní d'Ososmort, Barcelona, Spain.

Sows were randomly divided into three groups of 10 animals each, and each group was inseminated with extended, frozen-thawed control or frozen-thawed semen supplemented with 2mM GSH.

The experiment was repeated every month and up to five times, according to an insemination programming system of all-in/all-out production followed by the breeding farm.

Post-cervical insemination was used in all of the inseminations. Double insemination was performed in all cases, with an interval of 12 hours between both inseminations, thereby covering the insemination-to-ovulation interval recommended for extended and cryopreserved doses. Farrowing rate and litter sizes were recorded at parturition.

Statistical analyses consisted of a general linear mixed model with treatment as fixed-effects and boar as random-effects factors.

A post hoc Bonferroni's test was also run for multiple comparisons. In addition, farrowing rates were previously logit-transformed (logistic transformation) and data were checked for normality and homogeneity of variances.

The minimal level of significance was set at $P<0.05$ in all statistical analyses.

Results

As Table 1 shows (mean ± standard error of the mean, SEM), inseminations with frozen-thawed yielded significantly ($P<0.05$) lower farrowing rates.

In contrast, farrowing rates when using frozen-thawed sperm supplemented with 2mM GSH did not differ from those observed with extended semen.

In terms of litter sizes, total and alive born piglets were significantly higher when using extended semen and frozen-thawed semen supplemented with 2mM GSH than when using frozen-thawed unsupplemented sperm. The number of stillborn piglets did not differ between the three treatments (data not shown).

Table 1. Fertility results of sows inseminated with extended, frozen-thawed or frozen-thawed semen supplemented with 2mM GSH. Different numbers (^{1} ^{2}) mean significant differences (P<0.05) between treatments.

	Extended	Frozen-thawed	Frozen-Thawed + 2mM GSH
Farrowing rate (%)	92.8 ± 5.0^{1}	70.5 ± 3.9^{2}	90.4 ± 5.3^{1}
Total piglets born (n)	14.0 ± 1.0^{1}	8.1 ± 1.2^{2}	13.4 ± 1.2^{1}
Alive born piglets (n)	13.1 ± 0.9^{1}	7.3 ± 1.0^{2}	12.6 ± 1.1^{1}

Conclusions

This work, together with other previous works, confirms that supplementation of frozen-thawed sperm with 2mM GSH improves the fertilising ability of cryopreserved spermatozoa. This may thus help using this type of semen in some particular cases. ■

References are available from the authors on request