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Seminal plasma damages sperm during cryopreservation, but its presence during thawing improves semen quality and conception rates in boars with poor post-thaw semen quality

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Abstract

To determine the effects of seminal plasma during and after cyopreservation on post-thaw sperm functions in semen from poor freezability boars, seminal plasma was removed immediately after collection, and sperm was subjected to cooling and freezing. Removal of seminal plasma did not significantly affect post-thaw sperm motility in good freezability boars; however, in boars with poor freezability, it increased post-thaw motility relative to control sperm cooled with seminal plasma ($64.5 \pm 3.4\%$ vs. $30.9 \pm 3.1\%$, $P < 0.01$). Freezing sperm without seminal plasma increased both loss of the acrosome cap ($37.5 \pm 1.6\%$ vs. $18.4 \pm 2.8\%$, $P < 0.01$) and expression of a 15 kDa tyrosine-phosphorylated protein (capacitation marker) in thawed sperm relative to controls; the addition of 10% (v/v) seminal plasma to the thawing solution significantly suppressed both changes and increased conception rate to AI (70% vs. 9% in the control group, $P < 0.05$). In conclusion, our novel cryopreservation and thawing method increased the success of AI with frozen-thawed porcine semen, particularly from boars with poor post-thaw semen quality.

Keywords: Cryopreservation; Seminal plasma; Artificial insemination; Pig; Spermatozoa

1. Introduction

Cryopreservation of boar spermatozoa offers an effective means of long-term storage of important genetic material. Recently, we and other groups reported a high conception rate (70–80%) by AI using boar spermatozoa cryopreserved using a modification of previously described cryopreservation methods [1] and through the development of a novel sperm infusion method [2]. However, it is well known that the quality of frozen-thawed spermatozoa and the conception success rate are also dependent on unique characteristics of individual boars [3–6]. Thurston et al. [7] categorized sperm head morphology by Fourier descriptors, and detected a significant association between the head morphology of fresh spermatozoa and the motility of sperm after freezing and thawing. Additionally, they identified molecular markers linked to genes controlling semen freezability by amplified restriction fragment length polymorphism technology [8], suggesting that the freezability of boar sperm was affected by genetic factors.

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In a study in which semen was collected as two portions, the sperm-rich fraction and the post sperm rich fraction, in several boars there were significant differences between fractions in post-thaw sperm motility [9]. In addition, the freezability of epididymal sperm is higher than that of ejaculated sperm in boars [10]. Although the poor freezability of boar sperm is potentially affected by genetic background, we speculated that the effects of seminal plasma on sperm after ejaculation may be another factor.

Removing seminal plasma immediately after semen collection is one of the available techniques to improve motility of frozen-thawed sperm and *in vitro* fertility competence in miniature pigs [11]. However, frozen-thawed epididymal porcine sperm does not have high *in vivo* fertilization competence [Okazaki and Shimada, unpublished data, 12]. Additionally, it has been reported that seminal plasma is required to protect sperm against a spontaneous capacitation-like reaction during the thawing process [13]. Thus, we hypothesized that the presence of seminal plasma during freezing is not a critical determinant of post-thaw sperm quality in boars with good freezability, but has deleterious effects in those with poor freezability, and that seminal plasma is essential to maintain fertility competence during thawing. Consequently, we designed the following novel freezing and thawing method to preserve sperm from boars exhibiting poor sperm freezability. First, seminal plasma was removed from the sperm by centrifugation immediately after semen collection. Second, the sperm was frozen using a conventional method [1], and the cryopreserved sperm was thawed in a medium containing seminal plasma collected from a boar with proven high reproductive performance. The thawed sperm was then used for AI. To evaluate this freezing and thawing method, we analyzed frozen-thawed sperm motility, capacitation and acrosomal status *in vitro* and fertilization competence *in vitro* and *in vivo*.

2. Materials and methods

2.1. Media and seminal plasma

Routine chemicals and reagents were obtained from Nakarai Chemical Co. (Osaka, Japan) and Sigma Chemical Co. (Sigma; St. Louis, MO, USA).

The pre-treatment solution contained 0.33 M D-glucose, 12.8 mM trisodium citrate dihydrate, 14.3 mM sodium hydrogen carbonate, 9.9 mM EDTA-2Na, 1000 U/mL penicillin G potassium, and 1 mg/mL streptomycin sulfate.

Niwa and Sasaki freezing extender was used in this study, with some modifications (mNSF1, the osmolality was changed from 300 mOsm/kg to 400 mOsm/kg [1]). Orvus Es Paste (Miyazaki Chemical Sales, Ltd., Tokyo, Japan) at a concentration of 1.5% (v/v) and glycerol at a concentration of 4% (v/v) were added to mNSF1 (final glycerol concentration 2% (v/v)), which were then used for the second dilution (mNSF2) at 5 °C before freezing.

Modena solution [14] was used as the thawing solution. The sperm incubation medium for the investigation of spontaneous capacitation and acrosomal status was Modena solution without EDTA-2Na (pH 7.0).

To obtain seminal plasma for Experiment 3 (described below), spermatozoa recovered from high-fertility boars (>80% conception rate with fresh semen) were removed from the semen within 15 min after collection by centrifugation at 700 × *g* for 10 min. The supernatant was removed from the pelleted spermatozoa, centrifuged again at 1500 × *g* for 30 min, and used as the seminal plasma added to the thawing solution.

2.2. Semen collection and freezing and thawing procedure

Twenty mature Landrace and Large White boars, aged 15–28 mo, were used in this study. The sperm-rich fraction was collected weekly from each boar using the gloved-hand technique. The sperm-rich fraction was filtered thorough double gauze. The semen was directly diluted in pre-treatment solution (1:1, pre-treatment solution: semen) and held for 2 h at 15 °C. The semen was then centrifuged for 5 min at 800 × *g* to remove the pre-treatment solution. The precipitated spermatozoa were gently resuspended with mNSF1, and cooled slowly from 15 to 5 °C over 1.5 h. Then the sperm suspension was diluted in the same volume of mNSF2 (1×10^9 sperm/mL) and transferred into a 0.5 mL plastic straw. The straws were placed in liquid nitrogen vapor for 10 min, and finally stored in liquid nitrogen.

For thawing, the straw was transferred to water at 60 °C and kept there for 8 s, diluted in 4.5 mL of Modena solution, and then incubated at 37 °C for up to 6 h. The final concentration of the sperm was 1×10^8 spermatozoa/mL.

2.3. Evaluation of sperm motility, capacitation and acrosomal status

After 1-, 3- and 6-h incubations of frozen-thawed spermatozoa in Modena solution at 37 °C, sperm