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Short communication

Semen quality and reproductive performance after artificial insemination with boar sperm stored in a gelatin-supplemented extender

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ABSTRACT

This study tested the effects of inclusion of gelatin in an extender for cooled boar semen on sperm quality during storage and reproductive performance after artificial insemination (AI). In Experiment 1, ejaculates from four boars were stored in Beltsville Thawing Solution (BTS) and BTS including 1.5% and 3.0% gelatin. Sperm motility and morphology were similar among extenders during 72 h of storage ($P>0.05$). In Experiment 2, ejaculates from four boars were stored in BTS and BTS including 1.5% gelatin. Motility, normal morphology and membrane integrity declined over storage time for both extenders ($P<0.05$). Decrease on normal sperm morphology over time was less severe in semen stored in gelatin-supplemented BTS ($P<0.05$). In Experiment 3, AI was conducted in sows from a commercial farm using semen stored in BTS with or without 1.5% gelatin. Farrowing rate and total litter size did not differ ($P<0.05$), but AI with gelatin-supplemented BTS was shorter and presented less semen backflow ($P<0.05$). Semen stored in BTS including gelatin presented acceptable quality for longer periods and was efficiently used in AI with no losses in reproductive performance.

1. Introduction

The Beltsville Thawing Solution (BTS) (Pursel and Johnson, 1975) is the most commonly used extender for boar semen in artificial insemination (AI) programs in swine, keeping sperm viability for 1–3 d at 15 to 18 °C. After some time of storage at such temperatures, the sperm agglutinate, which influences negatively their viability (Rodríguez-Gil and Rigau, 1995). At farm level, the sperm is commonly re-suspended by slight agitation, after removing the AI doses from their thermal storage boxes, which exposes them to light and to temperature variation. Thus, extenders that prevent boar sperm agglutination are of interest for the swine industry. An alternative for such purpose is the use of sperm encapsulated within a semi permeable membrane that allows exchange of nutrients and metabolites among the spermatozoa and the medium (Vigo et al., 2009). So, the sperm remains viable within the membrane at normal body temperature for days, being gradually released inside the female reproductive tract.

The addition of gelatin to extenders for storage of rabbit semen was related to improve the sperm quality and prolonged the sperm viability (Nagy et al., 2002; López-Gatius et al., 2005). That was attributed to the viscosity of the gelatin-supplemented extender, which kept the sperm cells nearly paralyzed and evenly distributed in the medium. Nevertheless, the data about gelatin-supplemented extenders for boar semen are unknown. The objective of this study was to examine the effects of gelatin inclusion at distinct concentrations in an extender for cooled boar semen by evaluating the sperm quality in vitro during distinct storage periods and sow reproductive performance after AI.

2. Materials and methods

In experiment 1 (EXP1), semen donors were four sexually mature crossbred boars (Landrace × Large White) that were

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housed in individual pens in an experimental station, managed under similar conditions and presented acceptable semen quality according to conventional evaluations (motility, morphology and concentration). During three weeks, four ejaculates were collected per boar through the gloved hand method, in plastic bags with a disposable filter within a plastic bottle, and were extended in: BTS (Bretanha Suínos, Passo Fundo, RS, Brazil); BTS including 1.5% uncolored and unflavored commercially available gelatin (1.0% energy and 3.0% protein); and BTS including 3.0% gelatin. Sperm concentration was adjusted to 3.5×10^9 spermatozoa in 100 ml, using a Neubauer chamber. Sperm motility and morphology were evaluated by phase contrast microscopy. The reported values are the average of three evaluations per sample, at 24, 48, and 72 h of storage, always done by the same trained technician.

In experiment 2 (EXP2), six ejaculates were collected from four sexually mature crossbred boars (Landrace × Large White) from a commercial farm, during 45 d, as described for EXP1. The boars were housed in individual pens under similar management and had acceptable semen quality according to conventional evaluations. The ejaculates were extended in: BTS; and BTS including 1.5% gelatin. Sperm motility, morphology and concentration were evaluated as described for EXP1. Sperm motility was evaluated immediately after collection, but morphology and membrane integrity were evaluated after incubation of diluted samples at 24 °C for 1 h, during which they were transported to the laboratory. Membrane integrity was evaluated with an epifluorescent microscope (Olympus BX51), for samples having 5×10^4 spermatozoa in 0.05 ml and incubated at 37 °C for 10 min. In each slide, 200 cells were counted and classified as intact (green fluorescence) or not intact (red fluorescence) (Harrison and Vickers, 1990). Evaluations were also done at 24, 48, 72 and 96 h of storage.

In experiment 3 (EXP3), five ejaculates were collected from the same four boars from EXP2. Ejaculates from two boars were randomly pooled and extended in BTS and BTS including 1.5% gelatin. Sperm concentration was adjusted to 3.5×10^9 spermatozoa in 100 ml with a Karras spermdensimeter (Minitube of America, Verona, WI, USA). After incubation at 24 °C for 2 h, the semen samples were stored and used for AI of 26 crossbred sows per extender, with parities 1 to 8. Three AIs were done at 12 h intervals, using GD'Coll catheters (Bretanha Suínos, Passo Fundo, RS, Brazil), with the first AI done 12 h after estrus detection, which was characterized by the first positive response to back pressure in the presence of a boar. Semen backflow was collected into disposable human colostomy bags (KDL, Guarulhos, SP, Brazil), which were fixed around the vulva for up to 60 min after AI (Mezalira et al., 2005). The volume of the semen backflow was measured in a beaker and the AI duration was recorded. The farrowing rate was calculated and total litter size was recorded.

In both EXP1 and EXP2, the responses were analyzed by ANOVA with repeated measures considering: extenders; storage period; extenders per period interaction and the boar effect nested within extenders. Comparisons of means were done with the test of Tukey. In EXP1, the responses were submitted to arcsine transformation due to lack of normality, but in EXP2 all responses followed normal distributions, according to the Shapiro–Wilk test. In EXP2, a variation in responses over storage periods per extender was analyzed by linear regression. In EXP3, AI duration and volume of semen backflow were compared per extender by Kruskal–Wallis ANOVA. Farrowing rates were compared between extenders by chi-square tests. Total litter size was compared per extender by ANOVA, with comparisons of means done with the test of Tukey. In the three experiments, statistical analyses were done with Statistix® (2008).

3. Results

In EXP1, sperm motility and normal morphology did not differ ($P>0.05$) across storage periods for either extender (Table 1). In EXP2, motility did not differ ($P>0.05$) for BTS ($54.1 \pm 3.8\%$) and BTS including 1.5% gelatin (57.6 ± 3.8), but decreased for both extenders ($P<0.05$), as storage time increased (Fig. 1). Normal morphology and membrane integrity were both lower ($P<0.05$) for BTS ($86.3 \pm 0.9\%$ and $31.3 \pm 3.4\%$, respectively) than for BTS including 1.5% gelatin ($90.6 \pm 0.9\%$ and $47.5 \pm 3.4\%$, respectively). The decline in normal sperm morphology over time was more intense ($P<0.05$) for samples stored in BTS than for those including 1.5% gelatin (Fig. 1). Sperm membrane integrity declined with longer storage for both extenders ($P<0.05$).

In EXP3, AIs were longer ($P<0.05$) with samples stored in BTS (7.8 ± 0.4 min) than with gelatin-supplemented BTS (6.8 ± 0.4 min). Semen backflow was greater ($P<0.05$) for BTS samples (18.9 ± 1.3 ml) than for gelatin-supplemented BTS samples (10.1 ± 1.3 ml). After AI with semen stored in BTS or in BTS including 1.5% gelatin, no differences were observed ($P>0.05$) in farrowing rates (92.6% and 88.5%, respectively) and total litter size (12.0 ± 0.6 and 13.2 ± 0.7, respectively).

4. Discussion

This is the first study to test gelatin-supplemented extenders for boar sperm, both in vitro and in vivo. Although sperm motility and normal morphology did not differ for BTS including either 1.5% or 3.0% gelatin in EXP1, the lower gelatin concentration was used in EXP2 and EXP3 because tests conducted in our laboratory indicated that the greater viscosity of BTS including 3.0% gelatin hampered the flow of AI doses through the inseminating catheter. In EXP2, sperm motility declined from 85% to nearly 30% during storage for both extenders, but such decrease was slightly sharper for BTS samples, especially after 48 h, as indicated by the R^2 value

Table 1
Motility and normal morphology per storage period for semen stored in BTS with or without inclusion of gelatin in distinct concentrations (four ejaculates × four boars).

Extender	24 h	48 h	72 h
Sperm motility (%)			
BTS	70.0 ± 2.4	64.2 ± 2.4	61.4 ± 2.4
BTS + 1.5% gelatin	72.8 ± 2.4	69.2 ± 2.4	62.8 ± 2.4
BTS + 3.0% gelatin	71.4 ± 2.4	67.8 ± 2.4	64.2 ± 2.4
Normal sperm morphology (%)			
BTS	91.7 ± 0.7	90.3 ± 0.7	89.1 ± 0.7
BTS + 1.5% gelatin	89.4 ± 0.7	88.9 ± 0.7	87.7 ± 0.7
BTS + 3.0% gelatin	90.4 ± 0.7	89.9 ± 0.7	88.7 ± 0.7

Means ± SEM did not differ ($P>0.05$).