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Swine semen cooled at 5ºC with PIGPEL-5 extender: effects on semen quality *in vitro* and fertility estimators *in vivo*

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Abstract

This study consisted of two experiments. In Experiment 1, *in vitro* semen quality was assessed by comparing swine semen conditioned with either PIGPEL-5 extender at 5ºC or BTS extender at 17°C. Sperm motility was similar ($P > 0.05$) at 24 and 48 h post-dilution for PIGPEL-5 (67.9% and 59.3%, respectively) and BTS (73.9% and 64.0%, respectively). The frequency of sperm vigor equal to 4 was higher for PIGPEL-5 ($P < 0.05$) than for BTS after 24 h (70.8% and 29.2%, respectively) and 48 h (87.9% and 12.1%, respectively). After a thermal stress test, the frequencies of sperm vigor equal to 3 and 4 were higher ($P < 0.05$) for PIGPEL-5 than for BTS in both periods, but motility was not different between extenders ($P > 0.05$) in any period. Acrosome integrity was lower ($P < 0.05$) for PIGPEL-5 than for BTS in both periods, but head and tail morphology did not differ ($P > 0.05$). In the hypoosmotic swelling test, the frequency of tail rolling or bending was higher ($P < 0.05$) for BTS than for PIGPEL-5 after 24 h (33.1% and 8.2%, respectively) and 48 h (24.3% and 7.2%, respectively). In Experiment 2, 60 pre-pubertal gilts were artificially inseminated following induction of ovulation and using semen conditioned with both extenders ($n = 30$, per extender). There was no difference ($P > 0.05$) in the number of recovered or fertilized oocytes or in the fertilization rate for BTS (6.6 ± 0.6, 6.0 ± 0.6, and $83.7\% \pm 4.4$, respectively) and for PIGPEL-5 (4.6 ± 0.9, 4.3 ± 0.8, and $87.3\% \pm 6.3$, respectively). These results indicate that the PIGPEL-5 extender can be used to condition swine semen at 5ºC because parameters of semen quality and *in vivo* fertility following its use were generally similar to those of a conventional extender at 17°C.

Keywords: swine semen, cooling, extender, semen quality, fertilization rate.

Introduction

The use of artificial insemination (AI) in swine has largely increased over the last decades due to genetic improvement, optimization of reproductive performance monitoring, availability of qualified labor, greater facility efficiency, and benefits for health status (Bortolozzo and Wentz, 1997; Almond *et al.*, 1998; Deschamps *et al.*, 1998; Corrêa *et al.*, 2001; Gerrits *et al.*, 2005). As the use of AI increases, so does the search for improvements in both boar management and semen processing.

Most of the AI in the swine industry is conducted using diluted, fresh semen conditioned at 15-18°C whereas nearly 85% of all inseminations are conducted on the collection day or at most 24 h post-collection (Johnson *et al.*, 2000). The most-used extender for swine semen is the Beltsville Thawing Solution (BTS). The BTS was initially developed for frozen semen and later adapted to condition fresh swine semen at 15-18°C (Pursel and Johnson, 1975) although some extenders can increase semen storage up to 3 d (Johnson *et al.*, 1988) or even 5-7 d (Levis, 2000). Conditioning swine semen at 15-18°C requires use of refrigerators equipped with a thermostat for temperature adjustment. Nevertheless, in regions where temperatures are either highly variable or extremely high throughout the year, such equipment may not appropriately maintain set temperatures. Also, such equipment can be expensive for small swine operations. Thus, keeping swine semen cooled at temperatures around 5ºC would be a cheaper alternative to help with increasing the use of AI in swine. Another benefit is that bacterial growth is reduced at 5ºC, which would improve the quality of the semen (Althouse and Lu, 2005).

The use of swine semen, either cooled at temperatures below 15-18°C or frozen, is limited because of dramatic reductions in fertility and prolificacy, mainly due to reduced motility and loss of the membrane integrity (Dziuk and Henshaw, 1958; First *et al.*, 1963; De Leeuw *et al.*, 1990; Watson and Green, 2000). Thus, swine semen could only be used at 5ºC following the development of an extender capable of maintaining its viability at that temperature (Pursel *et al.*, 1973; Deschamps *et al.*, 2000, Pérez-Llano and García-Casado, 2005) such as the PIGPEL-5 extender, developed at the Biotechnology Center of the Universidade Federal de Pelotas (UFPEL), RS, Brazil (Corrêa *et al.*, 2004).

The objective of this study was to evaluate the efficiency of the PIGPEL-5 extender in conditioning swine semen at 5ºC in comparison to the BTS extender used for conditioning swine semen at 17°C by assessment of parameters of semen quality and *in vivo* fertility.

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Materials and Methods

Experiment 1

Four crossbred boars were used in this experiment. The boars were kept at UFPEL's experimental station and fed 2 kg of a diet containing 3000 kcal/kg metabolizable energy and 14% crude protein twice daily (National Research Council, 1998). Eighteen, weekly semen collections were performed for each boar following the recommendations of Hemsworth *et al.* (1983) using the hand gloved method with a pre-heated bottle covered by triple-layer gauze (Hancock and Hovell, 1959).

Ejaculates were diluted in isothermal conditions with one of two different extenders: Beltsville Thawing Solution (BTS) at 17°C (Pursel and Johnson, 1975) or PIGPEL-5 at 5ºC (Corrêa *et al.*, 2004). The AI doses were submitted to a cooling curve, which maintained semen samples at 22-24°C for 3 h (after dilution at 35ºC). After this period, the samples were conditioned according to the extender used. Beltsville Thawing Solution doses were kept at 17°C in conditioning boxes (Johnson *et al.*, 2000) whereas PIGPEL-5 doses were kept in a refrigerator with the thermostat adjusted to 5ºC. In both cases, temperatures were assessed during the conditioning period by a digital high-pressure thermometer. Sperm concentration was determined using a spermdensimeter (Busch *et al.*, 1991), and each semen sample, in both treatments, contained 4 x 10^9 spermatozoa/ml.

Evaluations of sperm motility, vigor, and morphology were performed immediately after collection and also at 24 and 48 h after conditioning. Only ejaculates with those parameters above the minimum standards required for swine semen (Almond *et al.*, 1998; Corrêa *et al*., 2001) were used in this experiment. Sperm motility was evaluated as the percentage of motile sperm cells by observing a semen drop on a slide previously heated at 37°C using a phase-contrast microscope. Sperm vigor was classified according to scores from 1 to 5 (Almond *et al.*, 1998). Evaluation of sperm morphology was conducted after adding 3-5 drops of semen to bottles containing 2-3 ml of salt-formalin solution (Hancock, 1957) as long as a waxing-aspect sample was obtained. After homogenization, a small drop of the sample was placed on a slide with a drop of immersion oil and examined using a phase-contrast microscope (1000x) at 37°C so that 200 cells would be counted for evaluation of acrosome, head, and tail morphology. Acrosome morphology was considered abnormal when particles in the apical crest region, damage in the acrosome outline integrity, absence of the apical crest, and/or absence of the acrosomal hood were present (Pursel *et al.*, 1972).

Semen samples were submitted to a thermal stress test (TST) at 24 and 48 h post-collection. For this test, 5 ml semen samples were placed in a 15-ml conical tube and incubated for 45 min in a water-bath at 42.5ºC. After this period, sperm motility and vigor were evaluated for both treatments (Fiser *et al.*, 1991).

Spermatozoa functional integrity after collection and conditioning was evaluated by the hypoosmotic swelling test (HOST). First, two solutions were prepared, one of sodium citrate and another of fructose and both in 300 mOsm/L of distilled water. Then, the two solutions were mixed up to produce a hypoosmotic solution with an osmolarity of 150 mOsm/L. Furthermore, 100-µl of each semen sample was added to 900 µl of the hypoosmotic solution, and the combined solution was homogenized and incubated in a waterbath at 37°C for 60 min. After the incubation period, a 15-µl sample was deposited in a Neubauer chamber, and 100 cells were counted using a phase-contrast microscope (400x) to record the number of spermatozoa with tail swelling that was revealed by tail rolling or bending (TRB). An identical procedure was conducted using an isoosmotic solution (ISO) in a control group for BTS semen samples. Therefore, the HOST value used in the statistical analysis corresponded to the difference between the number of TRB observed in the test with the hypoosmotic and ISO solution (Vazquez *et al.*, 1997).

Descriptive statistics were performed for sperm motility and acrosome, head, and tail morphology, after 24 and 48 h, sperm motility after TST, and TRB after the HOST. The effect of the two extenders on those variables for each period (24 and 48 h) was evaluated by analysis of variance (ANOVA) with repeated measures, including the estimation of the effects of each collection and effect of boar nested within extender. Comparisons of means were conducted using the least significant difference method (LSD) and the GLM procedure of SAS (SAS®, 1991). The percentage of ejaculates classified in each score of sperm vigor was compared across treatments within each period (24 and 48 h) by qui-square tests (x^2). Comparisons were conducted using the Fischer's exact test for categories with numbers of observations lower than 5.

Experiment 2

Six boars were used in this experiment: three purebred (one Duroc, one Large White and one Pietrain) and three crossbred. The boars were kept in UFPEL's experimental station under the same management conditions described in Experiment 1. Six ejaculates were used (one for each boar). The procedures regarding semen collection, dose preparation, and evaluation of sperm concentration, motility, vigor, and morphology were conducted as mentioned above in Experiment 1.

After semen collection, heterospermic doses were produced by pooling ejaculates from two boars. Three different semen pools were used, and all pools were made of semen collected on the same day, always

from the same two boars. Each pool was split in two treatments: BTS at 17°C and PIGPEL-5 at 5ºC. Twenty-four hours post-collection, six doses, randomly selected from each collection, were submitted for evaluation of sperm motility, vigor, and morphology in addition to TST and HOST, following the procedures described in Experiment 1, to check whether such samples were within the minimal standards recommended for swine (Almond *et al*., 1998; Corrêa *et al*., 2001).

Sixty pre-pubertal gilts from a commercial farm were inseminated with semen conditioned with both extenders (30 gilts per extender) to estimate *in vivo* fertility. The gilts weighed on average 93.5 kg and had mean age of 160 d. Gilts were treated with 1000 IU of eCG (CAL, Cientistas Associados Produtos Biológicos Ltda., Pelotas, RS, Brazil) and received 500 IU of hCG (Vetecor; Serono Produtos Farmacêuticos Ltda., São Paulo, SP, Brazil) 70-72 h later to induce ovulation. Both hormones were administrated intramuscularly. All gilts were inseminated within 26-30 h after the hCG injection regardless of signs of estrus because they were expected to ovulate nearly 38 h after hCG injection (Huhn *et al*., 1996). All gilts were checked for estrus using back pressure by an experienced technician in the presence of a mature boar.

All semen samples were conditioned for 24 h and were taken to the farm in thermal boxes with the temperature adjusted to the requirements of each extender. Before AI, sperm motility and vigor were checked for each sample. Samples were wrapped in a paper towel and transported to the AI facility. The inseminations were performed with melrose-type catheters. There were three different semen pools among the 60 AI doses, with a total of 20 doses per pool. Groups of 10 gilts were randomly assigned to receive each semen pool in each treatment.

All gilts were identified by ear tags and slaughtered 66-68 h after AI. Reproductive tracts were collected at the slaughterhouse and transported to the Biotechnology Center's Reproduction Lab. Number of corpora lutea in each ovary was counted. Uterine horns and oviducts were flushed with 0.9% saline solution at room temperature using 10 ml of solution for each oviduct and 40-50 ml for each uterine horn. The volume flushed was filtered with millipore filters that had 0.2 mm pores. Then, 90% of the collected volume was poured through the filter`s opening into Petri dishes. The search for oocytes (fertilized and non-fertilized) was performed using a stereomicroscope.

Descriptive analyses were performed for sperm motility, number of corpora lutea (CL), fertilized (FO) and non-fertilized oocytes (NFO), recovered oocytes (RO), recovery rate (RR), recovery structures (RS) and fertilization rate (FR).

The ovulation rate was determined by the formula: (number of gilts with CL/total number of synchronized gilts) x 100. Recovery rate was determined by the formula: (number of recovered oocytes/number of counted CL) x 100. Fertilization rate was determined by the formula: (number of fertilized oocytes/number of recovered oocytes) x 100. Embryos were considered fertilized when they had 2, 4 or 8 cells, which would be consistent with the number of hours post-AI.

The effect of the extenders on the number of FO, RO, RR, and FR was analyzed by ANOVA, with comparisons of means by the LSD method using the GLM procedure of SAS (SAS®, 1991). Effects of semen pools and categories of semen samples classified according to sperm motility and vigor after 24 h post-conditioning were also analyzed for use as a quality control test.

Results

Experiment 1

Mean sperm motility before and after dilution was 84.8 ± 5.1% and 80.1 ± 9.6%, respectively. Sperm motility was higher for BTS ($P < 0.05$) in both periods (Table 1). Sperm motility of semen cooled with PIGPEL-5 and BTS after 24 h was 67.9 ± 0.9% and 73.9 ± 0.9%, respectively. Sperm motility after 48 h was 59.3 ± 1.2% for PIGPEL-5 and 64.0 ± 1.2% for BTS.

Table 1. Sperm motility of swine semen cooled in PIGPEL-5 at 5°C and in BTS at 17°C before and after dilution and at 24 and 48 h post-dilution.

Extender	Before dilution	After dilution	24 h	48 h
PIGPEL-5	85.2 ± 0.9[a]	80.3 ± 1.0[a]	67.9 ± 0.9[b]	59.3 ± 1.2[b]
BTS	85.5 ± 0.9[a]	81.3 ± 1.0[a]	73.9 ± 0.9[a]	64.0 ± 1.2[a]
Mean ± SD	84.8 ± 5.1	80.1 ± 9.6	70.6 ± 9.4	61.1 ± 11.3

[a,b]Means within column with different superscripts differ ($P < 0.05$).

The percentage of semen samples with a vigor score equal to 3 was 16.4% and 26.5%, before and after dilution, respectively. Samples conditioned with PIGPEL-5 had higher frequencies ($P < 0.05$) of vigor scores equal to 4 after 24 and 48 h post-conditioning (70.8% and 87.9%, respectively) than those conditioned with BTS (29.2% and 12.1%, respectively) as shown in Table 2.

Table 2. Frequency of sperm vigor scores of swine semen samples cooled in PIGPEL-5 at 5°C or BTS at 17°C at 24 and 48 h post-conditioning.

Extender	24 h			48 h		
	Score (%)			Score (%)		
	2	3	4	2	3	4
PIGPEL-5	62.5[a]	34.7[a]	70.8[a]	50.0[a]	32.4[a]	87.9[a]
BTS	37.5[b]	65.3[b]	29.2[b]	50.0[a]	67.6[b]	12.1[b]
Total	6.3	56.3	37.5	15.6	57.8	26.6

[a,b]Frequencies within column with different superscripts differ (P < 0.05).

Mean sperm motility after TST did not differ (P > 0.05) between extenders for any of the conditioning periods (Table 3). Motility after 24 and 48 h were equal to 48.0 ± 1.9% and 39.1 ± 2.0%, respectively, for PIGPEL-5 samples, and 46.0 ± 1.9% and 38.0 ± 2.0, respectively, for BTS samples.

Table 3. Sperm motility for swine semen cooled in PIGPEL-5 at 5°C or in BTS at 17°C after a thermal stress test at 24 and 48 h post-conditioning.[a]

Extender	24 h	48 h
PIGPEL-5	48.0 ± 1.9	39.1 ± 2.0
BTS	46.0 ± 1.9	38.0 ± 2.0
Mean ± SD	46.0 ± 17.1	37.6 ± 19.4

[a] There were no differences (P > 0.05) between PIGPEL-5 and BTS extenders

The frequency of samples with vigor scores after TST equal to 3 and 4 were higher (P < 0.05) for PIGPEL-5 samples than for BTS samples (Table 4). The percentage of semen samples conditioned with PIGPEL-5 with vigor scores equal to 3 were nearly two-thirds of the total samples after both periods. Furthermore, all of the samples having vigor equal to 4, after both 24 and 48 h, were conditioned with PIGPEL-5. On the other hand, no samples conditioned with PIGPEL-5 were classified with vigor scores equal to 0 at either 24 or 48 h post-conditioning, and no PIGPEL-5 samples had a vigor score equal to 1 after 24 h.

Table 4. Frequency of sperm vigor scores of swine semen samples cooled in PIGPEL-5 at 5°C or BTS at 24 and 48 h post-conditioning after a thermal stress test.

Extender	Score (%)				
	0	1	2	3	4
24 h					
PIGPEL-5	0.0[b]	0.0[b]	35.7[b]	63.3[a]	100.0[a]
BTS	100.0[a]	100.0[a]	64.3[a]	36.7[b]	0.0[b]
48 h					
PIGPEL-5	0.0[b]	13.3[b]	50.0[a]	66.0[a]	100,0[a]
BTS	100.0[a]	86.7[a]	50.0[a]	34.0[b]	0.0[b]

[a,b]Frequencies within column with different superscripts differ (P < 0.05).

The frequency of acrosome abnormalities and head and tail morphology by extender after 24 and 48 h post-conditioning are described in Table 5. There was no difference in head and tail morphology among semen samples conditioned with PIGPEL-5 and BTS (P > 0.05) for either period. However, the percentage of acrosome abnormalities was lower (P < 0.05) for BTS samples (1.0 ± 0.3 and 2.6 ± 0.4, respectively) than for PIGPEL-5 samples (3.7 ± 0.3 and 4.1 ± 0.4, respectively) after both conditioning periods.

The mean percentage of sperm cells showing TRB after HOST was 44.3 ± 14.5%. Samples diluted with BTS had 33.1 ± 0.9% of TRB (Table 6) after 24 h post-conditioning, which was higher (P < 0.05) than the TRB from PIGPEL-5 samples (8.2 ± 0.9%). The TRB for BTS samples (24.3 ± 1.1%) was also higher (P < 0.05) than for PIGPEL-5 samples (7.2 ± 1.1%) after 48 h. The interaction between extender and collection was not significant (P > 0.05) for all dependent variables.

Table 5. Abnormalities in sperm morphology of swine semen cooled in PIGPEL-5 at 5ºC or BTS at 17°C at 24 and 48 h post-conditioning.

Abnormalities	24 h (%)		48 h (%)	
	PIGPEL-5	BTS	PIGPEL-5	BTS
Acrossome	3.7 ± 0.3[a]	1.0 ± 0.3[b]	4.1 ± 0.4[a]	2.6 ± 0.4[b]
Head	7.3 ± 0.2[c]	6.3 ± 0.2[c]	6.1 ± 0.3[c]	8.1 ± 0.3[c]
Tail	8.3 ± 0.4[d]	9.3 ± 0.4[d]	7.6 ± 0.4[d]	6.1 ± 0.4[d]

[a,b]Means within row with different superscripts differ (P < 0.05).

Table 6. Frequency of sperm tail rolling or folding in swine semen cooled in PIGPEL-5 at 5ºC or BTS at 17°C before dilution and 24 and 48 h post-conditioning.

Extender	Before dilution (%)	24 h (%)	48 h (%)
PIGPEL-5	44.9 ± 1.5[a]	8.2 ± 0.9[b]	7.2 ± 1.1[b]
BTS	44.8 ± 1.5[a]	33.1 ± 0.9[a]	24.3 ± 1.1[a]
Mean ± SD	44.3 ± 14.5	20.3 ± 15.9	15.5 ± 13.7

[a,b]Means within column with different superscripts differ (P < 0.05).

Experiment 2

Mean sperm motility of the semen samples used for AI was 64.2 ± 8.0%. Among all samples, 86.7% had sperm motility ≥ 60%. The percentage of samples having vigor scores equal to 4 was also 86.7%. Thus, most samples were within the standards recommended for AI in swine.

Among the 60 synchronized gilts, only 11 (18.3%) showed signs of estrus detected by the back pressure test, but 59 (98.3%) ovulated. Only one gilt did not show an ovarian response to hormonal treatments.

The mean number of CL was 9.6 ± 5.4. The mean number of recovered oocytes was 6.0 ± 4.0. As shown in Table 7, gilts inseminated with BTS or PIGPEL-5 samples had no difference (P > 0.05) in the number of RO (6.6 ± 0.6 and 4.6 ± 0.9, respectively). The mean RR was 62.2 ± 29.8%. Recovery rate was not different (P > 0.05) between the BTS and PIGPEL-5 extenders (61.4 ± 4.7% and 63.7 ± 6.9%, respectively).

Only four gilts (6.6% of the total) had non-fertilized oocytes (two in each treatment). The mean number of FO was 5.4 ± 4.0 whereas the mean number of NFO was 0.5 ± 1.1. The number of FO did not differ (P > 0.05) for BTS (6.0 ± 0.6) and PIGPEL-5 samples (4.3 ± 0.8), as shown in Table 7.

Mean fertilization rate was 85.0 ± 28.0%. Fertilization rate was similar (P > 0.05) between PIGPEL-5 and BTS treatments (87.3 ± 6.3% and 83.7 ± 4.4%, respectively). No differences were observed for RR, and FR considering the effects of sperm motility and vigor and semen pool (P > 0.05).

Table 7. Total number of recovered oocytes, fertilized oocytes, and fertilization rates for each semen extender after 24 h post-conditioning.[a]

Extender	Total oocytes	Fertilized oocytes	Fertilization rate (%)
BTS (17°C)	6.6 ± 0.6	6.0 ± 0.6	87.3 ± 6.3
PIGPEL-5 (5ºC)	4.6 ± 0.9	4.3 ± 0.8	83.7 ± 4.4
Mean ± SD	6.0 ± 4.0	5.4 ± 4.0	85.0 ± 28.0

[a] There were no differences (P > 0.05) between PIGPEL-5 and BTS extenders.

Discussion

Both extenders were capable of preserving sperm activity post-conditioning. Although sperm motility was higher in semen conditioned in BTS at 17°C, the motility obtained with PIGPEL-5 at 5ºC is promising since the differences, although statistically significant, were not large enough to prevent PIGPEL-5 samples from being used in the field. These results somewhat contradict the assumption that swine semen conditioned at temperatures under 12°C, even with an enriched extender such as Androhep® (Weitze, 1990), would not be feasible for commercial use due to irreversible structural damage and a decrease in sperm motility (Althouse *et al.*, 1998). Nevertheless, mean sperm motility observed across treatments in this study was below those usually considered acceptable at AI centers (Glossop, 1996; Almond *et al.*, 1998). This could be due to the suboptimal processing conditions because UFPel's experimental station is quite far from the semen processing lab. Therefore, a certain time lag occurred between semen collection and conditioning.

Despite the time lag, no differences in sperm motility were observed between pre- and post-dilution samples. Because sperm motility is an indicator of metabolic activity of spermatozoa and membrane integrity, it is extremely important for semen quality control and for evaluation of semen resistance to conditioning (Gadea and Matás, 2000).

The percentage of samples with sperm vigor classified as 4 was higher for PIGPEL-5 than for BTS samples after both conditioning periods. Thus, semen conditioned with PIGPEL-5 at 5ºC not only had vigor estimates within the standards for swine semen (Glossop, 1996; Almond *et al.*, 1998; Corrêa *et al.*, 2001) but also had superior vigor in comparison with a conventional extender at a higher temperature. The observed reduction in the frequency of samples with vigor scores of 4 and the increase in samples with vigor scores of 2 as conditioning time increased are consistent with other reports (Waberski *et al.*, 1994). That advantage of PIGPEL-5 over BTS in both post-conditioning periods indicates that PIGPEL-5 was beneficial for the maintenance of sperm cells metabolic capacity at cooling temperatures, which is associated with the maintenance of fertilizing capacity (Johnson *et al.*, 2000).

Sperm motility was similar between extenders for both periods after the TST, but the percentage of samples having vigor classified as 3 or 4 was higher for PIGPEL-5 than for BTS. Considering the sensitivity of the TST in evaluating resistance to thermal variation (Fiser *et al.*, 1991), swine semen conditioned in PIGPEL-5 was able to resist sudden temperature variation, which could reflect a potential improvement in sperm survival in the female reproductive tract post-AI and in its further fertilizing capacity.

PIGPEL-5 samples had a frequency of sperm morphological abnormalities within the levels accepted by AI centers (Pursel *et al.*, 1972; Almond *et al.*, 1998). Although the frequency of acrosome abnormalities in PIGPEL-5 samples was higher than for BTS samples, the frequency was below the maximum accepted limit of 5% (Waberski *et al.*, 1994; Corcuera *et al.*, 2000) and was numerically lower than that reported by Pursel *et al.* (1973) for semen conditioned at 5ºC. These results also indicate an advantage in comparison with the use of frozen semen, in which the frequency of abnormal acrosome morphology is usually between 20-40% (Pursel *et al.*, 1972). The percentage of abnormal head and tail morphology did not differ between extenders, although the frequency of both apparently increased with conditioning time, which could be due to the temperature, regardless of the extenders (Johnson *et al.*, 2000).

Although the percentage of TRB in response to the HOST was in agreement with some reports that used fresh semen (Vazquez *et al.*, 1997; Gadea and Matás, 2000), it was lower for both extenders after 24 and 48 h post-conditioning if compared with the findings of Pérez-Llano *et al.* (2001). Spermatozoa with biochemically active membranes will swell during the HOST as a result of hypoosmotic stress due to water influx. This is more evident in the tail than in the head because the membrane of the tail seems to be more sensitive to hypoosmotic stress (Jeyendran *et al.*, 1984). The TRB response was higher in BTS samples, and this indicates that sperm cells conditioned in PIGPEL-5 at 5ºC for 24 and 48 h could suffer damage to their membrane functional capacity. However, HOST results can vary in different trials without expressing characteristic associations with spermatozoa fertilizing capacity (Gadea and Matás, 2000). Furthermore, in the mentioned studies, swine semen was conditioned at temperatures other than 5ºC (Pérez-Llano *et al.*, 2001). Thus, organizational alterations in the sperm membrane caused by low temperature could negatively influence the HOST results.

In conclusion, the results of Experiment 1 indicate that PIGPEL-5 extender is capable of maintaining the viability of swine semen cooled at 5ºC at levels similar to those observed with conventional conditioning at 17°C. However, it would be important to compare the performance of swine semen conditioned with PIGPEL-5 stored in controlled temperature-refrigerators with the performance achieved with storage in domestic refrigerators.

Samples conditioned with either BTS at 17°C or PIGPEL-5 at 5ºC had a similar FR. Thus, the *in vivo* response with PIGPEL-5 was in agreement with the *in vitro* results of Experiment 1. The FR observed for both extenders was consistent with those reported elsewhere (Hughes and Varley, 1984). The FR obtained from semen conditioned in BTS is consistent with the FR reported for conditioning at temperatures between 15ºC and 18°C (Levis, 2000). The FR obtained with PIGPEL-5 was better than those reported elsewhere for semen conditioned at 5ºC in which unsatisfactory results were attributed to damage of the sperm membrane and reduction of sperm motility (Dziuk and Henshaw, 1958; First *et al*., 1963; De Leeuw *et al*., 1990; Hofmo and Almlid, 1991). The overall mean FR of 85% indicates that the use of pre-pubertal gilts and synchronization treatments did not negatively influence the results and would probably result in acceptable farrowing rates, which is in agreement with Brüssow *et al*. (1996) who reported that estrous synchronization protocols for gilts would result in FR at desirable levels.

The numerical advantage observed for BTS in the number of recovered structures (2.0) and fertilized oocytes (1.7) is likely a consequence of the numerically higher number of CL obtained with that treatment (1.2). However, none of those differences were statistically significant indicating that the response was similar for both extenders. The RR observed in this study is consistent with the results obtained in other studies whereas the number of structures and fertilized oocytes were lower than the levels generally reported for gilts

(Hughes and Varley, 1984), and the number of structures can be considered lower than that obtained by Bordignon *et al*. (1996). The relatively low number of follicles that ovulated, as indicated by the low number of CL obtained in this study, was certainly responsible for the low number of total oocytes and fertilized oocytes, which would certainly result in a reduced total litter size. This could be related to the nutritional and health status of the pre-pubertal gilts. The gilts used in the experiment were from a commercial farm and destined to slaughter; therefore, they were not selected according to reproductive traits.

In conclusion, the results of this experiment indicate that swine semen conditioned with PIGPEL-5 at 5ºC was capable of producing a fertilization rate similar to that obtained with conventional extender at 17°C in pre-pubertal synchronized gilts.

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