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Short Communication

Insulin addition to swine semen diluted and cooled at 15 °C

Evandro César Pereira Cunha[1], Márcio Gilberto Zangeronimo[1], Luis David Solis Murgas[1], Douglas Evangelista Braga[1], Bárbara Azevedo Pereira[1], Luiz Gustavo Pessoa Rocha[1], Bruno Generoso Faria[1], Luciano José Pereira[1]

[1] Departamento de Medicina Veterinária, Universidade Federal de Lavras, Caixa Postal 3037, 37200-000, Lavras, MG.

ABSTRACT - The objective of this study was to evaluate the effect of adding different doses of insulin to swine semen processed and stored at 15 °C. The experiment used sixteen ejaculates from four commercial breeding pigs, distributed in a randomized block design (ejaculate) with split plot along time (0, 24, 48 and 72 hours of storage) with four treatments (insulin levels - 0.0 4.0 8.0 and 12.0 IU per dose) and 16 repetitions. The experimental unit was made of two insemination doses of 100 mL each, with 3×10^9 spermatozoids. Insulin used was NPH-human, added at the time of processing the doses. The addition of insulin did not affect motility, sperm viability, the percentage of abnormal cells, the osmotic resistance or the degradation rate of motility in 120 minutes. There was a linear decrease in semen quality over storage time, regardless of insulin levels. The addition of insulin at the mentioned concentrations does not influence the quality of insemination dose in pigs.

Key Words: artificial insemination, boar, hormone, metabolism, sperm, reproduction

Introduction

The production of pig meat has been steadily tracking the growth of the world population and the development of this production depends on factors such as nutrition, health, management, genetics and reproduction. In the latter aspect, artificial insemination becomes an important factor in improving the reproductive rates of a facility by reducing the concentration of sperm insemination doses and longer storage of diluted semen, optimizing the use of sires in the squad.

The technique of artificial insemination requires the use of high-quality semen, for better control and monitoring during processing (Waberski et al., 2008). The determination of the sperm quality is essential, since infertility problems cause an increase of return to estrus rate and decreased birth rate, besides the reduction in average litter size (García et al., 2002). In this regard, several studies aimed at improving the quality of boar semen have been conducted (Vianna et al., 2004; Martins et al., 2009; Alvarenga et al., 2009).

One of the main causes of the drop in fertility of stored boar semen is the decline of sperm metabolic energy (Ford & Waites, 1986), which is directly related to the amount of energy used. Currently, the biggest challenge is to know the components that directly influence the metabolism of sperm cells, especially in swine. The presence of insulin in human semen has been recently demonstrated, specifically in the sub-acrosomal region, the middle piece and the entire length of the flagellum (Aquila et al., 2005). Moreover, according to the authors, when the capacitation is stimulated, sperm is able to secrete insulin and this may be a signaling mechanism for sperm metabolism.

Recently, the presence of specific receptors for insulin in porcine sperm (Carpino et al., 2010) has been detected. Thus, the addition of this hormone in cooled semen could be related to increased activity of the sperm cell after storage, improving the quality of semen used in artificial insemination.

Thus, the objective of this study was to evaluate the quality of boar semen diluted and cooled at 15 °C, added to different concentrations of insulin during sperm activation in the period of 72 hours of storage.

Material and Methods

The experiment was conducted in the Physiology and Pharmacology Laboratory, at Universidade Federal de Lavras. Sixteen ejaculates were used from four commercial breeding pigs from Fazenda São Paulo, a full cycle commercial farm with approximately 4500 matrices in the city of Oliveira, Minas Gerais, Brazil.

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Corresponding author: zangeronimo@dmv.ufla.br

Whole ejaculates (without the gelatinous fraction) were collected by the gloved-hand method, always in the morning. Semen was initially evaluated for appearance (macroscopic aspects) and then diluted 1:1 BTS Minitube do Brasil® (Beltsville Thawing Solution) and transported in isothermal box to the Laboratory of Physiology and Pharmacology of Lavras. In the laboratory, sperm quality was assessed according to the macroscopic quality standards: motility, sperm concentration and morphology according to the CBRA (1998). The motility was performed by two trained people by placing a drop of semen between a coverslip and slide at 37 ºC and evaluated under a microscope with 200x magnification, where the percentage of motile cells was determined subjectively. Each examiner performed three assessments. The final value was set at the average between the raters. The sperm concentration was determined with the spectrophotometer Spermacue (Minitube do Brasil®). For sperm morphology, the wet method in formalin-citrate 2.9% at room temperature was used and observed in contrast phase microscope with 1000-fold increase in oil immersion, characterizing the morphological abnormalities in proportion to 200 cells. To be considered normal ejaculate, it should present the normal appearance and smell, over 90% motility and morphological changes in total below 10%.

The ejaculate was then further diluted in BTS, so as to form insemination doses of 100 mL containing three billion sperm cells each. The doses were then added to different doses of insulin at 10 IU/ mL (0, 0.4, 0.8 and 1.2 mL in each insemination dose), which were represented, each, by two insemination doses of 100 mL (experimental plot). Sperm motility, viability and morphology at 0, 24, 48 and 72 hours of storage at 15 °C were evaluated.

Sperm viability was evaluated from the observation of a smear of a drop of semen mixed with eosin and negrosina using an optical microscope with 400 times magnification. The percentage of live sperm cells (white) and dead (pink) was checked in a total of 200 cells (Mies Filho, 1982).

At the beginning (time 0) and at the end of the storage period of insemination doses (72 hours) osmotic resistance (ORT) and degradation of motility (DMT) tests were performed. The osmotic resistance test was performed in 1.0 mL of semen mixed in 1.0 mL of the hyposmotic 150 mOs/ L (50% solution BTS and 50% distilled water) and incubated for 30 minutes was at 37 °C. In light microscopy, 200 sperm cells were counted, differentiating cells that showed a straight tail (with membrane rupture) and curly tail (membrane intact) (Scheid, 1993). The degradation of motility test was performed by incubation for two hours and the evaluation of 2.0 mL of semen at 37 ºC. The following equation proposed by Salgueiro et al. (2003) was used: DTM (%) = force (5 minutes) - force (2 hours) × 100/ vigor (5 minutes).

The experimental design was randomized blocks with split plots along time (time of assessment) with four treatments (insulin levels) and 16 repetitions of two insemination doses, each.

Data were submitted to analysis of variance after normality test and to regression analysis for evaluation of insulin doses and times of assessment. All statistical analyses were performed using the statistical package Sisvar (version 5.3) (Ferreira, 2008).

Results and Discussion

The addition of insulin to the swine cooled semen did not influence (P>0.05) sperm motility and viability, the percentage of morphological sperm or the degradation rate of motility or osmotic resistance of sperm cells in the different storage times (Tables 1 and 2).

The function of insulin on growth regulation, differentiation and metabolism is well known (Shrivastav et al., 1989). In addition, insulin has demonstrated a central role in regulating the gonadal function (Nakayama et al., 1999). However, the role of insulin in male fertility is not well understood (Carpino et al., 2010).

Silvestroni et al. (1992) demonstrated that insulin is able to bind to membrane receptors and also in the acrosome, suggesting the presence of receptors that were involved in sperm capacitating. In turn, Nakayama et al. (1999) demonstrated that insulin, along with IGF-I and II act in the differentiation of spermatogonia into spermatocytes by binding to IGF receptors. In humans, it has been shown that the presence of insulin-dependent diabetes mellitus is related to lower motility, higher incidence of abnormal cells (Baccetti et al., 2002) and therefore less able to penetrate the oocyte spermatozoids (Shrivastav et al., 1989).

Regarding the source of seminal insulin, Aquila et al. (2005) demonstrated the ability of sperm to produce and secrete this hormone, suggesting an autocrine regulation of its secretion related to the consumption of energy compounds by the sperm. According to the authors, the insulin concentration in the ejaculate is dependent on the number of cells and the concentration of glucose in the medium, and they found values ranging between 4 and 12 µUI/mL.

More recently, Carpino et al. (2010) confirmed the presence of insulin receptors in pig sperm. This hormone has the ability to modulate the influx of cholesterol and acrosome reaction. This, somehow, is related to sperm quality and its resistance to the cooling process. In domestic

Table 1 - Motility, viability and morphological changes (mean ± standard deviation) in boar semen stored at 15 °C, added to different concentrations of insulin

Concentration (UI/100 mL)	Time of evaluation after adding insulin (hours)				Mean
	0	24	48	72	
	Sperm motility (%)				
0.0	81.6±5.7	78.2±4.2	75.1±5.8	70.9±5.2	76.5±6.5
4.0	79.7±7.3	76.7±6.1	71.9±8.1	68.4±6.6	74.2±8.4
8.0	80.9±5.1	78.3±4.8	74.6±6.1	70.8±4.9	76.1±6.4
12.0	81.2±6.0	79.9±5.5	74.6±5.7	70.4±5.2	76.5±7.0
Mean[1]	80.9±5.9	78.3±5.2	74.1±6.5	70.1±5.6	
CV (%)	4.18				
	Sperm viability (%)				
0.0	96.4±2.0	93.5±2.5	91.1±3.6	88.7±3.9	92.4±4.2
4.0	96.0±2.4	93.5±2.4	91.0±3.6	88.0±4.3	92.1±4.4
8.0	95.9±2.2	93.2±3.0	90.8±3.7	89.0±4.5	92.2±4.3
12.0	96.1±1.9	93.8±2.0	90.6±3.6	88.4±3.9	92.2±4.2
Mean[1]	96.1±2.1	93.5±2.5	90.9±3.6	88.5±4.1	
CV (%)	2.32				
	Morphological changes (%)				
0.0	9.7±5.6	11.3±5.2	13.2±8.0	12.6±6.9	11.7±6.5
4.0	10.2±7.8	11.0±7.3	12.2±8.6	14.6±8.5	12.0±8.1
8.0	10.3±6.0	11.0±7.4	12.6±8.9	13.5±6.7	11.8±7.3
12.0	7.4±5.3	10.9±7.7	12.8±8.3	11.9±6.2	10.8±7.1
Mean[1]	9.4±6.2	11.0±6.8	12.7±8.3	12.9±7.1	
CV (%)	11.90				

[1]Significant linear regression (P<0.05).

Table 2 - Degradation rate of motility (DMT) and hypoosmotic test of boar semen stored at 15 °C, added to different concentrations of insulin

Concentration (UI/100 mL)	Time of evaluation after adding insulin (hours)		Mean
	0	72	
	Degradation rate of motility		
0.0	0.152±0.08	0.281±0.10	0.217±0.11
4.0	0.161±0.06	0.299±0.11	0.230±0.11
8.0	0.163±0.08	0.297±0.10	0.230±0.11
12.0	0.141±0.07	0.334±0.18	0.238±0.17
Mean	0.154±0.07b	0.303±0.12a	
CV (%)	19.66		
	Hypoosmotic test (%)		
0.0	13.0±5.6	15.9±4.0	14.5±4.9
4.0	12.1±5.3	15.6±5.2	13.8±5.7
8.0	12.8±5.5	15.5±5.1	14.2±5.1
12.0	11.5±5.4	13.1±4.7	13.5±5.3
Mean	12.3±5.3b	15.0±4.9a	
CV (%)	14.46		

[a,b] Means followed by different letters indicate significant difference using F test (P<0.05).

animals, particularly pigs, there are few studies aiming to detect the presence of the hormone in seminal plasma, its receptors and its importance in sperm quality.

Considering the processing of semen, Tilburg et al. (2008) suggests that dilution by seminal plasma reduces insulin levels in semen, reducing the ability of the sperm cell to metabolize energy compounds present in seminal plasma. In the present study, the insulin concentrations tested were above those observed for humans in the literature.

Linear decrease (P<0.05) was observed in sperm motility and viability and the percentage of abnormal cells during the storage time, regardless of insulin concentration added in the semen (Figures 1, 2 and 3).

With respect to motility, it is known that this is an event highly dependent on the glycolytic pathway, responsible for much of the ATP production in sperm (Mukay et al., 2004). Over time, degradation of sperm is expected since the cooling does not stop metabolism and constant production

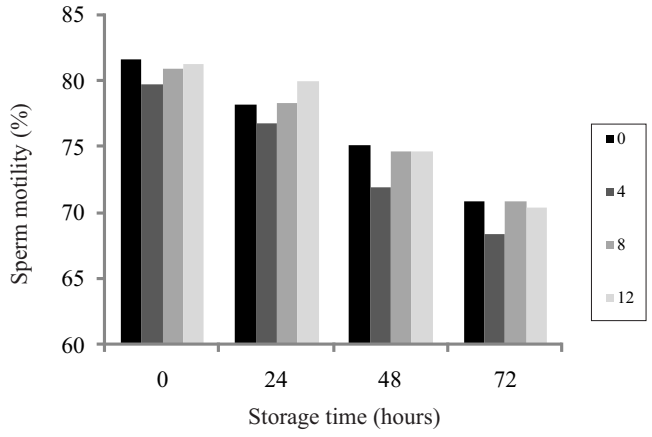

Figure 1 - Motility of boar semen diluted with different insulin concentrations (0, 4, 8 and 12 UI/80 mL) and stored for 72 hours at 15 °C.

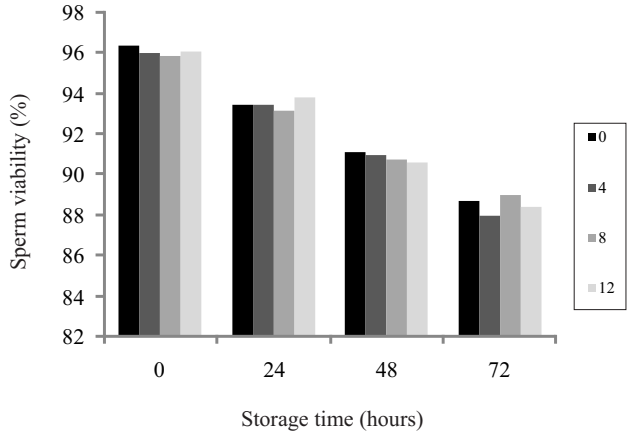

Figure 2 - Sperm viability of boar semen diluted with different insulin concentrations (0, 4, 8 and 12 UI/ 80 mL) and stored for 72 hours at 15 °C.

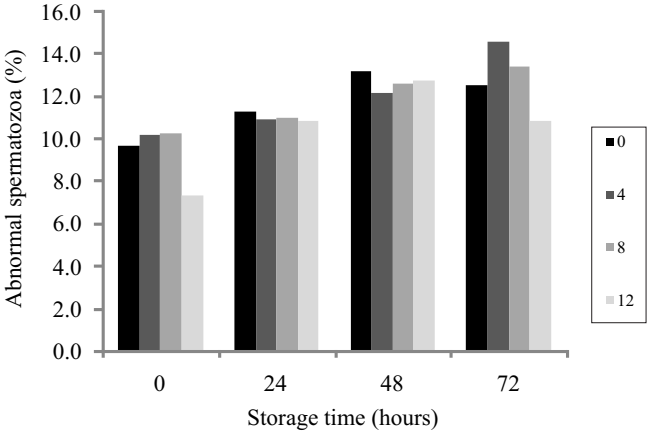

Figure 3 - Total sperm abnormalities (%) of boar semen diluted with different insulin concentrations (0, 4, 8 and 12 UI/ 80 mL) and stored for 72 hours at 15 °C.

of reactive oxygen substances resulting from energetic metabolism. Besides, sperm viability and osmotic resistance of the cell are also reduced, increasing the number of abnormal cells in semen (Johnson et al., 2000). In this sense, the presence of higher concentrations of insulin in the boar semen could reduce sperm quality at the end of the storage period, since the energy metabolism of sperm would be more active. The results of this experiment reinforce the idea that the concentration of insulin was not sufficient to alter sperm metabolism.

The results of this study reveal that the addition of insulin at the level of 12 UI/100 mL immediately after dilution for boar semen does not alter the semen quality of insemination doses. However, based on results of the literature, more studies should be conducted with higher doses of insulin or the same doses should be added after the storage period, i.e., right before its use in artificial insemination programs.

Conclusions

The addition of insulin until 12 IU/100 mL to cooled swine semen immediately after dilution does not alter the semen quality of insemination doses.

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