

# SPERM CONCENTRATION ON THE INTRAUTERINE ARTIFICIAL INSEMINATION IN SWINE

## Concentrações espermáticas na inseminação artificial intra-uterina suína

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### ABSTRACT

The aim of this work was to evaluate the efficiency of the intrauterine insemination (IUI) in swine, considering the conception rate, farrowing rate, litter size (alive born pigs). For the IUI, the females had been inseminated at 24 and 48 hours after the estrus detection, and the inseminating doses of 500 million, 1 billion, 1.5 billion and 2 billion spermatozoa in 20 mL extender had been used. The procedure of catheter insertion through the cervical canal was successfully performed in 97.9% of the females. The conception rate was 6.3% in the IUI. The farrowing rate in IUI was 87.2% but the farrowing rate was 100% for the sperm concentration of 500 million. Regarding the number of born pigs and alive born pigs observed in females inseminated with IUI, no significant difference was observed ( $p > 0.05$ ). The concentration of  $500 \times 10^6$  spermatozoa in 20 mL extender in the intrauterine insemination resulted in an optimal reproductive performance.

**Index terms:** Intrauterine insemination, reproductive performance, sperm cell dose.

### RESUMO

Conduziu-se este estudo, com o objetivo de avaliar a eficiência da inseminação intra-uterina (IIU) em suínos, considerando as taxas de retorno ao estro, aborto, parto, além do tamanho da leitegada (número de leitões nascidos e nascidos vivos). Na IIU, as fêmeas foram inseminadas nos tempos de 24 e 48 horas após a detecção do estro, utilizando-se as concentrações de 500 milhões, 1 bilhão, 1,5 bilhão e 2 bilhões de espermatozoides, em 20mL de diluente. A passagem do cateter de IIU através da cérvix foi possível em 97,9% das fêmeas. Foi realizado diagnóstico de retorno ao estro a partir do 18º dia e diagnóstico de gestação por ultrassonografia transcutânea entre o 28º e 30º dias após a inseminação. A taxa de retorno ao estro foi de 6,3% na IIU. A taxa de parto na IIU foi de 87,2%, sendo a taxa de parto para a concentração de 500 milhões de 100%. Com relação ao número de leitões nascidos totais e nascidos vivos, não houve diferenças, entre as diferentes concentrações espermáticas ( $P > 0,05$ ). A utilização da concentração de  $500 \times 10^6$  espermatozoides em 20mL de diluente, com inseminação intra-uterina, obteve-se um bom desempenho reprodutivo.

**Termos para indexação:** Inseminação intra-uterina, desempenho reprodutivo, concentração espermática por dose.

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### INTRODUCTION

Nowadays, over 50% of the total females in production in the world are artificially inseminated. This technique allows the reduction of sexually transmitted diseases, the increase of efficiency of the reproducer, the ejaculated control and an increase on the litter's genetic (Roca et al., 2006; Lima et al., 2007).

The techniques and applications of artificial insemination (AI) have been improved since the first viable technique to inseminate females in 1948 (Levis, 2002). During the 50's and 60's, researches on spermatozoon transport, in females and multiparas inseminated with doses differing in

volume and number of spermatozoon, suggesting that it was necessary a dose of  $5-10 \times 10^9$  spermatozoon in 100 mL of diluter to reach a great fertility rate (Belstra, 2002).

On these decades it was also considered the possibility of reduction of volume and spermatozoon on the inseminating dose through direct deposition inside the uterine horn. (Dallanora et al., 2003).

In the end of the 90's, studies about intrauterine insemination (IUI) restarted, both in the surgical and non-surgical forms, making possible the use of reduced number of spermatozoon and volume, with no loss of the reproductive efficiency (Vasquez et al., 2003).

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The intrauterine insemination (IUI) is a process consisted in placing the spermatozoon directly into the uterine body, contrary to intra-cervical, traditionally used, and its benefits are: reduction of total spermatozoon number per dose; higher number of doses per ejaculated; reduction of boar number and necessary installations; reduction of costs and maintenance of the males; higher utilization of genetically superior reproducer (Gil et al., 2001).

With the use of UIU, there is the possibility to increase the use of genetically superior males, making possible the production of 2 to 3 times more doses per ejaculated, resulting in a reduction of males on the stud. Instruments were developed to make viable the deposition of the semen as close as possible to the fecundation location, in a non-surgical form, making possible the use of a lower number of spermatozoa in the AI process. However, there are few comparative studies between the two techniques, in field conditions, conducted in commercial grange. This work aimed to evaluate the efficiency of intrauterine insemination (IUI) in swine, considering the reproductive husbandry values (return to estrus rate, abortion, farrowing, litter's size (born and born alive pigs).

#### MATERIAL AND METHODS

The experiment was conducted in a commercial grange, with swine production in a complete cycle, in the west region of the state of Minas Gerais, between March and December of 2006.

In a completely random delineation, there were used 48 multipara females from the Camborough 22 lineage (Agrocers – PIC) with order of farrowing from two to ten, gap of wean – estrus between three and six days, lactation duration from 18 to 21 days and average litter size on previous birth superior to eight piglets. After the estrus beginning, the females were randomly distributed, considering the characteristics quoted before, with 12 animals per treatment, totalizing 48 females in four treatments. In IUI, the females were inseminated with disposable AI pipettes (polypropylene tube with a polyurethane sponge) and a polypropylene semi-rigid catheter with external diameter of 3 millimeters and intern diameter of 2 millimeters (lumen), which slides inside the pipette, stretching 20 cm beyond the cervical folds reaching the region of the horns bifurcation or even to one of the uterine horn, allowing intrauterine deposition of the inseminating dose.

The semen collection from the 8 male donators was realized two times a week, by the gloved hand method. The concentration was checked by the direct spermatozoon count in hemocytometric chamber. The ejaculated were diluted in Beltsville Thawing Solution

(BTS), in doses of 500 million, 1 billion, 1.5 billion and 2 billion spermatozoon in 20 mL. All inseminating doses were produced by *split sample*, in other words, the used doses for the four treatments were produced from the same ejaculated.

After the wean, it was realized the estrus detection, twice a day, by reflex of tolerance of man in presence of the male (Martins et al., 2008). Two inseminations were realized, in 24 and 48 hours after estrus detection, similar to the commercial grange handling. The estrus return diagnostic occurred from 18 to 23 days after the AI, by reflex of tolerance of man in presence of the male test. The gestation diagnostic was realized 28 to 30 days after the AI, by transcutaneous ultrasonography in real time, with a 5 MHz sectorial transducer on all females subdued to IUI. On labor, data was collected referring to litter size (number of born piglets, born alive).

The values referring to litter size, females that delivered went through variance analyses by SAS, with averages compared by the Turkey test. The significance level was 5% in all analyses.

#### RESULTS AND DISCUSSION

It was possible to insert the catheter into the uterine lumen in 97.9% of females submitted to intrauterine insemination (47/48). This difficulty for the introduction of the catheter may be associated with failure to detect estrus in females, with late detection and at the moment of insemination, the female was no longer in estrus. Watson and Behan (2002) used a catheter with 2 mm internal diameter, the catheter could be introduced in 46 of 51 females (90.2%) without difficulty. Dallanora et al. (2003), obtained 100% success rate in the pipette passing, a total of 268 females inseminated with commercial pipette specifically developed for the practice of IUI.

The repetition of estrus after insemination occurred in 3 of 47 inseminated females (6.3%). Abortion also occurred in 3 of 47 inseminated females (6.3%). The farrowing rate was 87, 2% (Table 1). The spermatozoa concentration of  $500 \times 10^6$  per insemination dose had 100% farrowing rate, with no repetition of estrus or abortion. In the other concentrations, the number of females that repeated estrus and abortion were the same. This indicates that the intrauterine spermatozoa deposition of  $500 \times 10^6$  allowed the efficient formation of the spermatozoa reservoir at the junction uterus - tubes and the adequate fertilization. Mezalira et al. (2005) reported that intrauterine insemination can be successfully performed using only  $500 \times 10^6$  of spermatozoa cells that are inseminated in a 24 to 48 hours rate after the beginning of estrus.

The pregnancy rate observed by Wolken (2001), quoted by Dallanora (2004), was relatively low (77.3%) with a dose of 500 million spermatozoa in 20 mL, compared with the pregnancy rate obtained in this work (100%). Mezalira et al. (2005) used IUI and obtained a pregnancy rate of 85.5% with 500 million spermatozoa in 20 mL, with significant reduction to 77.1% when the dose contained 250 million spermatozoa. Martinez et al. (2002) obtained a farrowing rate of 76.2% and 82.9% using 50 and 150 million spermatozoa in 5 mL of diluents, with single insemination.

The farrowing rate in the concentrations of 1 and 2 billion spermatozoon were 83.4% and 81.8%, respectively. The values obtained by Watson and Behan (2002) were 86.9% and 92.5% for 1 and 2 billion spermatozoon, respectively. Dallanora (2004) used a concentration of 1.5 billion spermatozoon with the IUI technique and got the rates of return to estrus and delivery of 3.6% and 92.8%, respectively. The values observed by the author were better than the present work, which has values of 8.3% and 83.4%. The return to estrus and farrowing rate obtained by Dallanora (2004) may be

related to the high frequency of inseminations per female in a single estrus.

Mezalira et al. (2005) conducted a intrauterine deposition technique using concentrations of 500 and 250 million and observed that the pregnancy rate was significantly reduced from 85.5% to 77.1%, with decreasing spermatozoa concentration in the insemination dose.

The results obtained related to litter are presented on Table 2. The data show that, related to the farrowing number and total born alive, the concentrations used in the IUI did not differ among themselves ( $P > 0.05$ ).

The values obtained using concentrations of 1 and 2 billion spermatozoon for litter size were 10.7 and 9.67 piglets born and 9.4 and 8.7 born alive, respectively. Watson and Behan (2002) found values of 12.1 and 12.3 for total farrowing, and 10.9 and 10.8 for born alive, in 1 and 2 billion spermatozoon, respectively.

Dallanora (2004) obtained 11.6 born piglets using a concentration of 1.5 billion spermatozoon with the IUI technique, similar value obtained on the present work (11.1 piglets).

Table 1 – Coverage performance on females inseminated with intrauterine deposition technique (IUI).

	IUI				Total
	Dose (x 10 <sup>6</sup> spermatozoon)				
	500	1000	1500	2000	
Estrus repetition (%)	0.0	8.3%	8.3%	9.1%	6.3%
N = 47	(0/12)	(1/12)	(1/12)	(1/11)	(3/47)
Abortion (%)	0.0	8.3%	8.3%	9.1%	6.3%
N= 47	(0/12)	(1/12)	(1/12)	(1/11)	(3/47)
Farrowing rate (%)	100%	83.4%	83.4%	81.8%	87.2%
N= 47	(12/12)	(10/12)	(10/12)	(9/11)	(41/47)

Table 2 – Results related to litter number in the four degrees of concentration used on IUI.

Dose (x 10 <sup>6</sup> spermatozoon)	NT <sup>1</sup>	NV <sup>1</sup>
500	11.1	9.9
1000	10.7	9.4
1500	11.1	10.1
2000	9.67	8.7
CV (%)	27.8	29.3
P=	0.1311	0.1097

<sup>1</sup> Averages do not differ among themselves, on the columns by the Turkey test ( $P > 0.05$ ).

NT – Total born

NV – Born alive

### CONCLUSION

The use of  $500 \times 10^6$  of spermatozoon in 20 mL of diluents, with intrauterine insemination, allowed an efficient formation of spermatozoa reservoir in the junction uterus – tubes and proper fertilization, obtaining a reproductive performance similar to the other used doses.

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