Reproductive performance of sows submitted to intrauterine insemination

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ABSTRACT - The objective of this study was to evaluate the reproductive performance of sows artificially inseminated by the intrauterine (IAIU) technique. The experiment was arranged in a randomized complete block design, with 300 sows being distributed in five insemination techniques: control, intra-cervical (IAIC) with $3x10^9$ spermatozoa/100 mL; intrauterine (IAIU) with $1x10^9$ spermatozoa/100 mL; intrauterine with com $1x10^9$ spermatozoa/50 mL; intrauterine with $5x10^8$ spermatozoa/100 mL; and intrauterine com $5x10^8$ spermatozoa/50 mL. The sows submitted to intrauterine insemination presented a farrowing rate of 90.8% and return to estrus rate of 9.2%, which did not differ from the rates obtained by the intra-cervical technique (90.0% and 10.0%, respectively). Total litter size did not differ between the techniques, with the means being from 11.4 to 11.9 piglets at farrowing. Although 4.6% of the sows submitted to intrauterine artificial insemination had difficulty with pipette insertion into the cervix, 100% of them were inseminated. When evaluating semen backflow, no difference was found between the intra-cervical and intrauterine insemination techniques. However, total semen backflow was higher in sows submitted to inter-cervical insemination. No difference was found in the presence of blood between the two methods evaluated. Hence, any of the intrauterine insemination techniques can substitute inter-cervical artificial insemination without damaging the reproductive performance of the animals.

Key Words: intra-cervical insemination, intrauterine insemination, semen backflow, swine

Desempenho reprodutivo de matrizes suínas inseminadas pela técnica intrauterina

RESUMO - Um experimento foi realizado com o objetivo de avaliar o desempenho reprodutivo de matrizes suínas inseminadas pela técnica intrauterina (IAIU). Em um delineamento inteiramente casualizado, 300 fêmeas foram distribuídas em cinco técnicas de inseminação: controle – intracervical (IAIC) com $3x10^9$ espermatozoides/100 mL; intrauterina (IAIU) com $1x10^9$ espermatozoides/100 mL; intrauterina com $1x10^9$ espermatozoides/50 mL; intrauterina com $5x10^8$ espermatozoides/100 mL; e intrauterina com $5x10^8$ espermatozoides/50 mL. As fêmeas inseminadas pela técnica intrauterina apresentaram taxa de parto de 90,8% e taxa de repetição de estro de 9,2%, que não diferiram das taxas obtidas pela técnica intracervical (90,0% e 10,0%, respectivamente). O total de leitões nascidos não diferiu entre as técnicas, com média geral de 11,4 a 11,9 leitões por parto. Apesar da dificuldade na passagem da pipeta em 4,6% das fêmeas submetidas à inseminação artificial intrauterina, 100% das fêmeas foram inseminadas. Na avaliação da ocorrência de refluxo de sêmen, não houve diferença entre as técnicas de inseminação intracervical e intrauterina. Entretanto, o total de espermatozoides refluídos foi maior nas fêmeas submetidas à inseminação intracervical. A ocorrência de sangramento não diferiu entre as técnicas estudadas. Portanto, qualquer uma das técnicas de inseminação intrauterina pode substituir a inseminação artificial intracervical, pois não comprometem o desempenho reprodutivo dos animais.

Palavras-chave: inseminação artificial intracervical, inseminação artificial intrauterina, refluxo seminal, suínos

Introduction

Swine culture has a great social and economic importance in many countries. There has been a 2.8% per year growth in pork consumption during the last 10 years (ROPPA, 2006). Brazil's production increased sharply after 1990 and presently accounts for 2.71% of the total pork produced worldwide, ranked today as the fifth largest pork producer, with 3,130,000 million t in 2007 (FAOSTAT, 2009).

The increase in domestic swine culture productivity is related to the development of new technologies in practically all areas, including genetics, nutrition, management, plant sanitation and reproduction (ROPPA, 2006). There is no doubt that in the area of reproduction, artificial insemination represents an enormous advance in swine production. Artificial insemination increase is related, in many countries, to the expansion of swine production on an industrial scale, leading to reproductive management on a large scale, with operational advantages over natural mating (Silveira & Scheid, 2005).

Artificial insemination has great advantages, such as genetic gains by using genetically superior males, mating cost reduction per sow, reduced number of boars on the farm, leading to better installation use, sanitary control, improved mating hygiene and spermatozoa quality control as a result of eliminating ejaculates inadequate for use (Hansen, 2004; Silveira & Scheid, 2005).

A new artificial insemination technique has recently been developed: intrauterine artificial insemination (IAIU), with the advantage of allowing a reduction in spermatozoa number and semen cell dose volume. Thus, besides the reduction of the cost of sperm cell dose, it is possible to use genetically superior swines, increasing genetic gain (Williams, 2003; Bennemann et al., 2003; Weber et al., 2003; Hansen, 2004).

This study was carried out to evaluate the spermatic volume and concentration decrease in the reproductive performance of sows inseminated by the intrauterine technique compared to the intra-cervical (IAIC) technique.

Material and Methods

The experiment was conducted in a commercial swine farm in the Zona da Mata in Minas Gerais, from March to November 2006. Three herd reproducers, commercial line hybrids were used, with a mean age of 540 days, previously submitted to semen collections and andrological exams, and considered fit for reproduction. Collections were made between 7:30 am and 8:30 am and 4:00 to 5:00 pm.

Immediately after collection, the semen was transported to the laboratory. The ejaculate was evaluated to determine the processing viability and number of sperm cell doses produced. Only the ejaculates with a minimum of 80% motility, a minimum vigor of 4 (0-5) and a maximum of 20% morphological alterations were used.

After determining the spermatic concentration, the ejaculate was diluted with Beltsvile Thawing Solution" (BTS) (Minitub®) combined with 2.5 IU oxytocin (Prolacton® – Tortuga)/dose (PODDA, 1998). After dilution, the sperm

doses were maintained at ambient temperature (20-24°C) for 120 minutes, and stored at 15-18°C for up to 48 hours.

Camborough 22® sows with 17 to 18 mm fat thickness (point P2) were inseminated and randomly grouped into the treatments according to parity order (from 0 to 8 parities). Two inseminations were conducted: the first immediately after estrus detection (0 hours) and the second 24 hours later. Before the project started, the technicians in charge of the artificial insemination had been trained for 6 months in intrauterine insemination in a pilot project.

Artificial insemination was carried out on 300 sows grouped into the following 5 different techniques: control, intra-cervical (IAIC) with $3x10^9$ spermatozoas/100 mL; intrauterine (IAIU) with $1x10^9$ spermatozoa/100 mL; intrauterine with com $1x10^9$ spermatozoa/50 mL; intrauterine with $5x10^8$ spermatozoa/100 mL; and intrauterine com $5x10^8$ spermatozoa/50 mL.

Intra-cervical artificial insemination was performed using a Melrose (Minitub®) pipette. After being cleaned and dried, the vulvar lips were opened and the artificial insemination pipette was introduced and rotated repeatedly anti-clockwise towards the cervix. At this point, the sperm cell dose was infused for around 10 minutes. Insemination was concluded by removing the pipette in a clockwise movement.

Intrauterine artificial insemination was performed after dry cleaning the vulva and posterior opening of the vulvar lips and introduction of an intra-uterine catheter (Verona - Minitub®). The pipette was fixed in the cervix and a catheter was slowly but firmly introduced through the cervix into the uterine body (20 to 25 cm after the cervix). At this point, the sperm dose was infused for around 10 minutes. At the end of the insemination, the pipette and the catheter were removed.

The intrauterine insemination technique was compared to the intra-cervical technique to evaluate ease of pipette passage, the presence of blood, backflow at the moment of artificial insemination and over a period of 120 minutes, spermatic backflow volume and concentration, return to estrus rate and litter size per parity.

The quantitative variables were submitted to the normality (Lilliefors) and homocedasticity (Cochran) tests followed by analysis of variance. If significant, the means were compared by the Duncan test. If the normality and homogeneity premises were not met, even after the appropriate transformations, the data were submitted to the non-parametric Wilcoxon or Kruskal-Wallis tests (SAEG, 1999). The qualitative variables were compared in contingency tables and analyzed by the Chi-square test at 5% probability (Sampaio, 2002).

Results and Discussion

The results of this study indicated that pipette fixing was adequately performed in 100% of the animals, for both the IAIC and IAIU techniques. Similar IAIU pipette fixing results were obtained by Dallanora et al. (2004a), 97.4%;Bennemann et al. (2004); 100%; Bennemann et al. (2006), 98.1%, and Diehl et al. (2006), 95.9%. However, none of these authors inseminated nulliparous sows and the success reported by Bennemann et al. (2004) of 100% was obtained by inseminating only pluriparous sows.

The intra-cervical insemination pipette was introduced adequately with no difficulty in all the 60 sows. However, the intrauterine insemination pipette was introduced with a small degree of difficulty (4.6%), i.e., out of the 480 inseminations, 22 were performed with a small degree of difficulty when introducing the catheter through the cervical protuberances (Table 1). According to Williams (2003), intrauterine insemination is more invasive, rendering its execution difficult. However, in this study, only a small number of animals presented some difficulty, on account of -the previous training undertaken by the technicians.

The small degree of difficulty for the intrauterine pipette insertion in the 22 inseminations performed was related to the 12 young sows included in the experiment. Despite this fact, the catheter was introduced in the uterus and the intrauterine insemination was successfully performed. Using the same insemination pipette, Diehl et al. (2006) observed that it was not possible to introduce the intrauterine catheter in 4.5% of the sows. This incapacity was probably related to the short insemination time used by the authors (an average of 2.3 minutes/insemination), especially in the primiparous sows, which concentrated most cases of incapacity and difficulty in introducing the intra-uterine catheter. Patience and constant sow stimulation by massaging the back region, at the moment the catheter was introduced, allowed 100% insemination in this study, but no cases such as those described by Diehl et al. (2006) were recorded.

The introduction of the intrauterine insemination pipette in young sows, as performed in this experiment, overcame the difficulties presented by other researchers. Thus, Bortolozzo et al. (2005) characterized the artificial intrauterine technique as impossible to be applied in commercial sows. In addition, Levis et al. (2002) did not recommend intrauterine insemination for either nulliparous or primiparous sows due to the difficulty in introducing the catheter into the cervix, because of the small development of their genital tract, compared to the pluriparous sows. However, Martínez et al. (2001) used a flexible catheter for

Table 1 - Degree of difficulty in the inseminations performed

Description	Insemination technique	
	Intra-cervical	Intrauterine
Number of inseminations	120	480
Degree of difficulty	0a	22b
Number of inseminations with the presence of blood (n)[1]	1.6% (2)	7.7% (37)
Number of sows with the presence of blood (n)[2]	1.6% (2)	6.0 (29)

A difference (P<0.01) was found between the insemination techniques by the Wilcoxon test.
[1] Percentage of inseminations with the presence of blood out of the total performed (Each sow received two inseminations per estrus).
[2] Percentage of sows with the presence of blood in at least one of the inseminations, out of the total performed.

embryo transfer in a procedure very similar to intrauterine artificial insemination and verified that a catheter could be successfully introduced in 10.3% of the sows. In this study, the inseminations presenting a small degree of difficulty in fixing the pipette were observed in primiparous and, mainly, nulliparous sows, confirming the reports by Levis et al. (2002) and Diehl et al. (2006).

In this experiment, the presence of blood was observed in two out of the 120 intra-cervical inseminations (1.6%) performed. In the intrauterine insemination, it was observed in 37 inseminations (7.7%) and 29 sows (Table 1), but these values did not differ (P>0.05) among the techniques studied.

Similar results were obtained by Dallanora et al. (2004b) and Bennemann et al. (2006), who verified blood rates of 9.5 and 8.4% in the sows inseminated, respectively.

Lower values were reported by Watson & Behan (2002), with 1.8% blood presence in females artificially inseminated by the intrauterine technique, and by Bennemann et al. (2004) who reported 1.7%. However, in the study by Watson & Behan (2002), the presence of blood was reported only at the time of intrauterine catheter removal, while in this study, bleeding lasted for up to 120 minutes after insemination. However, both Watson & Behan (2002) and Bennemann et al. (2004) inseminated only pluriparous sows. According to Levis et al. (2002), sows with a higher parity number presented a more developed genital tract than the primiparous and nulliparous sows that facilitated the introduction of the catheter into the cervix, thus reducing lesions.

High bleeding rates were reported by Diehl et al. (2006), who evaluated two different intrauterine insemination pipettes, one formed by a single catheter and the other similar to the one used in this study, consisting of a pipette and an internal catheter. Of the females inseminated, 20.6 and 15.2% presented bleeding when the first and second catheter were used, respectively, in disagreement with the

Table 5 - Total number of spermatozoa during backflow in millions and number of backflows collected using the different insemination techniques

Insemination technique	Number of spermatozoids	Backflow volume in mL (%[1])	Total of backflow sptz in millions (%[1])	Number of backflows collected
Intra-cervical	3×10^9/100 mL	85.8 (85.8%)	782.4 (26.0%)a	23
Intrauterine	1×10^9/100 mL	83.2 (83.2%)	164.0 (16.4%)b	25
Intrauterine	1×10^9/50 mL	41.5 (83.0%)	111.4 (11.1%)b	25
Intrauterine	5×10^8/100 mL	87.8 (87.8%)	80.5 (16.1%)b	28
Intrauterine	5×10^8/50 mL	45.3 (90.6%)	58.0 (11.6%)b	30

[1] Correspond to percentage in the backflow, considering the volume or the total number of spermatozoids of the insemination dose.
No differences (P>0.05) occurred between the insemination techniques by the Kruskal - Wallis test, regarding the collected volume.
There was difference (P<0.05) between the IAIC technique and the IAIU technique by the Kruskal - Wallis test, regarding spermatic concentration.

spermatozoids was lower in the females submitted to intrauterine insemination, compared to those inseminated by intracervical insemination. The deposition of the spermatozoids at the initial/medium third of the uterus probably facilitated their fast progression towards the spermatic reservoirs, thus allowing for higher retention of cells in the genital tract (Dallanora et al., 2004a). Another hypothesis is that the presence of the catheter in the uterine horn can induce the release of hormones that stimulate the uterine contractibility, therefore allowing for more efficient spermatic transport (Martínez et al., 2002).

Among the females under study, 20% received intracervical insemination and 80% received intrauterine insemination. The values obtained at general rate for both techniques were 90.7% and 9.3% for farrowing and estrus repetition, respectively (Table 6). Similar results were reported by Panzardi (2006), who observed percentages corresponding to 90% farrowing and 10% estrus repetition, as well as by Dahl et al. (2006) who obtained an average farrowing rate of 88.2% and 11.8% estrus repetition.

Panzardi (2006) and de Dahl et al. (2006) accomplished three inseminations per dam, whereas two inseminations were performed in the present study. Despite the similar results concerning reproductive performance, the reduced number of inseminations per estrus improved the use of the male on the farm and reduced the costs per inseminated dam without reducing the reproductive indexes (Costa et al., 2003).

Neither farrowing and estrus repetition rates among the inseminated sows were affected compared to inseminations with one billion spermatozoids and that one with three billion spermatozoids. These results showed that the use of one billion spermatozoids in 50 mL can appropriately substitute the traditional technique (IAIC) without harming the reproductive performance of the inseminated animals. These observations corroborate the findings by Watson & Behan (2002), who applied both intracervical and intrauterine insemination under commercial conditions, with three numbers of spermatozoids per dose (1, 2 and 3

billion) and reported that it was possible to reach satisfactory results for parity rate with one billion spermatozoids per dose (86.9%) in intrauterine insemination, compared with two (91.8%) and three (91.1%) billion in intracervical insemination.

Dahl et al. (2006) and Panzardi (2006) inseminated sows using 2.0 and 1.5 billion spermatozoids respectively. They applied the intrauterine insemination technique and obtained a lower farrowing rate than that reported in the present study. In these studies, the sows submitted to the intrauterine insemination technique were inseminated at 12, 24 and 36 hours after detecting the estrus, whereas the inseminations in the present study were accomplished immediately after detecting the estrus and repeated 24 hours after detection. The accomplishment of the first insemination at 12 hours after detecting the estrus might have contributed to these lower rates, mainly when there were failures in the identification of the beginning of the estrus.

The use of 500 million spermatozoids per insemination dose showed farrowing rates corresponding to 90.3% and 90.0% for the volumes of 100 and 50 mL, respectively, that did not differ (P>0.05) from the intracervical insemination. According to Mezalira et al. (2003, 2005) and Bennemann et al. (2006), the use of 500 million spermatozoids per intrauterine insemination technique showed a similar result to intracervical insemination, even with 20 mL volumes (Mezalira et al., 2005; Bennemann et al., 2006). However, Mezalira et al. (2005) studied under experimental conditions with control of the external factors.

In compensation, Dahl et al. (2006) obtained a low farrowing rate (81%) in females inseminated with 500 million spermatozoids in 50 mL volume. As previously mentioned, the inseminations were performed at 12, 24 and 36 hours after detection of the estrus. In the present study, however, the inseminations were performed immediately after detecting the estrus and were repeated after 24 hours. Another factor that probably contributed to obtaining satisfactory pregnancy rates in the present study was the

Table 6 - Farrowing and estrus repetition rates in each insemination technique

Insemination technique	Spermatozoid number	Calving rate % - (n[1])	Estrus repetition rate % - (n[1])
Intracervical	3×10^9/100 mL	90.0% (54)	10.0% (6)
Intrauterine	1×10^9/100 mL	93.3% (56)	6.7% (4)
Intrauterine	1×10^9/50 mL	86.7% (52)	13.3% (8)
Intrauterine	5×10^8/100 mL	93.3% (56)	6.7% (4)
Intrauterine	5×10^8/50 mL	90.0% (54)	10.0% (6)

No differences (P>0.05) occurred between the insemination techniques by Chi-square test.
n[1]: Number of animals which gave birth or which repeated the estrus according to each insemination technique.

use of oxytocin diluted in semen. According to Vera Alcaraz (1994), this allowed for increases from 5 to 7% in pregnancy rates.

The total number of piglets born per farrowing was not affected by the insemination techniques (P>0.05). Thus, under the studied conditions, any spermatic concentrations and tested volumes can substitute traditional insemination without causing damage (Table 7).

Watson & Behan (2002) inseminated sows with one, two and three billion; Dallanora (2004a) and Panzardi (2006) used 1.5 billion spermatozoids per dose; and Bennemann et al. (2004) used one and two billion spermatozoids per dose. They accomplished the analyses by counting the number of embryos in the uterus of the females. These researchers found no differences in the number of piglets born to the animals submitted to the intrauterine and intracervical insemination methods. However, these studies showed differences concerning the amount of spermatozoids, the number of inseminations performed, insemination volume and/or insemination protocol. Similar to the present study, Dahl et al. (2006) inseminated animals by using 500 million spermatozoids. They also highlighted that there were no differences among the treatments, considering the total number of piglets born.

Mezalira et al. (2005) used fewer than than 500 million spermatozoids per dose (250 million spermatozoids) via IAIU under experimental conditions. They concluded that the number of embryos in the females inseminated with 500 million spermatozoids was superior, compared to those inseminated with 250 million. According to these authors, the treatments with 250 million spermatozoids rather damaged the formation of the spermatic reserve, and consequently the complete fecundation of the ovulated oocytes. This result showed that the minimum spermatozoid concentration per insemination dose that does not damage the reproductive performance of the inseminated females is 500 million spermatozoids per dose.

Mezalira et al. (2005) and Bennemann et al. (2005) carried out an evaluative test under field conditions. When using IAIU with 500 million spermatozoids in 20 mL and comparing the IAIC with three billion spermatozoids and found no difference for farrowing rate between the treatments. However, a considerable reduction was found in the total number of the piglets born by the IAIU method (P<0.05). In the present study, however, no differences (P>0.05) were found in the total number of piglets born, even when the animals were submitted to IAIU insemination with 500 million spermatozoids per 50 mL dose. According to Williams (2003), the protocols involving treatments with dose volumes corresponding to 30 mL lead to a decrease in the number of piglets born, even at concentrations higher than those used by Benneman et al. (2005). The use of semen-diluted oxytocin might have also contributed to the improvement of the born piglet numbers observed in the present study, compared to the results by Bennemann et al. (2005), since the use of this hormone at 2.5 UI concentration increases the number of piglets born per sow (Costa et al., 1999).

In this study, the employees previous training in new technology prior to performing the experiment appears to have been an important factor in obtaining the results. In this context, there is a need for skilled employees to apply the IAU techniques, especially in studies carried out under

Table 7 - Number of farrowings and total piglets born per farrowing

Insemination technique	Number of spermatozoids	Number of farrowings	Number of newborns by farrowing
Intracervical	3×10^9/100 mL	54	11.5 ± 3,4
Intrauterine	1×10^9/100 mL	56	11.7 ± 3,4
Intrauterine	1×10^9/50 mL	52	11.4 ± 3,2
Intrauterine	5×10^8/100 mL	56	11.8 ± 3,0
Intrauterine	5×10^8/50 mL	54	11.4 ± 3,6

There were no differences (P>0.05) between the insemination techniques by the Kruskal – Wallis test.

field conditions (Echegaray, 2003), and who are attentive to fundamental matters such as hygiene and complete ejection of the insemination dose (Bernardi et al., 2005).

The effect of the employees training was highlighted by Williams (2003), who evaluated the number of the pigs born per sow, relative to insemination weeks. As the experiment was carried out, there was a significantly increased number of the piglets born from the females submitted to IAIU, thus showing that both practice and follow-up of the employees lead to better results.

Conclusions

The application of the intrauterine artificial insemination technique using 500 million spermatozoids in 50 mL did not endanger the reproductive performance of the inseminated animals. Intrauterine artificial insemination did not affect the occurrence of backflow and made lower spermatic losses possible, compared with the traditional insemination technique. The bleeding caused by the intrauterine insemination technique did not affect the fertility or the number of piglets born.

Acknowledgements

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