

## NUMBER OF ARTIFICIAL INSEMINATIONS PER ESTRUS IN SWINE: ASSOCIATION WITH ESTRUS PROFILES AND IMPACT ON REPRODUCTIVE PERFORMANCE

MARCUS VINICIUS FIGUEIRA DE ALVARENGA,<sup>1</sup> IVAN BIANCHI,<sup>1</sup> ANTONIO SERGIO VARELA JÚNIOR,<sup>1</sup> ODIRLEI CALDERAM,<sup>1</sup> EDUARDO SCHMITT,<sup>1</sup> MARCIO NUNES CORRÊA,<sup>1</sup> JOÃO CARLOS DESCHAMPS<sup>1</sup> E THOMAZ LUCIA JR.<sup>1</sup>

1. PIGPEL – Biotechnology Center, Veterinary School, Pelotas Federal University. E:mail thomaz@ufpel.edu.br

### ABSTRACT

In swine, multiple artificial inseminations (AI) are commonly performed per estrus due to the long estrus duration. This observational study aimed to evaluate the association among the number of AI conducted per estrus, the estrus duration and the subsequent reproductive performance on a commercial swine farm. After evaluation of both estrus profile and follicle dynamics using ultrasound, by two independent teams, the number of AI per estrus was recorded, with no interference in the farm habitual management. The first AI was done at the time of estrus detection and subsequent AIs at 12 h intervals, as long as females showed estrus signs. The risk of conducting more than three AI per estrus as a function of estrus duration was also estimated. For females

receiving two AI per estrus, farrowing rate (92.0%) was higher ( $p < 0.05$ ) than for those receiving three (77.5%) or four to six AI (79.6%). Total litter size did not differ ( $p > 0.05$ ) for females receiving two, three or 4 to 6 AI per estrus (10.6, 12.0 and 12.0, respectively). For females having estrus longer than 78 h, the odds of receiving 4-6 AI were 6.1 times higher than for those having estrus shorter than 50 h, and 3.4 times higher than for those having estrus within 50 to 74 h (both  $p < 0.05$ ). Thus, although long estrus duration allows multiple AI per estrus, we concluded that more than three AIs per estrus are not recommended because there is no benefit for subsequent reproductive performance.

KEYWORDS: estrus duration, farrowing rate, litter size, swine..

### RESUMO

#### NÚMERO DE INSEMINAÇÕES ARTIFICIAIS POR ESTRO EM FÊMEAS SUÍNAS: ASSOCIAÇÃO COM O PERFIL ESTRAL E IMPACTO SOBRE O DESEMPENHO REPRODUTIVO

Em suínos, é comum realizar múltiplas inseminações artificiais (IA) por estro, em função da longa duração do estro. Este estudo observacional teve o objetivo de verificar a associação entre o número de IA realizadas por estro com a duração do estro e o desempenho reprodutivo subsequente, em uma granja comercial. Após avaliação do perfil estral e da dinâmica folicular por ultrassonografia, por equipes independentes, o número de IA por estro foi registrado, sem interferência no manejo da granja. A primeira IA era realizada no momento da detecção do estro e as IA subsequentes eram realizadas em intervalos de 12 h, enquanto

a fêmea mostrasse sinais de estro. A taxa de parição e o tamanho total da leitegada foram comparados em função do número de IA por estro. Também foi estimado o risco da realização de mais de 3 IA por estro, em função da duração do estro. A taxa de parição das fêmeas que receberam duas IA (92,0%) foi maior ( $p < 0,05$ ) do que a observada para fêmeas que receberam três (77,5%) ou 4 a 6 IA (79,6%). O tamanho total da leitegada não diferiu ( $p > 0,05$ ) entre fêmeas que receberam duas, três ou 4 a 6 IA (10,6, 12,0 e 12,0, respectivamente). Para as fêmeas com duração do estro superior a 78 h, o risco de receberem 4 a 6 IA durante o estro foi 6,1 vezes maior

do que o risco para fêmeas com duração do estro inferior a 50 h ( $p < 0,05$ ) e 3,4 vezes maior do que o risco para fêmeas com duração do estro entre 50 a 74 h ( $p < 0,05$ ). Ainda que a longa duração do

estro permita um maior número de IA por estro, pode-se concluir que um número de IA por estro superior a três não é recomendado, pois não há benefício para o desempenho reprodutivo.

PALAVRAS-CHAVES: duração do estro, taxa de parição, tamanho de leitegada, suínos.

## INTRODUCTION

Artificial insemination (AI) has been increasingly used in swine breeding because of the genetic, economic and sanitary benefits (DESCHAMPS et al. 1988; CORRÊA et al., 2001). Current assessments indicate that at least 50% of females reared in technological production systems are artificially inseminated (BORTOLOZZO & WENTZ, 2005). Therefore, the protocols used in AI may have an impact on the reproductive efficiency of these systems.

The implementation of AI in the period from 28 h before ovulation to up to 8 h after ovulation is associated to the obtainment of adequate indicators of reproductive performance (KEMP & SOEDA, 1996; NISSEN et al., 1997); thus, determining the time of ovulation is critical for the success of AI programs. In general, ovulation in sows occurs at the beginning of the final third of estrus, which is characterized by variable and relatively long duration, between 48 and 62 h (WEITZ et al. 1994; NISSEN et al. 1997; LUCIA et al., 1999; ALVARENGA et al., 2006). The duration of estrus can be estimated according to the weaning-to-estrus interval (WEI), considering that females with shorter WEI tend to stay longer in estrus (WEITZ et al. 1994; KEMP & SOEDA, 1996). However, as this association is not consistent, estimates of the ovulation timing based only on this negative association between duration of estrus and WEI are inaccurate (LUCIA et al. 1999; CORRÊA et al. 2002; ALVARENGA et al., 2006). By using ultrasound, the diagnosis of ovulation may be carried out with greater accuracy (WEITZ et al. 1994; KEMP & SOEDA, 1996; NISSEN et al. 1997; VIANA et al. 1999; ALVARENGA et al., 2006), but its routine use is still limited because the equipment is expensive and its handling requires specialized training.

Due to prolonged duration of estrus, multiple AI can be used to compensate the lack of precision in assessing the ovulation time. In general, in the AI protocols used on commercial farms, the procedure is performed twice to three times during estrus, each AI with a total sperm between 2.5 and 3.0 x10<sup>9</sup> (JOHNSON, 2000; CORRÊA et al. 2001; RATH, 2002; BORTOLOZZO & WENTZ, 2005). Nevertheless, in some commercial farms, the females are consecutively inseminated, while showing signs of estrus, and hence, they are often inseminated more than three times during this period. This study examined the association between the number of AI performed during estrus with the females' estrous profile and their subsequent reproductive performance.

## MATERIAL AND METHODS

This study was conducted in a commercial farm, located in the northwestern region of Rio Grande do Sul state, with a herd constituted of 2250 sows from the same genetic basis. Half of the weaned sows were randomly selected from each weekly group of weaning. Females which showed estrus at weaning or the ones which did not present it within 10 days after weaning were not evaluated, and those which were subsequently discarded because of reproductive problems were excluded. The study followed an observational design, and the data were collected without interference in the management performed at the commercial farm. After weaning, the evaluations of the follicular dynamics and estrous profiles were initiated by two teams which worked simultaneously, though each team was not aware about the data collected by the other. Estrus detection was based on the observation of the reflex of tolerance to man in the presence of a sexually mature male (RTMM), in three shifts (6:30 am, 2:30 and

10:30). Males were led by a trained technician, through the aisle between the rows of individual cages in front of the females. The onset of estrus was characterized by the first positive RTMM and the end of estrus by the first negative RTMM. The WEI was calculated by the interval between the weaning and the estrus onset, and the duration of estrus was calculated by the interval between the beginning and end of estrus (WEITZ et al. 1994; LUCIA et al. 1999; VIANA et al. 1999).

Evaluation of follicular dynamics was made by ultrasound in real time, through transcutaneous technique, with 5.0 MHz convex transducer (Anser Vet 485, Pie Medical ®) (SOEDA & KEMP, 1993; ALVARENGA et al., 2006). The females were examined in standing position, twice a day (6:30am and 2:30pm). At examination, carboxy-methylcellulose gel was used, at the contact surface of the transducer, to ensure the sound propagation. The transducer was positioned on the right flank, midway between femoro-tibio articulation (stifle joint) and the last rib, 10 cm above the udder. Bladder in caudal position and intestinal loops in cranial position were considered as reference points for locating the ovary. The ovulation was defined as the absence of pre-ovulatory follicles, or as a decreased number of follicles compared to the number found in the previous test, but these findings had to be confirmed by the next examination in order to avoid false positive diagnoses.

The inseminations were performed according to protocols for estrus detection routinely conducted by the farm staff, with estrus detection based on RTMM observation twice a day (8:00am and 4:30pm). During the evaluation period, 168 females were inseminated with semen doses of 100 mL and  $3 \times 10^9$  spermatozoa, diluted in Beltsville Thawing Solution (BTS) (Pursel & Johnson, 1975) and used within 48 h after collection. The first AI was performed at the moment of estrus diagnosis (0 h), and the other AI every 12 h until females stopped showing signs of estrus. The number of AI performed during estrus was recorded. The farrowing rate was calculated by dividing the number of births by the total number of inseminated females.

The total number of piglets born in the subsequent birth was extracted from the farm database. Since the maximum number of AI for estrus observed in this study was equal to six, females which received more than three inseminations were grouped into a single category (4-6 AI).

The association between litter size and number of AI was evaluated by analysis of variance, with comparisons of means by the method of least significant difference (LSD). The association between farrowing rate and the number of AI was assessed by the Chi-square test. A logistic regression analysis was performed to evaluate the association between the number of inseminations during estrus and estrus duration in females, considering control the females which received two or three AI during estrus, and in cases in which females received between 4-6 AI. Estrus duration was categorized as <50 h, 50-74 h and > 78 h. The risk of females classified into different categories of estrus duration undergoing 4 to 6 AI during estrus was estimated by the odds ratio, whose significance was determined by confidence intervals at a significance level of 95% and the Chi-square test. All statistical analyses were generated by the program STATISTIX ® (2003).

## RESULTS AND DISCUSSION

Females evaluated in this study had the same birth order of  $3.3 \pm 2.7$  with farrowing rate of 80.4% and total litter size equal to  $11.8 \pm 3.3$ . The effect of parity on farrowing rate and litter size was not significant ( $p > 0.05$ ). Even though the average litter size was within the desired level, the farrowing rate observed was below acceptable levels in modern swine production systems (DIAL et al., 1992). More than half of females inseminated received three AI by estrus (Table 1), showing lower farrowing rate ( $p < 0.05$ ) than the females which received two AI (77.5 and 92.0%, respectively). However, this advantage in farrowing rate might be considered questionable, due to the substantial numerical disadvantage between the litter size of these females (10.6) and of the females

inseminated three or more times (12.0).

As an example, if we considered the farrowing rate and the total size of the litter observed in this study, 25 females with two AI per estrus would have produced 244 piglets. But if, at the same farrowing rate, total litter size was equal to that one observed for females with three AI per estrus, they would have produced 276 piglets (i.e. 32 more piglets). Therefore, although the observed difference was not significant, it would be relevant in field conditions. The lack of statistical significance may be due to the relatively small number of females evaluated, justified by the observational model used in this study. A greater number of females could not be used in this study due to the lack of human resources, within the time intervals required for the evaluation, even though a greater number of animals could be advisable under a statistical point of view.

Less than 15% of the evaluated females received two AI per estrus, suggesting that at the farm, the number of AI per estrus is proportional to the length of estrus, as females who stayed longer in estrus were inseminated more times (Table 2). In this context, females with two AI per estrus would not have shown estrus long enough to be inseminated at least a third time. The carry-out of assessments by ultrasound in shorter intervals would allow greater precision in determining the time of ovulation; however, due to the lack of human resources mentioned above, performing such assessments in shorter intervals would be possible only by ending the independence of two teams involved in the study, which would undermine the established methodology. Still, the ovulation timing observed in this study (Table 2) is consistent with estimates obtained from assessments within shorter intervals (KEMP & SOEDA, 1996).

**TABLE 1.** Farrowing rate and total litter size in function of the number of artificial insemination (AI) during estrus.

AI	n (%)	Farrowing rate (%)	Total of piglets/birth
2	25 (14.9)	92.0a	10.6 ± 0.7a
3	89 (53.0)	77.5b	12.0 ± 0.4 <sup>a</sup>
4 to 6	54 (32.1)	79.6b	12.0 ± 0.5 <sup>a</sup>

a, b Average in the column differed statistically ( $p < 0.05$ ).

**TABLE 2.** Number of artificial insemination (AI) during estrus in function of the occurrence of estrus and ovulation (n = 168).

Parameter	AI during estrus			Average
	2	3	quatro a seis	
Weaning-estrus (h)	106.5 ± 8.8b	84.1 ± 3.3 <sup>a</sup>	83.0 ± 4.1a	87.3 ± 29.1
Estrus duration (h)	53.3 ± 3.4a	61.8 ± 1.8b	70.1 ± 2.3c	63.2 ± 17.8
Beginning of heat-ovulation (h)	50.7 ± 2.9a	45.7 ± 1.5 <sup>a</sup>	50.4 ± 1.9a	47.9 ± 14.3

a, b Average in lines differed significantly ( $p < 0.01$ ).

On the other hand, over 30% of females were inseminated four or more times during estrus (Table 1), presenting farrowing rate and litter size similar to those of the females inseminated three times ( $p > 0.05$ ). There-

fore, the use of more than three AI during estrus did not bring benefits to reproductive performance, producing only an increase in the cost per inseminated female. In some ways, these results are consistent with studies conducted with natural mating (XUE et al., 1998a, b),

which reported similar reproductive performance with only two or three matings per estrus, even though, in this study, the number of mating by females has been allocated at random, regardless of estrus length. The apparent benefit observed in litter size of females with three or four to six AI per estrus compared with those with two AI per estrus may not be relevant regarding the low farrowing rate observed in both categories.

For females receiving three AI per estrus, the lowest farrowing rate could be due to the protocol used for the procedure, because with the first AI occurring at the time of estrus detection and the others at 12 h intervals, the third AI would occur 24 h after estrus detection. Considering that the females that received 3 AI during estrus ovulated around 45 h after estrus detection (Table 2) and that ovulation occurs in the final third of estrus, regardless of the duration of estrus (KEMP & SOEDA, 1996; NISSEN et al. 1997, CORRÊA et al., 2002), it is possible that a different protocol, with the first AI 12 h after estrus detection, could be better synchronized with ovulation, which probably would be beneficial to reproductive performance, considering the total size of the litter observed for females receiving three AI per estrus. It is noteworthy that the information related to estrus detection and follicular dynamics collected by the teams who worked on this study were not known by the farm team that conducted the AI according to predetermined protocols for their routine work.

The estrus of the females which received 4-6 AI was longer than the one of the females inseminated less often (Table 2). The averages of WEI, estrus duration and ovulation time were consistent with other studies (WEITZ et al. 1994; SOEDA & KEMP, 1993; KEMP & SOEDA, 1996; LUCIA et al, 1999; VIANA et al.,

1999). Weaning-to-estrus interval (WEI) was longer in females inseminated twice, which also had shorter estrus. However, despite of the number of AI per estrus being higher as the estrus was longer, WEI did not differ for females inseminated three times or 4-6 times per estrus ( $p > 0.05$ ). These data confirm that, although there is a negative association between WEI and estrus duration (WEITZ et al. 1994; KEMP & SOEDA, 1996), the duration shows limited intensity and therefore cannot be used to accurately estimate ovulation timing (LUCIA et al. 1999; CORRÊA et al. 2002; ALVARENGA et al., 2006).

For the females with estrus duration longer than 78 h, there was a 6.1 times greater risk of receiving four or more AI per estrus ( $P = 0.0003$ ) than for females with estrus shorter than 50 h, and a 3.4 times greater risk ( $P = 0.0028$ ) than for females with estrus duration between 50 and 74 h (Table 3), although this risk was similar ( $p > 0.05$ ) for females with estrus duration between 50 to 74 h or shorter than 50 h. Thus, due to the great number of AI performed in females with longer estrus, it is likely that one or more inseminations were carried out in metaestrus, if estrus detection carried out by the farm staff was deficient. In such cases, leukocyte infiltration could occur in the female's genital tract to eliminate potential aggressors, such as infectious agents or sperm present in their interior, which is associated with a higher frequency of pathological processes, such as endometritis and vulval discharges, which can be associated with an increase in the rate of return to estrus (ROZEBOOM et al. 1997, 1999; ALTHOUSE et al. 2000; KAEOKET et al., 2005), explaining the decrease of farrowing rate observed in females with 4-6 AI during estrus.

**TABLE 3.** Risk of occurrence of four or more inseminations per estrus according to the estrus duration

Estrus duration		Estrus duration	
		50 a 74 h	> 78 h
< 50 h	Odds ratio	1.79	6.11
	Confidence interval (95%)	0.75 - 4.32	2.17 - 17.17
	P	0.1868	0.0003
50 a 74 h	Odds ratio	-	3.40
	Confidence interval (95%)	-	1.49 - 7.74
	P	-	0.0028

## CONCLUSIONS

Although the long estrus duration in swine allow the performance of multiple inseminations per estrus, the performance of more than three inseminations is not recommended because it does not show benefits regarding the farrowing rate and the litter size.

## REFERENCES

- ALTHOUSE, G. C.; KUSTER, C. E.; CLARK, S. G.; WEISIGER, R. M. Field investigations of bacterial contaminants and their effects on extended porcine semen. **Theriogenology**, v. 53, p. 1167-1176, 2000.
- ALVARENGA, M. V. F.; BIANCHI, I.; SCHMITT, E.; VARELA JUNIOR, A. S.; CALDERAM, O.; CORRÊA, M. N.; DESCHAMPS, J. C.; LUCIA, T. JR. Characterization of estrus profile in female swine and its accuracy in estimating ovulation time in comparison to ultrasound diagnosis. **Animal Reproduction**, v. 3, p. 364-369, 2006.
- BORTOLOZZO, F. P.; UEMOTO, D. A.; BENNEMANN, P. E.; POZZOBON, M. C.; CASTAGNA, C. D.; PEIXOTO, C. H.; BARIONI JR. W.; WENTZ, I. Influence of time of insemination relative to ovulation and frequency of insemination on gilt fertility. **Theriogenology**, v. 64, p. 1956-1962, 2005.
- BORTOLOZZO, F. P.; WENTZ, I. Situação da IA em suínos no Brasil e no mundo. In: BORTOLOZZO, F.P.; WENTZ, I. **Inseminação artificial na suinocultura tecnificada**. Porto Alegre, RS: Pallotti, 2005. 183 p.
- CASSAR, G.; KIRKWOOD, R. N.; POLJAK, Z.; BENNETT-STEWART, K.; FRIENDSHIP, R. M. Effect of single or double insemination on fertility of sows bred at an induced estrus and ovulation. **Journal of Swine Health and Production**, v. 13, p. 254-258, 2005.
- CORRÊA, M. N.; MEINCKE, W.; LUCIA, T. JR.; DESCHAMPS, J. C. **Inseminação artificial em suínos**. Londrina, PR: Printpar, 2001. 181 p.
- CORRÊA, M. N.; LUCIA, T. JR.; AFONSO, J. A. B.; DESCHAMPS, J. C. Reproductive performance of early-weaned female swine according to their estrus profile and frequency of artificial insemination. **Theriogenology**, v. 58, p. 103-112, 2002.
- DESCHAMPS, J. C.; CORRÊA, M. N.; LUCIA, T. JR. Impacto da inseminação artificial em suínos. **Revista Brasileira de Reprodução Animal**, v. 22, p. 75-79, 1998.
- DIAL, G. D.; MARSH, W. E.; POLSON, D. D.; VAILLANCOURT, J-P. Reproductive failure: differential diagnosis. In: LEMAN, A. D.; STRAW, B. E.; MENGELING, W. L.; D'ALLAIRE, S.; TAYLOR, D. J. (Ed.). **Diseases of swine**. 7. ed. Ames, IA: Iowa State University Press, 1992. p. 88-137.
- FOXCROFT, G. R.; DYCK, M. K.; RUIZ-SANCHEZ, A.; NOVAK, S.; DIXON, W. T. Identifying useable semen. **Theriogenology**, v. 70, p. 1324-1336, 2008.
- JOHNSON, L. A.; WEITZE, K. F.; FISER, P.; MAXWELL, W. M. C. Storage of boar semen. **Animal Reproduction Science**, v. 62, p. 143-172, 2000.
- KAEOKET, K.; TANTASUPARUK, W.; KUNAVONGKRIT, A. The effect of post-ovulatory insemination on the subsequent embryonic loss, oestrous cycle length and vaginal discharge in sows. **Reproduction in Domestic Animals**, v. 40, p. 492-494, 2005.
- KEMP, B.; SOEDE, N. M. Relationship of weaning-to-estrus interval to timing of ovulation and fertilization in sows. **Journal of Animal Science**, v. 74, p. 944-9, 1996.
- LUCIA, T. JR.; CORRÊA, M. N.; DESCHAMPS, J. C.; PERUZZO, I. A.; MATHEUS, J. E. M.; ALEIXO, J. A. G. Influence of equine chorionic gonadotropin on weaning-to-estrus interval and estrus duration in early-weaned primiparous female swine. **Journal of Animal Science**, v. 77, p. 3163-3167, 1999.
- NISSEN A. K.; SOEDE N. M.; HYTTEL, P.; SCHMIDT, M.; D'HOORE, L. The influence of time of insemination relative to time of ovulation on farrowing frequency and litter size in sows, as investigated by ultrasonography. **Theriogenology**, v. 47, p. 1571-1582, 1997.
- PURSEL, V. G.; JOHNSON, L. A. Freezing of boar spermatozoa: fertilizing capacity with concentrated semen and a new thawing procedure. **Journal of Animal Science**, v. 40, p. 99-102, 1975.
- RATH, D. Low dose insemination in the sow: a review. **Reproduction in Domestic Animals**, v. 37, p. 201-205, 2002.
- ROZEBOOM, K. J.; TROEDSSON, M. H. T.; SHURSON, G. C.; HAWTON, J. D.; CRABO, B. G. Late estrus and metestrus insemination after estrual insemination decreases farrowing rate and litter size in swine. **Journal of Animal Science**, v. 75, p. 2323-2327, 1997.
- ROZEBOOM, K. J.; TROEDSSON, M. H. T.; MOLITOR, T. W.; CRABO, B. G. The effect of spermatozoa and seminal plasma on leukocyte migration into the uterus of gilts. **Journal of Animal Science**, v. 77, p. 22201-22206, 1999.
- STATISTIX®. Statistix® 8 Analytical software. Tallahassee, FL., 2003.

SOEDE, M. N.; KEMP, B. In synchronized pigs, the duration of ovulation is not affected by insemination and is not a determinant for early embryonic diversity. **Theriogenology**, v. 39, p. 1043-1053, 1993.

VAZQUEZ J. M.; ROCA J.; GIL, M.; CUELLO, A.; PARRILLA, C. I.; VAZQUEZ, J. L.; MARTINEZ, E. A. New developments in low-dose insemination technology. **Theriogenology**, v. 70, p. 1216-1224, 2008.

VIANA, C. H. C.; SILVEIRA, P. R. S.; MORETTI, A. S.; RODRIGUES, P. H. M. Relationships between the characteristics of weaning-to-estrus interval, estrus duration and moment of ovulation by ultrasonography in sows. **Brazilian Journal of Veterinary Research and Animal Science**, v. 6, p. 205-211, 1999.

WEITZE, K. F.; WAGNER-RIETSCHER, H.; RICHTER, L. H.; WABERSKI, D. The onset of heat after weaning, heat duration, and ovulation as major factors in AI timing in sows. **Reproduction in Domestic Animals**, v. 29, p. 433-443, 1994.

XUE, J. L.; LUCIA, T. JR.; KOKETSU, Y.; DIAL, G. D.; MARSH, W. E. Influence of mating frequency and weaning-to-mating interval on sow reproductive performance. **Swine Health and Production**, v. 6, p. 157-162, 1998a.

XUE, J. L.; DIAL, G. D.; TRIGG, T.; DAVIES, P. R.; KING, V. L. Influence of mating on sow reproductive performance. **Journal of Animal Science**, v. 76, p. 2962-2966, 1998b.

---

Submitted on October 23, 2007. Accepted on January 26, 2010