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Reproductive performance of sows submitted to intrauterine insemination at different pre-ovulatory intervals

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Abstract

This study evaluated the effect of the insemination-ovulation (AIOV) interval and sperm cell dose (SD) on the reproductive performance of sows submitted to intrauterine artificial insemination (IUI). The experimental design involved a factorial 2x2 ($1x10^9$ spermatozoa (1 bi) and $2x10^9$ spermatozoa (2 bi) at 0-24 and 25-36h insemination-ovulation intervals). Estrus detection and time of ovulation (assessed by transcutaneous ultrasonography) were performed twice a day at 12 h intervals. After the onset of estrus sows were distributed into the four treatments receiving a single IUI. A total of 66 PIC Camborough 22 sows were inseminated with a flexible catheter and a 60 ml SD stored at 17°C up to 36 hours. Pregnancy was determined by transcutaneous ultrasonography 20-23 days after IUI. Pregnant sows (51/57) were slaughtered at 31.7±4.3 days of pregnancy and total embryos (TE) and corpora lutea were counted. Pregnancy rate was analyzed by chi-square test. TE and embryonic survival (ES) were analyzed by GLM procedure and means were compared by Tukey's test. No interaction was observed ($P>0.05$) between SD and AIOV interval. PR and ES did not differ between SD or AIOV intervals ($P>0.05$). TE was not affected by SD but it was lower ($P<0.05$) for the interval insemination-ovulation of 25-36h compared to 0-24h.

Keywords: intrauterine insemination, reduced sperm number, sows, insemination-ovulation interval

Introduction

Reproductive performance of sows is affected by many factors as artificial insemination-ovulation interval (AIOV) and site of semen deposition. According to Waberski *et al.* (1994), the AIOV interval has to be considered as a major factor of variation in fertility results not related to semen quality. Studies involving real-time ultrasonography showed that the optimal interval for insemination was 0 to 24 h before ovulation in sows (Soede *et al.*, 1995), being possible to extend it to 28 h before until 4 h after ovulation without affecting farrowing rate and litter size (Nissen *et al.*, 1997).

The site of semen deposition in the female genital tract is another aspect that contributes to an optimal fertilization rate. Although Hancock (1959) had described a non-surgical technique for semen deposition into the uterus, in the 50's, only in the 90's this technique was improved (Vasquez *et al.*, 1999). The intrauterine insemination (IUI) allows a reduction of sperm cell dose and volume without a negative effect to the fertility (Martinez *et al.*, 2001). However, the optimal AIOV interval and its interaction with different sperm numbers is poorly known. The aim of this study was to verify the reproductive performance of intrauterine inseminated sows with reduced sperm number at different intervals before ovulation.

Materials and Methods

Animals

A total of 66 PIC Camborough 22® sows was inseminated. Based on parity (3-9), lactation length (17-31 days), average of piglets born in life (>10 piglets), weaning-to-estrus interval (2-6 days) and visual body condition (2-5) sows were paired and assigned to one of the four treatments. After weaning the sows were housed in individual crates and received 2 kg daily of feed (3.100 Kcal DE and 14% CP) and water *ad libitum*.

Estrus detection

Estrus detection was performed at intervals of 12 h (08:30 and 20:30 h) using a mature boar. The onset of estrus was defined as the first time when the sow showed a standing response to the back pressure

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in the presence of the boar, minus 6 h. The end of estrus was defined as the last time when the sow showed a standing response to the back pressure test, plus 6 h.

Time of ovulation

Transcutaneous ultrasonography of ovaries (Weitze *et al.*, 1989) was performed during the estrus at 12 h intervals (08:30 and 20:30 h) beginning in the next turn after the onset of estrus. A 5 MHz sector scanner (Aloka Co., Ltd., Mure, Mitaka-shi, Tokyo 181-8622, Japan) was used to observe follicles presence and to determine the moment of ovulation. The time of ovulation was defined as the interval between the onset of estrus and the first time when no follicles were observed, minus 6 h. The ovulation was confirmed by one additional scanning 12 h later.

Insemination doses

Semen of three mature boars Agroceres PIC® was collected twice weekly. The semen was macro (color, odor, aspect and volume) and microscopically (motility, spermatic concentration and morphology) evaluated. Only ejaculates that presented at least 75% of motility were utilized. Spermatic concentration (sperm/mL) was evaluated by Neubauer Improved® chamber counting. The sperm cells in the semen doses (SD) were recounted after dilution. SD were prepared from a single boar. Doses contained 1 or 2 billion spermatozoa extended in BTS, in a total volume of 60 mL and were stored at 15-18°C up to 36 h.

Intrauterine Insemination

Intrauterine insemination was performed between 12-24 h after estrus onset. Sows were inseminated once with 1 or $2x10^9$ spermatozoa at 0-24 or 25-36 h before ovulation with a Verona (Minitub®) catheter model. After fixing the pipette in the cervix a catheter was slowly introduced into the uterine body and the SD was infused into one uterine horn (200 mm beyond the cervix). All IUI were performed in the presence of a mature boar.

Pregnancy detection

The pregnancy detection was performed through transcutaneous ultrasonography at 21-23 days after IUI. The sows were slaughtered at 31.7 ± 4.3 days of pregnancy and their genital tracts were removed. The corpora lutea (CL) and total embryos (TE) were counted.

Experimental design and statistical analyses

The semen was processed in a split-sample basis. Sows were separated according to AIOV intervals: 0-24 or 25-36 h before ovulation. Those with AIOV interval higher than 36 h were not included in the analysis. The variable pregnancy rate (PR) was analyzed by the Chi-square test. TE and embryonic survival (ES) were analyzed using the GLM procedure (SAS, 1998). Effects of boar, SD, AIOV intervals and of their interaction were considered in the model. Furthermore, number of corpora lutea and average of piglets born in life were included as co-variables. The means were calculated by the LSMEANS procedure (SAS, 1998) and compared by the Tukey's test.

Results

Nine sows were excluded of the analysis because their AIOV interval was higher than 36 h. The estrus length average of all the sows was 59.8 ± 16.3 h (24-120h) and average interval between estrus onset and moment of ovulation was 42.6 ± 7.7 h (24-60h). The average of SD storage at IUI moment was 13.7 ±1 3.7 h (0-36h). In this study it was possible to insert the flexible catheter through the cervix into the uterus in all sows. There was no semen backflow during insemination. The blood presence on the catheter tip was observed in 1.7% (1/57) of sows.

There was no effect of the interaction between spermatozoa numbers (1 or $2x10^9$ sperm) and AIOV intervals (0-24 or 25-36 h) on TE and ES (P>0.05). The average of piglets born in life and the CL remained as co-variables in the model of TE and ES analysis, respectively. Boar had no effect on pregnancy rate, TE and ES (P>0,05).

PR and ES were not affected neither by sperms number in the SD (P>0.05) nor by AIOV intervals (P>0.05). TE was not affected by sperms number in the SD (P>0.05), but it was affected (P<0.05) by AIOV interval (Tab.1).

Table 1. Pregnancy Rate (PR), Corpora Lutea (CL), Total Embryos (TE) and Embryo survival (ES) in sows submitted to one intrauterine insemination with 1 or $2x10^9$ spermatozoa at 0-24 or 25-36 h before ovulation.

		PR% (n/n)	n	CL*	TE*	ES (%)*
Dose (x 10^9)	1	82.1 (23/28)	21	20.6 ± 2.9	15.9 ± 2.4	69.8 ± 12.0
	2	96.5 (28/29)	27	22.3 ± 4.4	14.9 ± 4.2	64.7 ± 19.6
Interval (h)	0-24	88.2 (30/34)	29	21.3 ± 2.8	16.4 ± 3.3[a]	70.3 ± 13.6
	25-36	91.3 (21/23)	19	22.0 ± 5.2	14.4 ± 3.6[b]	64.2 ± 21.2

*Adjusted means ± Standard deviation; a, b in the column differ (P<0.05).

Discussion

Sows were intrauterine inseminated once with 1 or $2x10^9$ sperms at 0-24 or 25-36 h before ovulation. On average, ovulation occurred at 72% of the estrus period which is similar to the range of 68-72% reported previously for sows (Soede *et al.*, 1995; Nissen *et al.*, 1997; Steverink *et al*., 1997).

In this study it was possible to insert the flexible catheter through the cervix in all sows, corroborating with results of other studies in which 94.0 and 97.4% of success was achieved with this method (Roca *et al.*, 2003 and Dallanora *et al*, 2003a, respectively). The presence of blood in the catheter tip was observed in 1.7% of sows. Similar results (1.7%) were observed by Watson and Behan (2002).

Semen backflow during insemination did not occur what is consistent with other studies in which females were intrauterine inseminated with semen doses of 60 or 20 mL (Dallanora *et al.*, 2004). In females inseminated traditionally with 80 mL doses, Steverink *et al.* (1998) observed that more than 5% of the inseminated spermatozoa in backflow during insemination affected fertilisation negatively in those sows inseminated with 1 billion spermatozoa compared to those receiving 3 or 6 billion doses. However, backflow after insemination had no effect on fertilisation results. Recently, it has been observed, that even with intrauterine deposition, backflow semen after insemination is possible (Dallanora *et al.*, 2004). These authors reported that average volume of backflow ranged from 64% to 75% in females inseminated with 0.25 to 1 billion spermatozoa in 20 mL or 1.5 billion in 60 mL. The average percentage of spermatozoa in the backflow after insemination ranged from 12% for doses of 0.25, 0.5 or 1 billion spermatozoa in 20 mL to 23% for 1.5 billion in 60 mL. A combination of low dosage and loss of spermatozoa due to backflow during insemination, may lead to sub-optimal fertilisation results (Steverink *et al.*, 1998). Steverink *et al.* (1998) observed that the concentration of spermatozoa in backflow during traditional insemination is higher if compared to the backflow after insemination. Therefore, one of the advantages of intrauterine insemination would be the absence of backflow during insemination. The deposition of semen directly into the uterus associated to a low volume could result in a better and faster sperm distribution in the female genital tract and consequently avoiding semen backflow during insemination.

Although some authors describe a positive effect of higher sperm number on the fertilization rate (Baker *et al.*, 1968; Lefebvre and Suarez, 1996; Flowers, 2003), in the present study, PR did not differ between 1 and $2x10^9$ spermatozoa. In other studies using IUI it was not observed a detrimental effect on PR, litter size and total embryos with 1.5 billion (Dallanora *et al.*, 2003a), 1 billion (Watson and Behan, 2002) or 0.5 to 1 billion (Mezalira *et al.*, 2004) spermatozoa. Probably, in IUI, the sperm transport until the utero-tubal junction would be more efficient, allowing optimal fertility rates, even with a reduced sperm number. It has been suggested that deep intrauterine insemination produces a large distension of the cervix and uterine horn, which might induce a greater release of hormones implicated in uterine contractility and sperm transport compared with the traditional insemination method (Martinez et al., 2002). According to Watson and Behan (2002) the site of sperm deposition affects fertility results. The authors observed that 1 billion doses resulted in PR and litter size higher in IUI (88.7% and 12.1, respectively) than in traditional artificial insemination (66.2% and 10.3, respectively).

The TE was similar to 1 and $2x10^9$ spermatozoa. In other studies, TE or litter size were similar between 0.5 and 1.0 (Mezalira *et al.*, 2004) or between 1.0 and 3.0 billion spermatozoa (Watson and Behan, 2002). In the same way, Dallanora *et al*. (2003a), performing IUI with 1.5 billion dose, did not observe differences in the litter size when compared to control group (traditional artificial insemination with 3 billion dose).

It has been demonstrated that in the traditional artificial insemination with $3x10^9$ spermatozoa, the optimal AIOV interval is up to 24 h (Soede *et al.*, 1995; Kemp and Soede, 1997; Steverink *et al.*, 1997). However, in IUI, the optimal AIOV interval still remains unclear. In most of the experiments, the intrauterine insemination was performed in an AIOV interval of 0-24 h achieved by hormonal treatment (Kruger *et al.,* 1999; Martinez *et al.*, 2002), ultrasound monitoring of time of ovulation (Wolken *et al.,* 2002) or multiple inseminations during estrus (Watson and Behan, 2002). In the present study, with 1 and 2 billion spermatozoa, PR was not affected by the AIOV interval up to 36h. This could be explained by the fact that semen deposition beyond the cervix could reduce sperm losses by backflow during insemination, proportioning a higher sperm number in the utero-tubal junction. However, the insemination at 25-36h before ovulation resulted in a reduction of 2 embryos per AI ($P<0.05$). This could be due to a possible *in vivo* aging of spermatozoa at the moment of fertilization. Spermatozoa aging could lead to mitochondrial DNA damage or chromosomal abnormalities, and even if the sperm cell still preserves its capacity to penetrate the oocyte, this damage would compromise the viability of the conceptus (Vishwanath and Shannon, 1997). Nevertheless, to confirm the optimal AIOV interval in IUI, it would be necessary to perform more studies with a higher number of females inseminated with lower sperm cell doses.

ES average was 64.7 and 69.8% in sows inseminated with 1 and $2x10^9$ spermatozoa, respectively, which is in agreement to losses up to 40% until 35 days of pregnancy reported previously (Pope and First, 1985). Surprisingly, even when insemination was performed at an AIOV interval of 25-36h, 15.8% of sows showed an ES rate higher than 80%. Other authors also obtained a ES rate higher than 78% (Bortolozzo *et al.*, 2000) or fertilization rate above 90% (Soede *et al.*, 1995; Bracken *et al.*, 2003) when the insemination was performed at an AIOV higher than 24 h. This fact could be related to longer spermatic viability in the female genital tract. Nevertheless, the mechanism by which some sows present good fertility results at AIOV intervals higher than 24 h is still unknown. Xu *et al.* (1998) described a possible division of sperm populations in sub-populations which could lead to slower or faster capacitation. Based on *in vitro* results, these authors suggest that a sub-population that respond slowly would maintain the capability to fertilize oocytes *in vivo* for a longer period. In this way, these sub-populations could be responsible for the optimal results obtained in some females with AIOV interval higher than 24 h. Other authors comment the existence of boars that present a longer sperm lifetime in the female genital tract and this effect could be related to plasma seminal composition (Flowers, 1997). Furthermore, according to Rousseau and Ménézo (1993), inherent female aspects could be related to a better fertilization rate in some of them. The authors suggest that some females could have better conditions to embryonic survival in their genital tract and this could be associated to the uterine secretion and composition, which would benefit viability or sperm fertilizing ability.

In conclusion, in sows intrauterine inseminated with semen stored up to 36 h, pregnancy rate, number of total embryos and embryo survival are not affected by sperm cell dose when the artificial insemination-ovulation interval is up to 24 h. However, when this interval is between 25-36 h, the number of total embryos is reduced.

References

Baker RD, Dziuk PJ, Norton HW. 1968. Effect of volume of semen, number of sperm and drugs on transport of sperm in artificially inseminated gilts. *J Anim Sci,* 27:88-93.

Bortolozzo FP, Uemoto DA, Wentz I, Pozzobon MC. 2000. Reproductive performance of gilts submitted to artificial insemination in different intervals before ovulation. In: Abstract book of the 4th. International Conference on Boar Semen Preservation, 2000, Beltsville. Beltsville, USA: Allen Press. pp.239. (abstract).

Bracken CJ, Safranski TJ, Cantley TC, Lucy MC, Lamberson WR. 2003. Effect of time of ovulation and sperm concentration on fertilization rate in gilts. *Theriogenology*, 60:669-676.

Dallanora D, Mezalira A, Katzer LH, Bernardi ML, Bortolozzo FP, Wentz I. 2004. Volume and sperm number in the semen backflow after intrauterine or cervical insemination in sows. *In*: Abstracts of the International Congress on Animal Reproduction, 15, 2004, Porto Seguro,-BA. Belo Horizonte, MG, Brazil: CBRA. v.2, pp.387.

Flowers WL. 1997. Management of boars for efficient semen production. *J Reprod Fertil Suppl*, 52:67-78.

Flowers WL. 2003. Future reproductive technologies:- applied results of trans cenvircal insemination and other studies related to artificial insemination. *In*: 47th Annual North Carolina Pork Conference, 2003, NC, USA. Available in: http://mark.asci.ncsu.edu/NCPorkConf/2003/flowers.htm. Accessed in: July 2sd 2004.

Hancock JL. 1959. Pig insemination technique. *Vet Rec,* 71:527.

Kemp B, Soede NM. 1997. Consequences of variation in interval from insemination to ovulation on fertilization in pigs. *J Reprod Fertil Suppl,* 52:79-89.

Krueger C, Rath D, Johnson LA. 1999. Low dose insemination in synchronized gilts. *Theriogenology*, 52:1363-1373.

Lefebvre R, Suarez SS. 1996. Effect of capacitation on Bull sperm binding to homologous oviductal epithelium. *Biol Reprod,* 54:575-582.

Martinez EA, Vasquez JM, Roca J, Lucas X, Gil MA, Vazquez L. 2001. Deep intrauterine insemination and embryo transfer in pigs. *J Reprod Fertil Suppl*, 58:301-311.

Martinez EA, Vazquez JM, Roca J, Lucas X, Gil MA, Parrilla I, Vazquez JL, Day BN. 2002. Minimum number of spermatozoa required for normal fertility after deep intrauterine insemination in non-sedated sows. *Reproduction*, 123:163-170.

Mezalira A, Dallanora D, Bernardi ML, Wentz I, Bortolozzo FP. 2004. Reproductive performance of sows submitted to intrauterine insemination with different sperm cell dose. *In*: Proceedings of the 18th International Pig Veterinary Congress, 2004, Hamburg-Germany.. v.2, pp.477.

Nissen AK, Soede NM, Hyttel P, Schmidt M, D'hoore L. 1997. The influence of time of insemination relative to time of ovulation on farrowing frequency and litter size in sows, as investigated by ultrasonography. *Theriogenology*. 47:1571-1582.

Roca J, Carvajal G, Lucas X, Vazquez JM, Martinez EA. 2003. Fertility of weaned sows after deep intrauterine insemination with a reduced number of frozen-thawed spermatozoa. *Theriogenology*, 60:77-87.

Rousseau JP, Ménézo Y. 1993. Role of the female

genital tract in the transport and survival of gametes and the fertilized egg. *In*: Thibault C, Levasseur MC, Hunter RHF. *Reproduction in mammals and man*. Paris: Editions Marketing. pp.369-386.

SAS user's guide: statistical analysis system. 1998. Release 6.12. SAS Institut, Cary, NC, USA.

Soede NM, Wetzels CCH, Zondag W, Koning MAI, Kemp B. 1995. Effect of time of insemination relative to ovulation, as determined by ultrasonography, on fertilization rate and accessory sperm count in sows. *J Reprod Fertil*, 104:99-106.

Steverink DWB, Soede NM, Bouwman EG, Kemp B. 1997. Influence of insemination-ovulation interval and sperm cell dose on fertilization in sows. *J Reprod Fertil,* 111:165-171.

Steverink DWB, Soede NM, Bouwman EG, Kemp B. 1998. Semen backflow after insemination and its effect on fertilization results in sows. *Anim Reprod Sci,* 54:109–119.

Vazquez JL, Martinez EA, Vazquez JM, Lucas MA, Gil IP, Roca J. 1999. Development of a non-surgical deep intra-uterine insemination technique. *In*: Abstract book of the 4th. International Conference on Boar Semen Preservation, 1999, Beltsville, USA. Beltsville, USA: Allen Press. pp.262-263.

Vishwanath R, Shannon P. 1997. Do sperm cells age? A review of the physiological changes in sperm during storage at ambient temperature. *Reprod Fertil Dev,* 9:321-331.

Waberski D, Weitze KF, Lietmann C, Lubbert Zur Lage W, Bortolozzo FP, Willmen T, Petzoldt R. 1994. The initial fertilizing capacity of longterm-stored liquid boar semen following pre- and postovulatory insemination. *Theriogenology*, 41:1367-1377.

Watson PF, Behan JR. 2002. Intrauterine Insemination of sows with reduced sperm numbers: results of a commercially based field trial. *Theriogenology*, 57:1683-1693.

Weitze KF, Habeck O, Willmen R, Rath D. 1989. Detection of ovulation in the sow using transcutaneous sonography. *Zuchthygiene*, 24:40-42.

Wolken A, Rath D, Bortolozzo FP, Wentz I, Marquetti AN. 2002. A. Sows can successfully be inseminated non-surgically into the distal uterine horn with a highly reduced number of sperm cells. *Theriogenology*, 57:392. (abstract).

Xu X, Pommier S, Arbov T, Hutchings B, Sotto W, Foxcroft GR. 1998. *In vitro* maturation and fertilization techniques for assessment of semen quality and boar fertility. *J Anim Sci,* 76: 079-3089.