Effect of insemination-to-induced ovulation interval on fertilization rate, embryo viability and number of accessory sperms in sows

Carlos Henrique Cabral VIANA[1]
Pedro Henrique CANDINI[2]
Rogério Dantas GAMA[2]
Adriana CARBONE[2]
Renato Campanarut BARNABE[2]

1 - Faculdade de Medicina Veterinária da Fundação de Ensino Otávio Bastos, São João da Boa Vista – SP
2- Departamento de Reprodução Animal da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo - SP

Correspondência para:
CARLOS HENRIQUE CABRAL VIANA
Faculdade de Medicina Veterinária da Fundação de Ensino Octávio Bastos
Av. Dr. Octávio Bastos, s/n - Jardim Nova São João
13874-149 - São João João da Boa Vista
chcabral@usp.br

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Abstract

The ideal interval between AI and ovulation (OV) is not well determined yet, varying from 12 to 28 h before up to 4 h after ovulation. Utilization of gonadotrophins to synchronize ovulation would allow the pre-determination of the groups' size, according to the AI-OV intervals, and would contribute to determine a secure interval between AI-OV. 120 sows received 7.5 mg IM of Luprostiol, between days 12 and 17 of the estrous cycle, 600 IU of eCG IM 24 h after prostaglandin and 5.0 mg of LH IM 72 h after eCG injection. The moment of ovulation was diagnosed by transrectal ultrasonography at intervals of 6 h. There were 5 treatments according to IA-OV interval: T1- 48 to 36 h before OV; T2- 36 to 24 h before OV; T3- 24 to 12 h before OV; T4- 12 to 0 h before OV and T5- 0 to 12 h after OV. Sows were slaughtered 96.7±11.37 h after OV. Recovery rate (RR), number of corpora lutea (NC), total number of structures (ST), fertilization rate (FR), embryo viability (EV) and number of accessory sperm (AS) were analyzed. The synchronization protocol showed an homogenous distribution of the animals among treatments (LH-OV interval 39.22±7.6h), and it didn't influenced the results. FR and EV results suggest that 36 h is the time of sperm viability in sow genital tract. There was a strong decline of AS between T3 and T4.

Key-Words:
Swine.
Synchronization.
Insemination.
Ovulation.

Introduction

The ideal moment to perform artificial insemination (AI) in swine has attracted the researchers' attention since the 60's. Until the end of the 80's, the determination of this moment based on data obtained by the estrus observation[1], by induction of ovulation and fixed time insemination[2], and by determination of hormone levels (LH and progesterone) related to ovulation[3]. With the coming of the ultrasound, as an accurate and not invasive technique, for the diagnosis of ovulation[4], more consistent results surged in relation to insemination-to-ovulation interval. Waberski et al.[5] didn't observe significant differences in fertilization rate of gilts inseminated in intervals of 0 to 12, 12 to 24 and over 24 hours before ovulation, when the semen was stored for a period of up to 48 hours. However, the number of accessory sperms was quite larger, but not significant, when the inseminations were performed between 0 and 12 hours in relation to the other intervals.

The evaluation of the percentage of normal embryos, in sows inseminated from 48 hours before to 16 hours after ovulation, showed significantly higher results when the insemination happened between 24 and 0 hours before ovulation, in comparison to intervals from 48 to 24 hours before and 0 to 16 hours after ovulation[6].

According to the results of Nissen et al.[7], a safe insemination-to-ovulation interval would be from 28 hours before up to 4 hours after ovulation, according to the results of farrowing rate and litter size, and from 24 hours before up to the moment of ovulation, according to embryo recovery rate. Evaluation of the interval between inseminations showed that there were no significant differences between intervals of 12 and 24 hours, in relation to the results of conception rate, farrowing rate and litter size. However, there was a decrease of 1.3 inseminations per sow for the group of 24 hours compared to the group of 12 hours interval[7].

The classic protocol of synchronization of ovulation extols the eCG administration 24 hours after weaning, and the hCG[4] or porcine LH[8] administration 72 hours after eCG, with the occurrence of ovulation between 32 and 44 hours after the last injection. The use of gonadotropins to synchronize ovulation, making it possible to pre-determine the groups' size, according to the insemination-to-ovulation interval, associated to the ultrasonography for the diagnosis of ovulation, would complement the information about the determination of a safe insemination-to-ovulation interval.

The objectives of this experiment are:

To determine a sufficiently long interval between inseminations to facilitate management and sufficiently safe to guarantee appropriate farrowing rate and litter size, by the evaluation of fertilization rate and of the number of accessory sperms;

To evaluate the use of a protocol, which uses Luprostiol, eCG and LH, as a technique of synchronization of the ovulation, to elaborate fixed time AI protocols and make the equalization of the groups size possible, in experiments that evaluate the effect of insemination-to-ovulation interval on reproductive parameters.

Material and Method

The work was performed in an Agroceres-PIC farm, Minas Gerais State. 120 cyclic sows were used after the 3rd parturition. During lactation, sows received a lactation diet and water ad libitum, staying there for an average period of 5.35±1.13 days. Because of the early weaning management in this farm, the second post-weaning estrus was used to avoid possible influences in ovulation rate, fertilization rate and recovery of embryos. The 120 sows were divided into 12 weekly groups, of approximately 10 animals each, to make the execution of the work possible. For the diagnosis of ovulation, the technique of trans-rectal ultrasound was used with the Scanner 200 machine (Pie Medical©), equipped with a sector transducer of 7.5 MHz. The examinations started 24 hours after LH application with intervals of 6 hours, approximately at 08:00, 14:00, 20:00 and 02:00, continuing until the ovulation occurred. The diagnosis of ovulation was considered when no follicle was found or when the number of follicles was smaller than in the previous examination. The moment of ovulation (MO) was defined as the mean time between the last examination where preovulatory follicles were detected and the first examination where no follicles could be detected on the ovary.

Of the 120 examined sows, 16 were excluded from the experiment for having ovulated within 24 hours after LH application. In the first estrus (first cycle after weaning), the day of ovulation was determined and served as a reference for the beginning of the hormonal applications. Sows were injected with 7.5mg of Luprostiol (Prosolvin®, an $PGF_{2\alpha}$ analog), between 12 and 17 days after the first ovulation (D1); 600UI of eCG (Novormon®) 24 hours after the Luprostiol injection (D2); and 5.0mg of porcine LH (Lutropin®) 72 hours after the eCG injection (D5). The prostaglandin application was performed at 10:00, on Fridays and on Saturdays (D1) (two weekly groups of hormonal treatment), trying to achieve a larger number of sows, which were among 12 to 17 days after the first ovulation.

The eCG injection was applied approximately at 10:00, on Saturdays and Sundays (D2) and porcine LH at 10:00 on following Tuesdays and Wednesdays (D5) (Figure 1).

The sows were submitted to 5 treatments. Only one insemination was performed by sow. In treatment 1, sows were inseminated between 48 and 36 hours before the ovulation; in treatment 2, between 36 and 24 hours before; in treatment 3, between 24 and 12 hours before; in treatment 4, between 12 and 0 hours before and in treatment 5, between 0 and 12 hours after the ovulation. Heterospermic semen from 3 boars was used in the concentration of 3.5 billion sperm cells per dose. The sows were slaughtered 96.7±11.37 hours after the occurrence of ovulation and the number of corpora lutea (NCL) was counted in both ovaries. Each oviduct was flushed with 10 ml of solution of phosphate buffered saline (PBS). Later, the oviducts were separate from the uterus and each uterine horn was flushed twice, and in the first flushing, it was used 20ml and in the second 30ml of PBS, in two different plates to collect the embryos and oocytes. The recovery rate (TR) was determined by the total number of embryos and collected oocytes (ET) in relation to the number of corpora luteaa[9]. The evaluation of the embryos was performed using stereomicroscopy, in a magnitude of 40x and based, firstly, in the presence and uniformity of the perivitelline space with the cells evenly distributed, in the development stages according to the time between ovulation and collection[10], and in the presence of accessory sperms in the zone pellucida (EA), which were counted using optical microscopy in a magnitude of 400X. The embryos were deposited in a glass slide, where the zone pellucida was disrupted with 0.5% pronase solution. Later, it was covered with a slide to make possible the count of the sperms. Embryos, which didn't fit to the first two evaluation criteria, were classified as degenerate. The fertilization rate (TF) it was calculated dividing the number of embryos (degenerate and viable) by the total number of embryos more oocytes and the embryo viability rate (TEV), dividing the number of embryos with normal development by the total number of embryos more oocytes.

The data were analyzed using SAS (1999) and were presented as the average ± standard deviation and variation (minimum–maximum). All variables were submitted to the test of normality of the residues, to verify if they followed the normal distribution. The variables: NCL, TR and ET obeyed the normal distribution and were analyzed by ONEWAY procedure, according to the factor insemination-to-ovulation interval, divided in 5 classes (T1, T2, T3, T4 and T5).

The variables TF, TEV and EA didn't follow the normal distribution and they were analyzed by the non parametric procedure NPAR1WAY, according to the factor insemination-to-ovulation interval, divided in 5 classes (T1, T2, T3, T4 and T5).

Results

Of the 120 used sows, 16 were discarded of the experiment, in reason of precocious ovulation (before the expected interval, 9 sows) or metritis (7 sows). The protocol of synchronization of ovulation was satisfactory (LH application-to-ovulation interval of 39.22±7.6 hours) in the homogeneous distribution of the number of animals for each treatment of insemination-to-ovulation interval and it didn't influence the results, since there were no significant differences among the averages of the characteristics NCL, TR and ET, according to the treatments (Table 1). A significant difference was verified in the characteristics TF and TEV, and, in both, the insemination-to-ovulation interval from 48 to 36 hours before ovulation showed a smaller average than all of the other intervals, whose averages were not different between each other. The number of accessory sperms was significantly different, according to insemination-to-ovulation interval, so that the averages of the intervals 48 to 36, 36 to 24 and 24 to 12 hours before ovulation didn't

Table 1 - Average values, standard deviation and range NC, ET, EA, TR, TF and TEV, according to the insemination-to-ovulation interval, São Paulo, 2004

Variables	insemination-to-ovulation interval				
	48 – 36h	36 - 24h	24 - 12h	12 - 0h	0 - 12h
NC	23.0±8.2	23.6±3.8	25.4±5.7	23.1±5.6	25.3±4.0
	(2 – 34)	(16 – 29)	(17 – 45)	(5 – 30)	(16 – 31)
ET	19.3±7.2	21.0±4.2	20,8±5,1	18,6±5,7	22,1±4,7
	(2 – 27)	(15 – 29)	(10 – 28)	(7 – 27)	(8 – 28)
EA	31.7^{b}±68.5	26.5^{b}±53.9	47,9^{b}±47,7	187,8^{a}±139,3	163,4^{a}±138,1
	(0 – 263)	(0 – 222)	(0,2 – 153)	(48 - 516)	(20 – 444)
TR (%)	86.8±17.5	89.1±10.8	83,6±19,2	76,6±18,6	86,9±11,9
	(43.5 - 100.0)	(64.3 - 100.0)	(35,5 - 100,0)	(30,4 – 95,7)	(50,0 – 100,0)
TF (%)	75.5^{b}±19.4	85.5^{a}±21.1	84.0^{a}±22.2	90.8^{a}±13.5	87.3^{a}±26.4
	(29.4 -100.0)	(10 -100.0)	(28.6 -100.0)	(57.2 -100.0)	(0 -100.0)
TEV(%)	57.9^{b}±28.5	75.2^{a}±29.2	72.9^{a}±29.0	84.5^{a}±17.9	75.2^{a}±30.0
	(0 -100.0)	(5.0 -100.0)	(0 -100.0)	(28.6 - 100.0)	(0 - 100.0)

Means followed by similar superscripts, within the same line don't differ statistically among each other (P < 0,05)

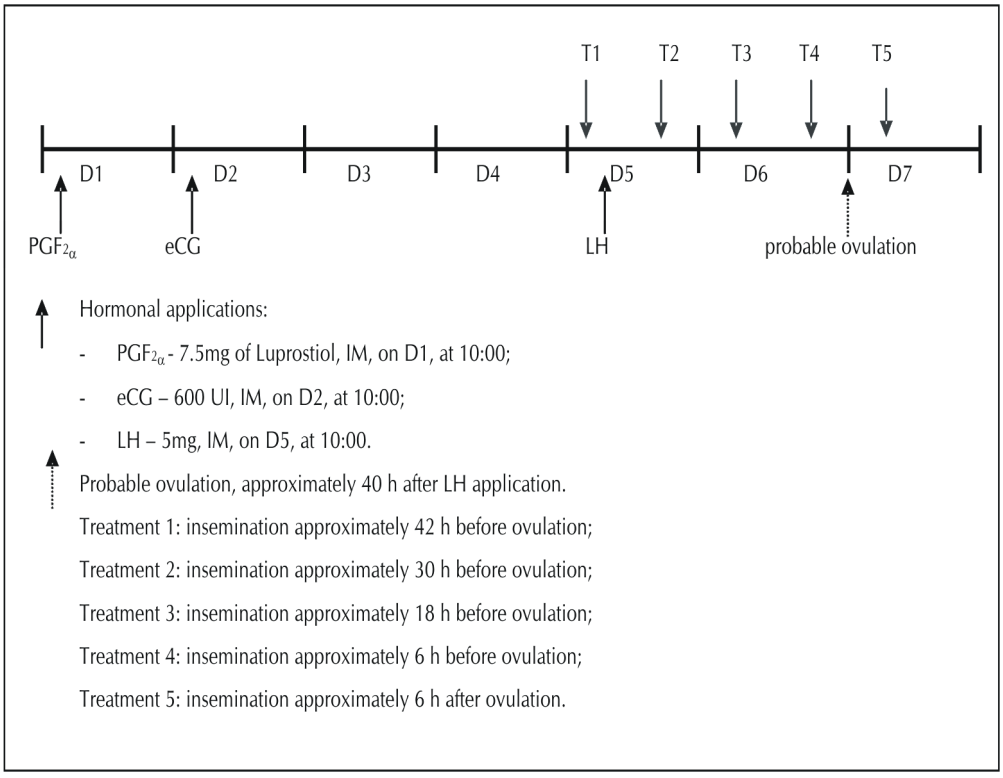

Figure 1 - Schedule of synchronization of ovulation and treatments

Discussion

The LH application-to-ovulation interval of 39.22±7.6 hours is in agreement with the protocols of synchronization ovulation developed before, which used LH[8,11] or hCG and GnRH[2,12,13,14], and it made possible the formation of groups of homogeneous size according to insemination-to-ovulation interval.

Weaning works very well as starting point for protocols of induction of ovulation in swine. However, in some situations, as certain experiments[13] or in of embryo[15] transfer programs, it is necessary to use cyclic sows. In the present experiment cyclic sows were used to avoid possible effects of the short lactation period on reproductive results[16,17], showing satisfactory results.

The average TR, TF, TVE and the number of embryos of all treatments together were satisfactory according to data obtained previously, so much in experiments that used the induction of the ovulation[18] as in experiments with spontaneous ovulation[6,9]. NCL and TR were similar among the classes of interval insemination-ovulation (Table 1), and therefore, they didn't influence the results of the fertilization rates and of embryonic viability, confirming once again, the usefulness of the synchronization of the ovulation for the equalization of the size of the groups.

According to the results of fertilization rate and embryonic viability, an interval of up to 36 hours can be considered as the sperm viability in the sow reproductive tract. According to the literature, the longest minimal sperm viability considered was of 24 hours[6,7], and other authors considered this time 16[19] or 12 hours[5]. A possible cause for the longer sperm viability observed in the present experiment related to the results of Soede et al.[6] and Nissen et al.[7] is that these authors used a semen dose of 3 and 2 x 10^9 sperms against 3.5 x 10^9 in this experiment. However, Steverink et al.[9] didn't see any effect of the number of sperms cells per dose on fertilization rate and nor on the number of accessory sperms.

The accessory sperm count technique has been used as parameter to evaluate experiments related to artificial insemination, by expressing the amount of sperms capable of fertilization, at the place and moment of occurrence of ovulation and for being correlated positively to embryo viability[5,6,9,20,21]. As in all the consulted experiments, which used accessory sperm count as a parameter, in the present experiment this parameter was highly variable, what is observed by the standard deviations in table I. The abrupt decrease in the average accessory sperms among the classes of insemination-to-ovulation interval from 0 to 12 and 12 to 24 hours also happened in the experiment of Waberski et al.[5]. However, this decrease didn't reflect a decrease in the fertilization rate and in the embryonic viability (Table 1), in disagreement to the positive correlation between the number of accessory sperms and these variables, described in other experiments[21]. Steverink et al.[9] reported that several sows showing 100% of normal embryos had a relatively low number of accessory sperms and found negative correlation (R^2=0,24; $P < 0,0001$) between the number of accessory sperms and the insemination-to-ovulation interval. These authors suggest that the relationship between accessory sperms and fertilization rate and embryonic viability can be indirect, once there is a clear effect of the insemination-to-ovulation interval on these variables.

Conclusions

It was not possible to show significant differences in the results of fertilization rate and of embryonic viability when the sows were inseminated between 36 hours before and 12 hours after ovulation. There was a decrease in the number of accessory sperms when the insemination-to-ovulation interval was longer than 12 hours.

The use of the synchronization of ovulation technique in cyclic sows (Luprostiol, eCG and LH) and fixed time insemination, resulted in the expected pattern of synchronization, making possible the equalization of the group's size, without influence on the reproductive parameters.

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Efeito do intervalo inseminação-ovulação induzida sobre a taxa de fecundação, viabilidade embrionária e número de espermatozóides acessórios em porcas

Palavras-chave:
Suínos.
Sincronização.
Inseminação.
Ovulação.

Resumo

O intervalo ideal entre a AI e ovulação (OV) não está bem determinado ainda, variando entre 12 a 28 h antes até 4 h depois da ovulação. A utilização de gonadotrofinas para sincronizar ovulação permitiria a pré-determinação do tamanho dos grupos, de acordo com os intervalos IA-OV, e possibilitaria determinar um intervalo seguro entre IA-OV. 120 porcas receberam 7.5 mg IM de Luprostiol, entre os dias 12 e 17 do ciclo estral, 600 IU de eCG IM 24 h após o Luprostiol e 5.0 mg de LH IM 72 h após a injeção de eCG. O momento de ovulação foi diagnosticado pela ultra-sonografia trans-retal a intervalos de 6 h. Definiu-se 5 tratamentos de acordo com o intervalo IA-OV: T1 - 48 a 36 h antes da OV; T2 - 36 a 24 h antes da OV; T3 - 24 a 12 h antes da OV; T4 - 12 a 0 h antes da OV e T5 - 0 a 12 h após a OV. O abate ocorreu 96.7±11.37 h após a OV. A taxa de recuperação (RR), número de lutea de corpos (NC), número total de estruturas (ST), taxa de fecundação (FR), viabilidade embrionária (EV) e número de espermatozóides acessórios (AS) foram analisados. O protocolo de sincronização mostrou uma distribuição homogênea dos animais entre os tratamentos (intervalo LH-OV de 39.22±7.6h), e não influenciou os resultados. A FR e os resultados de EV sugerem que 36 h seja o tempo de viabilidade do espermatozóide trato genital da porca. Houve um forte declínio do AS entre T3 e T4.

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