Follicular Development and Ovulation in Sows: Effect of hCG and GnRH Treatment

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Nissen, A.K., H. Lehn-Jensen, P. Hyttel and T. Greve: Follicular development and ovulation in sows: Effect of hCG and GnRH treatment. Acta vet. scand. 1995, 36, 123-133.

– Follicular growth, chronology of ovulation and embryo morphology were compared in sows ovulating spontaneously and sows, in which the ovulation was attempted induced by hCG or GnRH.

Indwelling catheters were placed on day 1 (weaning = day 0) in the ear veins of 18 sows, which were then randomly divided into 3 groups: a control group (N = 6), a group (N = 6) given 750 iu hCG (Physex®) im 76h after weaning (hCG group) and a group (N = 6) given 500 µg GnRH (Fertagyl®) im 76h (N = 3) or 100h after weaning (N = 3) (GnRH group). Follicular diameter and time of ovulation were monitored by ultrasonography every 4h from day 3 until ovulation or development of cysts by means of a sector scanner fitted with a 5.0/7.5 MHz multiangle probe. Heat detection was performed every 8h from day 3 until ovulation. On day 13, the sows were slaughtered, the number of corpora lutea (CL) was counted, and embryos were flushed from the uteri. The control group showed clear heat symptoms, and on day 3, the follicles were typically 3-7 mm and grew up to 7-10 mm over 2 days, where they remained for approximately 24h until ovulation took place 41h ± 9h after first sign of standing heat. The hCG group exhibited no signs of heat, and the follicles only reached 5-8 mm in diameter at time of ovulation, which occurred 40h ± 1h after hCG-injection. The GnRH group exhibited inconsistent signs of heat, and the follicles reached a maximum size of 7-12 mm in diameter where they remained for more than 24h. Only 2 sows in this group ovulated within 84-92h after the GnRH injection, and development of bursa cysts and cystic follicles was a common finding. The average number of CL was 18.2 ± 5.7 per sow (N = 16, range: 3-27) with no significant difference between the groups. Total embryo recovery was 79 ± 13 % with no significant difference between groups. The embryo diversity calculated as standard deviation of the maximum diameter was higher in the hCG group as compared with the control group.

It is concluded that (1) transrectal ultrasonography can be used in sows for accurate assessment of follicular growth and ovulation; (2) the use of hCG results in lack of heat symptoms and reduced follicle size at the time of ovulation when injected 76h after weaning; (3) administration of a single injection of GnRH, if given before the first signs of heat, results in inconsistent heat symptoms and no or late ovulations.

Ultrasonography; embryonic development; heat.

Introduction

Porcine embryonic mortality prior to implantation is approximately 20-50% (Pope & First 1985). The patho-physiological background for this phenomenon still remains an enigma, but a number of experiments suggest that embryo diversity may be causally involved (Bazer et al. 1988, Pope et al. 1990). Within a litter more advanced embryos will cause changes in the uterine secretion through their accelerated oestrogen production on days 11.5 to 13.0, and these environmental changes are thought to have a detrimental effect on the least developed embryos (Pope & First 1985, Pope et al. 1990). On the other hand, if the least developed embryos are transferred to a less advanced uterine environment they survive as well as the larger ones indicating that the embryos themselves are viable (Pope & First 1985).

It is still beyond our present knowledge fully to explain the origin of embryonic diversity which is evident during the early development of the porcine embryo. Factors such as oocyte and follicular maturation, duration of the ovulation- and fertilization period (Pope et al. 1988a & b, Wilde et al. 1988, Xie et al. 1990a & b, Soede et al. 1992), the time of insemination (Cárdenas & Pope 1993), oviductal transport, moment of hatching, and genetic aspects have been implicated (Oxenreider & Day 1965, Ford et al. 1988). Recent studies strongly suggest that also the maternal as well as embryonic genotype are of importance for embryonic diversity (Galvin et al. 1993, Pope et al. 1990).

An essential question is whether the developmental diversity is established already while the oocytes still reside in the ovary, or it is imposed after ovulation and are exposed to the oviduct and uterine environments. In other words: Is embryo diversity inherent to oocyte diversity before ovulation, or is it inherent to

embryonic and/or maternal factors after ovulation.

In answering these questions, monitoring of the time and duration of ovulation is essential. Until recently, timing of ovulation could only be monitored by means of laparotomy or laparoscopy (Pope et al. 1988a, Brüßow & Ratky 1992, Hunter et al. 1993, Xie et al. 1990b), and because of the inherent traumatic and sedative/anaesthetic nature of these procedures, they may interfere with the ovulation process. The rapid merge of transrectal ultrasonography in animal reproduction has made it possible to monitor the time of ovulation not only in cattle and horses, but also in sows, although the accuracy depends on the acquired skills of the operator and the frequency of scanning within each sow (Weitze et al. 1989, Wagner-Rietschel 1991, Soede et al. 1991 & 1992).

In experimental studies of embryo diversity it is important to be able to predict and control the exact time of ovulation. Although ultrasonography offers a unique way of estimating the time of spontaneous ovulation, this method does not allow prediction and control of the timing of this event. Neither do intense heat detection and plasma LH-determinations. Human chorionic gonadotropin (hCG) has been used extensively for induction of ovulation mostly in combination with Regumate® and equine chorionic gonadotropin (eCG). When injecting hCG approximately 80h after eCG, ovulations occur at an average of 40h after the hCG-injection (Spalding et al. 1955, Hunter & Polge 1966, Cran 1985, Pope et al. 1988a, Wiesak et al. 1990, Soede et al. 1992, Hunter et al. 1993). Presently a combination of 400 I.U. eCG and 200 I.U. hCG is the most commonly used hormone treatment for induction of ovulation in anestrus gilts and sows.

The exact influence of hCG on the follicular

dynamics of the ovary is still poorly understood. Wiesak *et al.* (1990) demonstrated that follicular volume and oestradiol concentrations decreased in eCG/hCG treated prepuberal gilts, while the progesterone concentrations increased. Moreover the diversity in oocyte maturation based on stage of meiosis was more pronounced in hormone treated than in naturally cyclic gilts.

Gonadotropin releasing hormone (GnRH) has also been used to induce ovulation in gilts pretreated with eCG (Georg 1985, Lutz *et al.* 1985, Brüßow & Ratky 1990 & 1992). The time of ovulation varied from 36 to 42h after the GnRH treatment dependent on the dose. The effect of hCG and GnRH on weaned sows with respect to follicular dynamics and embryo diversity is unknown. The purpose of this study was therefore to compare follicular dynamics, time of ovulation, and degree of embryonic diversity in spontaneously ovulating sows versus sows in which ovulation was induced by use of hCG or GnRH.

Materials and methods

Animal treatment

Danish Landrace X Yorkshire (LY) sows (N = 18, 200-250 kg bdw, 2-8 parity) were weaned (day 0). On day 1 they were anaesthetized with thiopental (15 mg/kg bdw), and an indwelling catheter was placed in the ear vein. The sows were divided into the following 3 experimental groups:

The control group (N = 6) was monitored by ultrasonography at 8 am on day 3 and 4 and from 24h after first signs of heat every 4h until ovulation. The sows were inseminated 24h after first sign of standing heat and again 24h later or immediately after ovulation, whichever came first.

The hCG group (N = 6) was given 750 iu hCG (Physex®, Leo, Ballerup, Denmark) im at 12

am on day 3. Ultrasonography was carried out at 8 am on day 3 and from 12 am on day 4 every 4h until 52h after hCG injection. The sows were inseminated 24h after the hCG-injection and again after ovulation had been observed.

The GnRH group (N = 6) was given 500 µg GnRH (Fertagyl®, Intervet, Scandinavia A/S, Skovlunde, Denmark) im at 12 am on day 3 (N = 3) or on day 4 (N = 3). Ultrasonography was carried out at 8 am on day 3 and from 24-28h after the GnRH-injection every 4h for 72h or until ovulation. The sows were inseminated between 24h and 36h after the GnRH-injection dependent on the intensity of heat and again after 24h or after ovulation whichever came first.

Heat detection

Heat detection was carried out at 8h intervals from day 3 until ovulation. Occurrence of heat was based on the following parameters: swelling and reddening of the vulva, viscosity of the vulva mucus, standing reflex (backpress-human) alone and in front of a boar. Beginning of heat was defined as first standing reflex in front of a boar, with the other parameters as support.

Ultrasonography

Transrectal ultrasonography was performed with a sector scanner fitted with a 5.0 and 7.5 Mhz multiangle probe (Basic Scanner 150, Die Medical®, Maastricht, The Netherlands). Movement of the sow was restrained using a barrier placed at the rear of the sow. Some sows were fed during the scanning session to stand still. After removal of the faeces, the hand with the probe was carefully brought 35-45 cm into the rectum. By turning the hand with the probe from lateral to ventral, the position of the ovaries was found. The size of the follicles was measured after freezing the pic-

Table 1. Comparison between number of follicles estimated by ultrasonography and number of Corpora Lutea (C.L.) and cysts counted at slaughter. No significant difference was found (95%, Wilcoxon's test, SAS®).

Treatment	hCC group						GnRH group						Control group					
Sow no.	1	2	3	10	11	12	4	5	6	13	14	15	7	8	9	16	17	18
No. of follicles (B)	0	14	18	17	20	13	15	20	11	15	3	15	18	20	16	20	16	20
No. of C.L. (A)	1*	22	19	18	18	13	0	26	11	15	3	21	22	23	16	17	27	20
No. of Cysts. (A)	0	1	6	1	2	0	18	0	5(a)	1	9(b)	1	0	0	1	0	1	0
Difference (C)	0	9	7	3	0	0	3	6	0(5)	1	0(9)	7	4	3	1	-3	12	0

(a)5 and (b)9 cysts apparently developed after termination of the ultrasonography.

*) An C.L. was found at the first scanning and no follicles developed during the scanning period.

(A): found at slaughter, (B): found by ultrasonography. (C): Findings at slaughter – findings by ultrasonography.

ture on the monitor. All scanning sessions were recorded on video.

The following parameters were recorded: 1) the diameters of the 1-3 largest follicles, 2) the estimated general size of the remaining follicles 3) the estimated total number of follicles and 4) any features deviating from the expected normal ultrasonography findings like cysts or follicles growing to a diameter over 20 mm. Time of ovulation was defined as the first observed reduction in number of follicles between 2 consecutive scanings.

Embryo collection and evaluation

The sows were slaughtered on day 13, and the number of corpora lutea was counted, and other findings like ovarian cysts, fluid or infection in uterus and bursa cysts were noted. Immediately following slaughter, each uterine horn was flushed with 60 ml of phosphate buffered saline (PBS) added 1% foetal calf serum (FCS) at 35°C. The medium collected after flushing was packed in a styrofoam box to prevent the temperature of the medium falling below 20°C and transported to the laboratory within 1h. Subsequently, the embryos were isolated in PBS + FCS under a stereomicroscope and photographed within the next hour. The maximum diameter of each embryo was measured from the photographs taken. The

data were analysed (SAS Institute Inc. 1989) using the procedures Univariate and GLM for each sow separately to test for the normal distribution of the embryo sizes (95% confidence), and the standard deviation was calculated for each sow as a measurement of the diversity of the embryos.

Results

Ultrasonography

Within each sow the position of the ovaries remained almost constant from one scanning to the next and often the ovaries were located very close to each other. The accuracy in assessing the number of follicles by ultrasonography was inversely related to their number, in particular when exceeding 10 follicles per ovary (Table 1 and Fig. 1). The number of ovulations, based on the numbers of corpora lutea, was 18.2 ± 5.7 per sow among ovulating animals (N = 16, range: 3-27), with no significant difference between groups.

Follicle growth

The follicles detected at the initial scanning were normally from 4 to 6 mm in diameter. They did, however, develop differently in the 3 experimental groups throughout the scanning period. As a general feature in each

Figure 1. Difference between number of follicles estimated by ultrasonography and number of Corpora Lutea (C.L.) and cysts counted at slaughter.

ovary 1 to 2 follicles were 1-2 mm larger than a major and more homogeneous group, with 1 to 2 follicles being even smaller (3 mm).

In the control group the follicles reached a maximum diameter of 7-10 mm, at which size they remained for about 24h until ovulation. In the hCG group the follicles grew continuously until ovulation. The follicle diameter at the last scanning before ovulation was normally 6 to 7 mm in this group. The follicles in the GnRH group reached 7-12 mm in diameter and maintained this size for more than 24h before they ovulated or developed into cysts.

Time of ovulation

In the control group, ovulation occurred 41 ± 9 h (N = 6, range: 32-52h) after the first signs of heat. In the hCG group, ovulation was observed at 40 ± 1 h (N = 5, range: 40-42h, Fig. 2) following the hCG injection in all but one sow, in which the presence of an apparently active corpus luteum was found at the first scanning. In the GnRH group, 2 out of 6 sows

ovulated within the scanning period, i.e. 84-92h after the injection of GnRH. One of these sows had only 3 ovulations and developed 9 cysts (>25 mm in diameter) after ovulation, while in the other sow 11 follicles ovulated and 5 developed into cysts. At slaughter it was found that 1 had failed to ovulate, and 3 had ovulated after the termination of the scanning period.

Additional ultrasonography findings

In one sow from the hCG group, a corpus luteum was found at the first scanning, and no follicles developed during the scanning period. Development of ovarian cysts (>20 mm in diameter) or follicles that did not ovulate was noted in 2 sows of the control group, and in 4 and 5 sows belonging to the hCG and GnRH groups, respectively. Permanent fluid accumulation (i.e. cystic dilation of the wall of the bursa ovarii found at slaughter) was observed around 1 ovary in 1 sow in the hCG group, in 4 sows in the GnRH group and in 1 sow in the control group.

Table 2. Maximum embryo diameter in the hCG and control groups.

Sow no	age of embryo (hour)	N	Embryo diameter		
			range (mm)	mean (mm)	SD
hCG: 2	198	19	0.37 - 1.09	0.79	±0.22
– 10	190	9	0.43 - 0.78	0.60	±0.12
– 11	198	11	0.46 - 0.80	0.63	±0.10
– 12	197	10	0.41 - 0.72	0.58	±0.08
average	196±4	49	0.37 - 1.09	0.68	±0.13 (a)
Control: 7	175	18	0.26 - 0.46	0.39	±0.05
– 8	163	17	0.17 - 0.37	0.28	±0.06
– 9	157	14	0.24 - 0.35	0.29	±0.03
– 17	167	21	0.22 - 0.46	0.33	±0.07
– 18	170	17	0.22 - 0.39	0.29	±0.04
average	166±6	87	0.17 - 0.46	0.32	±0.05 (b)

(a) and (b) are significantly different ($p < 0.03$).

Heat detection

In general, the backpress test did not provide a satisfying response when the sows were on their own. However, in the presence of a boar, about half of the sows exhibited signs of heat. Clear vulva signs were only found in the control group, where all sows showed increasing vulva swelling and reddening until full graded positive backpress test. None of the sows in the hCG group responded to the backpress test. The GnRH group showed inconsistent vulva signs from one heat control to the next, and only 4 out of 6 sows showed a positive backpress test.

Embryo evaluation

In 6 out of 18 sows, the uterine horns were not flushed due to lack of ovulation, ovulation occurring after termination of the scanning period (1 in each group) or due to infection in the uterus (1 in the hCG group and 2 in the GnRH group).

The total embryo recovery-rate was $79 \pm 13\%$, with no significant difference between groups

(69, 88 and 81% in the hCG, GnRH and control group, respectively).

The collected embryos were contracted with diameters ranging from 0.16-0.46 mm (day 7 after ovulation) to 0.37-1.09 mm (day 8 after ovulation, Fig. 3). The embryos recovered in the GnRH group were not included in the evaluation of the embryo size-data due to indistinct photos. Embryonic diversity was significantly higher in the hCG group compared with the control group (Table 2).

Discussion

The present study demonstrated that transrectal ultrasonography provides a reliable method to assess the time of ovulation in sows. The agreement between the number of ovulations estimated by ultrasonography and the number counted at slaughter was high up to around 10 ovulations per ovary. Other conditions such as cysts and fluid accumulations in the bursa ovarii could also be accurately diagnosed.

The transrectal approach was considered to

Figure 2. Intervals from first standing reflex to ovulation in control sows and from injection of hCG or GnRH to first standing reflex and ovulation in hormone treated sows (day 0 = weaning).

be the method of choice, since the transcutane techniques require much more practice, up to 2000 scanings before satisfactory results can be expected on sows (Wagner-Rietschell 1991). The transrectal methods used in this study provides satisfactory results after no more than 50-100 scanings (Soede *et al.* 1991 & 1992). It was observed that the 3 groups of animals showed different patterns of follicular growth up to ovulation. In the control group, the follicular growth as observed by scanning

from day 3 was uniform, and ovulations were observed approximately 24h after the follicles had reached their maximum size (7-10 mm). In the hCG group, the follicles only reached a maximum diameter of 5-8 mm, and the ovulation occurred at 40 ± 1 h after the hCG-injection. This is in correspondence with the reports of Hunter & Polge (1966) and Cran (1985). Ovulation in the hCG group occurred approximately at the time when the sows in the control group showed their first signs of

Figure 3. Litter of hatched blastocysts flushed from a sow in the hCG group on day 8 after ovulation. Note the variation in embryonic size and the contracted appearance of the embryos. X 30.

heat. This feature may explain the smaller size of the preovulatory follicles of the hCG group: They were apparently induced to ovulate before reaching their maximum size. The presumable still limited production of oestrogen in these smaller follicles may also explain why heat symptoms were absent in this group. Accordingly, *Hunter et al.* (1976) gave hCG injection to mature gilts on days 4, 3, 2 and 1 before expected ovulation and found 0%, 11%, 87%, and 93% showing heat symptoms, respectively. Taking the time of ovulation in the control group into consideration, the time of injection in the hCG group in this study was carried out about 3 days before the spontaneous ovulation. This indicates that only a low percentage (11%) of the sows could be expected to show heat.

In the GnRH group, the follicles grew approximately to the same size (7-12 mm) as in the

control group. However, ovulation was not detected until 36h after reaching the maximum follicular diameter, and a high proportion of the follicles did not ovulate but developed into cysts. This may be due to the lack of a proper LH-surge in this group of animals. The ability of GnRH to induce ovulation has been substantiated in other experiments (*Georg* 1985, *Brüssow & Ratky* 1990 & 1992). It is, however, also clear that the time of injection and the dose of GnRH are factors of great importance (*Georg* 1985). Both Fertagyl® (Intervet), as used in this experiment, and Gonavet®, as used by *Georg* (1985) and *Brüssow & Ratky* (1992), are synthetic products in which the potency and dose response can not be compared directly. In addition, *Brüssow* (1994) showed that injections of GnRH-agonists in midcycle result in a reversible inhibition of gonadotropin release in

gilts and the early GnRH injection in this experiment can be the source of the poor result in the GnRH group. The use of weaned sows versus Regumate®- and eCG-treated gilts as used by *Georg* (1985) may be another important factor.

The high frequency of cysts in the hCG and GnRH groups may indicate again that the timing and dosage of the drugs may not have been correctly adjusted. The frequency of cysts or non-ovulated follicles in spontaneously ovulating sows is approximately 10% (*Wagner-Rietschell* 1991, *Thorup* 1990). However, the present material is not substantial enough to calculate any significant difference between the groups.

The recovery rate (79%) of the embryos was close to that reported in other experiments (*Soede et al.* 1992).

The higher degree of embryo diversity in the hCG group may be due to the hormonal influence on the oocyte maturation and/or due to the fact that the hCG group embryos were 30h older than the control group embryos. The recovered embryos displayed a certain degree of contraction. After hatching, porcine embryos are very fragile, and in the period until implantation, the embryos are sensitive to handling and changes in the environment caused by flushing, manipulation, and ambient temperature, which may explain the embryonic contraction.

Conclusion

The use of transrectal ultrasonography on sows was found accurate in assessing follicular growth and numbers as well as the timing of ovulation. At day 3 (weaning: day 0), the follicles in untreated sows had an average size in diameter of 4-6 mm, and over the following 48h they grew to the maximum size of 7-10 mm, and the follicles remained at that size ap-

proximately 24h up to ovulation. The use of hCG, when given on day 3, resulted in ovulation at a follicular diameter of 6-7 mm and in lack of heat signs. The use of GnRH, if given before first signs of heat, resulted in a high proportion of anovulatory follicles.

Acknowledgement

Thanks to Erika Busch, Inger Heinze and Hanne Tinggård for technical assistance. Nicoline Soede is thanked for comments and encouragement.

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Sammendrag

Follikeludvikling og ovulation hos søer: Effekt af hCG og GnRH behandling.

Follikelvækst, ovulation og embryomorfologi blev sammenlignet hos spontant ovulerende søer og søer, hvor ovulationen blev induceret med hCG eller GnRH.

Atten søer fik på dag 1 (fravænning = dag 0) ilagt et kateter i ørevenen og søerne blev opdelt tilfældigt i 3 grupper. En kontrolgruppe (N = 6), en gruppe (N = 6) som fik 750 ie hCG (Physex®) im 76 timer efter fravænning (hCG-gruppe) og en gruppe (N = 6) som fik 500 µg GnRH (Fertagyl®) im 76 timer efter fravænning (N = 3) eller 100 timer efter fravænning (N = 3) (GnRH-gruppe). Folliklernes vækst og ovulationstidspunktet blev fastlagt ved transrectal ultralydscanning hver 4. time fra dag 3 indtil ovulation eller udvikling af cyster. Scanneren var en sektorscanner påmonteret et lydhoved (multiangle probe m. frekvens 5,0/7,5 Mhz). Brunstkontrol blev udført hver 8. time fra dag 3 til ovulation. På dag 13 blev søerne slagtet, antallet af corpora lutea (C.L.) talt og uterus skyllet for embryoner.

Kontrolgruppen udviste normale brunstsymptomer. Ved den første scanning på dag 3 var folliklerne

typisk 4-6 mm store. I løbet af de næste 2 dage voksede de til 7-10 mm i diameter og bibeholdte denne størrelse ca. 1 døgn indtil ovulationen 41 ± 9 timer efter første stående brunst. HCG-gruppen udviste ingen brunstsymptomer, og folliklerne opnåede kun en diameter på 6-7 mm ved ovulationen, som fandt sted 40 ± 1 timer efter hCG-injektionen. GnRH-gruppen havde meget variable brunstsymptomer, og folliklerne voksede til 7-12 mm i diameter i løbet af 2 dage, hvorefter de bibeholdte denne størrelse i mindst 24-36 timer. Kun 2 af 6 søer ovulede indenfor 84-92 timer efter injektionen af GnRH og udvikling af cyster i bursa ovarii og cystiske follikler var almindelige fund. Den totale embryo genfindelsesprocent var 79 ± 13 uden signifikant forskel mellem grupperne. Embryodiversiteten regnet som standardafvigelsen i embryonernes maksimale diameter var højere i hCG-gruppen end kontrolgruppen.

Det kan konkluderes, at 1) transrectal ultralydsscanning kan bruges hos søer til bestemmelse af follikelvækst og ovulationstidspunkt; 2) injektion af hCG resulterer i manglende brunstsymptomer og reduceret follikelstørrelse ved ovulationen, hvis givet 76 timer efter fravænning; 3) en enkel injektion af GnRH givet før brunstsymptomer resulterede i variable brunstsymptomer og ingen eller sen ovulation.

(Received May 25, 1994; accepted November 29, 1994).

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