Acta vet. scand. 2000, *41,* 321-328.

Ovulation and Embryonic Developmental Rate Following hCG-stimulation in Sows

By *A. K. Nissen*[1,2,3], *M. Schmidt*[2], *P. Hyttel*[1] and *T. Greve*[2]

[1]Department of Anatomy and Physiology, and [2]Department for Clinical Studies, Reproduction, The Royal Veterinary and Agricultural University, Frederiksberg, and [3]National Committee of Pig Breeding, Health and Production, Copenhagen, Denmark.

Nissen AK, Schmidt M, Hyttel P, Greve T: Ovulation and embryonic developmental rate following hCG-stimulation in sows. Acta vet. scand. 2000, 41, 321-328. – The hCG induced ovulation in sows was studied by use of ultrasonography, and an investigation of the development and diversity of the zygotes/embryos was performed at 24 h after ovulation. Crossbred sows (N=48) were weaned (day 0) and checked for heat twice daily from day 3 onwards. From day 4, the ovaries were transrectally scanned twice daily. On day 4, the sows were given an injection of 750 iu hCG im and inseminated 27 ± 2 h (X ± SD) and 38 ± 1 h later. From 38 to 48 h after the hCG injection, the ovaries were scanned at 60 to 90 min intervals. At 24 h after ovulation the oviducts were surgically flushed in 18 sows. Out of the 48 sows, 34 showed heat at 12-36 h after the hCG-treatment and 14 showed heat before the hCG treatment. In the former group of sows, 20 (59%) ovulated within the interval of 38 to 48 h after the hCG treatment, and the follicular size immediately before ovulation was 7.8 ± 0.6 mm. Among the sows which showed heat before hCG treatment only 7 (50%) ovulated within the above interval and the preovulatory follicle size was larger (8.3 ± 0.5, $p<0.05$) than in the former group of sows, which showed heat after the hCG treatment. The flushing of 18 sows yielded a total of 243 ova, 70 (29 %) 1-cell stages, 160 (66 %) 2-cell stages and 13 (5%) 4-cell stages. A pronounced difference in the degree of variation in embryonic development was seen between sows: 4 animals yielded 1- to 4-cell stages, one exclusively 2-cell stage. In conclusion, the control of ovulation in sows by hCG treatment will affect the follicular growth and the exact timing of ovulation can not always be relied on. It is strongly recommended to use ultrasonography to monitor the time of ovulation if this parameter is important. Ova recovered at 24±1 h after the median time of ovulation revealed a pronounced diversity (1- to 4- cell stage) within sows. No obvious relation with this embryonic diversity and the follicular size at ovulation was seen in these data.

embryonic diversity; transrectal ultrasonography; follicular growth; embryo collection; onset of heat.

Introduction

In pig production the litter size is of great economic importance. This crucial parameter is mainly dependent on the rates of ovulation and embryonic mortality, as the fertilization rate is assumed to be 95% to 100% (*Hunter* 1967 & 1972, *Polge* 1978).

The frequency of embryonic mortality is reported to be 30% to 50% (*Pope & First* 1985), but the mechanisms behind it are poorly understood. Experiments indicate that developmental asynchrony, known as diversity, within the litter may disturb the embryo-uterus interaction for

some embryos, causing the loss of the least developed embryos (*Pope et al.* 1990).

This concept has stimulated research in mapping of both the developmental rates of the porcine embryo and of the degree of diversity within a litter at different stages of development. The degree of diversity varies greatly between as well as within breeds and is seen at all stages of development (*Van der Lende et al.* 1994). Further insight into causes and the degree of embryonic diversity during embryonic development might add to the understanding of the background of this phenomenon.

Pope et al. (1990) evaluated the different factors, which could affect embryo development and lead to diversity and concluded that the oocyte and follicular maturation, the process of ovulation, and the expression of the embryonic genotype, were the factors most likely to be involved. The process and duration of ovulation have been found not to have any measurable affect on embryonic diversity (*Soede et al.* 1992, *Van der Lende et al.* 1994). This is in agreement with *Xie et al.* (1990), who showed that the diversity found during oocyte maturation was comparable to that found among zygotes after fertilization. However, they used onset of heat as their reference point, which can give age variation of the zygotes due to the variation in time of ovulation after onset of heat (*Soede et al.* 1992, *Nissen et al.* 1997). As the age variation can have an impact of the degree of development of the zygotes, exact timing of the ovulation is crucial when investigating degree of diversity in zygotes between sows.

It is generally assumed that ovulation takes place about 40 h after injection of human chorionic gonadotropin (hCG). However, if the hCG is not given shortly before the endogenous surge of LH, deviations such as lack of heat signs and impaired follicular growth resulting in reduced follicular size at ovulation can be seen (*Hunter* 1976, *Soede et al.* 1992, *Nissen et al.* 1995). The reduced follicular size is likely to affect the final oocyte and follicular maturation and the subsequent quality of the ovulated oocytes (*Hunter* 1979, *Grant et al.* 1989, *Wiesak et al.* 1990), and according to *Pope et al.* (1990) it may have an impact on embryonic diversity. By giving the hCG injection late, around the onset of heat, the suppression of the final follicular growth and the possible effect on final oocyte and follicular maturation may be avoided (*Nissen et al.* 1995). On the other hand, this approach is combined with the risk that the endogenous LH-surge has already initiated follicular and oocyte maturation.

The objective of this study was to monitor the hCG induced ovulation and the size of the ovulatory follicles by the use of ultrasonography and to investigate the development and diversity of the zygotes/embryos at 24 h after the ovulation.

Materials and methods

Animal treatment, heat detection and ultrasonography

Danish crossbred sows (Yorkshire, Landrace and Duroc, parity 2 to 9, 180 to 250 kg bdw of which 48 were chosen on the basis of history of normal fertility and ultrasonography findings such as normal ovaries without cysts or fresh corpora lutea (CL)), were weaned 3 weeks after farrowing (day 0) and transported to the stable in experimental groups of 3-5 sows. Heat detection was carried out twice daily from days 3 to 6. The onset of heat was defined as the first time the sow showed standing reflex in front of a boar in combination with swelling and reddening of the vulva. On day 4, the sows were given an im injection of 750 iu hCG (Physex®, Leo Pharmaceutical, Ballerup, Denmark) to induce ovulation.

From day 4, the ovaries of the sows were scanned morning and late afternoon by transrectal ultrasonography (Basic Scanner 150,

Table 1: Time of ovulation and onset of heat in relation to hCG injection.

	Onset of heat before or at hCG injection, number (%)	Onset of heat after hCG injection, number (%)	Total number (%)
Time of ovulation before expected interval (before 38 h after hCG injection)	7[a] (50)	13[b] (38)	20 (42)
Time of ovulation in expected interval (38 to 48 h after hCG injection)	7 (50)	20 (59)	27 (56)
Time of ovulation after expected interval (48 to 64 h after hCG injection)	0	1 (3)	1 (2)
Total	14 (100)	34 (100)	48 (100)

[a,b)] The numbers of sows ovulating before 38 h after the hCG injection are significantly different from zero.

multiangle probe 5.0/7.5 Mhz, Pie Medical®, Maastricht, The Netherlands) as described by *Nissen et al.* (1995). The number of follicles and the average diameter of 3-5 follicles, representative for the ovary, were noted together with any features deviating from the expected normal ultrasonographic findings. From 38 to 48 h after the hCG injection the ovaries were scanned at 60 to 90 min intervals until the time and duration of the ovulation wave had been determined. The duration of the ovulation wave was roughly defined as the interval between the last scanning at which the maximum number of follicles was recorded and the scanning with no presumptive ovulatory follicles left. The median time of ovulation was defined as the midpoint of this interval. For sows ovulating before 38 h post hCG treatment, the time and duration of the ovulation was not monitored. If no ovulation had taken place at 48 h after the hCG injection, the sows were scanned once daily until ovulation had occurred.

The sows were inseminated twice at 27 ± 2 h and 38 ± 1 h after hCG injection with semen from the same Landrace boar. The semen was collected and controlled at the AI-station (2.0×10^9 sperm per dose).

Collection of presumptive zygotes and embryos

The sows which ovulated in the interval 38 to 48 h after hCG injection were operated on at 24 ± 1 h after median time of ovulation (day 7). The sows (N=18) were given azaperonum 800 mg IM (Sedaperone® vet., Janssenpharm, Birkerød, Denmark, 40 mg/ml) as a sedative, and 30 min later the sows were submitted to general anaesthesia with thiopental (Pentothal®, Abbot, North Chicago, USA, 15mg/kg bdw). Each oviduct was flushed with 2x20 ml of phosphate buffered saline (PBS, Dulbeccos) supplemented with 1% heat inactivated fetal calf serum (flushing medium) at 38 °C through a flank incision. The uterus wall was penetrated by a blunt needle (16 or 18 G) which was further pushed through the utero-tubal junction. The needle was connected to a syringe containing 20 ml of flushing medium, which was flushed from the uterus tubal junction to the infundibulum, where a catheter collected the flushing medium.

Following flushing, the presumptive zygotes and embryos were isolated and evaluated under a stereomicroscope (100× to 320×). Their total number and their stage of development (1-4 cells) were recorded. Ova displaying shrinkage

Table 2: Medium time of ovulation and duration of the ovulation wave in sows ovulating 38 to 48 h after hCG injection (mean ± standard deviation [range]).

	Number of sows (N)	Interval from onset of heat to medium time of ovulation (h)	Interval from hCG injection to medium time of ovulation (h)	Max. Size of follicles (mm)	Duration of ovulation wave (h)
Onset of heat before hCG injection	7	42.4 ± 3.1 [39 to 48]	40.6 ± 1.8 [38½ to 44]	8.3 ± 0.5 [8 - 9]	1.9 ± 0.8 [1 to 3]
Onset of heat after hCG injection	20	24.4 ± 3.2 [18 to 28½]	41.2 ± 1.6 [38½ to 44]	7.8 ± 0.6 [7 - 8]	2.4 ± 1.2 [1 to 4]
Total	27	29.1 ± 8.6 [18 to 48]	41.0 ± 1.6 [38½ to 44]	7.9 ± 0.6 [7 - 9]	2.3 ± 1.1 [1 to 4]

or excessive granulation were considered as degenerated. Ova without spermatozoa in the zona pellucida were considered as being non-fertilized.

Statistics

The distributions of ovulation frequencies were compared using the chi-square test. The data in the text are indicated as X ± SD [range]. All other data are only presented descriptively as mean (X) and [range].

Results

Heat control and ultrasonography

In 14 out of the 48 sows monitored, the onset of heat was seen before or at the time of hCG injection, whereas in 34 sows heat was not detected until 12 to 36 h after the injection (Table 1). Noticeably, only 27 sows ovulated at 38 to 48 h after the hCG injection, and in these animals the time and duration of the ovulatory wave was monitored and showed that median time of ovulation occurred 41.0 ± 1.6 h after the hCG injection and the duration of the ovulation

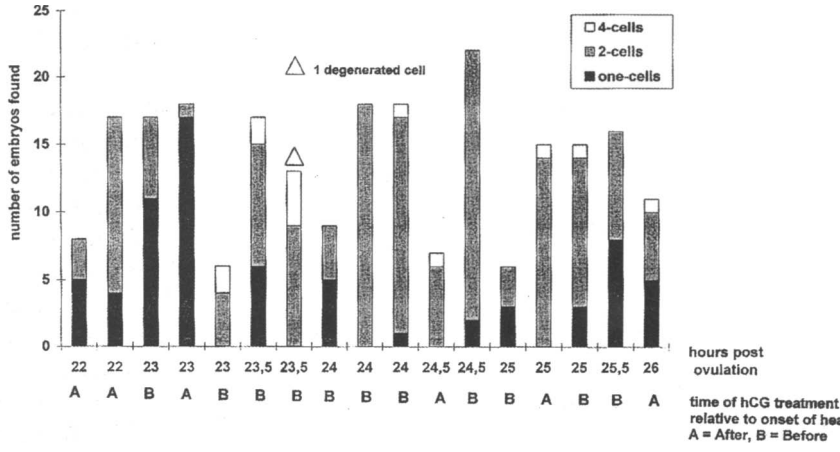

Figure 1: Developmental stages of embryos recovered 22-26 h post ovulation from 17 sows. (Each column represents 1 sow).

Table 3: Connection between duration of ovulation, follicle size at ovulation and developmental stage of ova recovered approx. 24 h after ovulation.

Sow No.	Time of hCG treatment relative to onset of heat	Time after ovulation (h)	Developmental stage (number)				Size of follicles at ovulation (mm)	Duration of ovulation wave (h)
			one-cell (N)	2-cell (N)	4-cell (N)	total (N)		
16[a)]	After	22 h	5	3	-	8	8 [8]	1
19	After	22 h	4	13	-	17	7.3 [7-8]	1½
14	Before	23 h	11	6	-	17	7.3 [7-8]	2
45	After	23 h	17	1	-	1	8 [8]	1
10	Before	23 h	-	14	2	16	7.3 [7-8]	4
13	Before	23½ h	6	9	2	17	7.3 [7-8]	1
383	Before	23½ h	-	9	4	13	7 [7]	1½
24	Before	24 h	5	4	-	9	8 [7-9]	2½
21	Before	24 h	-	18	-	18	7 [7]	2½
47	Before	24 h	1	16	1	18	7.3[7-8]	1½
26[b)]	After	24½ h	-	6	1	7	7.7[7-8]	3
29	Before	24½ h	2	20	-	22	7 [7]	1½
23	Before	25 h	3	3	-	6	7.3 [7-8]	3
41	After	25 h	-	14	1	15	8 [7-9]	1½
4140	Before	25 h	3	11	1	15	7 [7]	1½
2	Before	25½ h	8	8	-	16	7.3 [7-8]	4
4054	After	26 h	5	5	1	11	7.6 [7-9]	1

[a)] The left oviduct was not flushed due to a block. [b)] Only cysts were observed at the right ovary.

wave was on average 2.3 ± 1.1 h (Table 2). The maximum number of follicles counted by ultrasonography per sow was on average 17.2 ± 3.8. The average maximum follicle size monitored at the last scanning before ovulation was 7.9 ± 0.6 mm. However, when hCG was given at or after the onset of heat the follicles were significantly ($p<0.05$) larger than when hCG was given before onset of heat (8.3 ± 0.5 mm vs 7.8 ± 0.6 mm respectively).

In 20 sows ovulating before and 1 sow ovulating after the expected time frame of 38 h to 48 h after the hCG treatment, the time and duration of the ovulatory wave was not monitored.

Embryo collection

A total of 18 sows were operated 24 ± 1 h after median time of ovulation. The flushing of 17 of the 18 sows yielded a total of 243 embryos, all with spermatozoa in the zona pellucida. A single ovum was considered generated (Fig.1). The average recovery rate in these sows was 89% ± 17%, and of these embryos 70 (29%) were at the 1-cell stage, 160 (66%) at the 2-cell stage and 13 (5%) at the 4-cell stage (Table 3 and Fig. 1). Flushing of the remaining sow resulted in the recovery of 12 unfertilised 1-cell stages without spermatozoa in the zona pellucida. Examination of the uterus revealed block of the lumen in both uterine horns.

A pronounced difference in diversity of embryonic development was noticed between sows. Thus, 4 animals yielded 1-4-cell embryos while one animal yielded exclusively 2-cell stage (Table 3 and Fig. 1).

Discussion

The time from hCG injection to ovulation is reported to vary both between and within breeds depending upon several factors (*Polge* 1978, *Hunter* 1979). In general, ovulation is expected to occur 38 to 46 h after injection. We gave the hCG injection on day 4 to assure growth of the follicles to a normal preovulatory size (*Nissen et al.* 1995). But, when giving the hCG injection late i.e. after the onset of heat, a large proportion of the sows will ovulate at an unpredictable time, before the expected hCG induced ovulation. However, our use of ultrasonography with the 60-90 min. interval allowed determination of the time of ovulation with similar accuracy, despite the late hCG injection. We found that, if the hCG injection was given in proestrus, 12 to 36 h before onset of heat, only 59% of the sows ovulated within the interval 38 to 48 h after injection. If the hCG injection was given as late as at the beginning of oestrus, after the onset of heat, only 50% of the sows ovulated within this interval.

The considerable number of sows ovulating before the expected time in both groups underline the fact that the relative late hCG treatment of sows is inaccurate for precise prediction of the time of ovulation, eg. for synchronous embryo transfer or production of zygotes for genetic manipulation. In experimental design where timing of the ovulation is essential it is highly recommended to use ultrasonography to monitor the time of ovulation.

The unpredictability of the timing of the ovulation after a late hCG injection, i.e. injection after the onset of heat, is probably due to the fact that the endogenous LH surge has already induced the ovulation and the final follicular maturation. On the contrary, when the hCG treatment is administered early, i.e. before the onset of heat, final follicular maturation is induced by the hCG.

In the latter cases final follicular maturation is induced at a smaller follicular size, resulting in consequently smaller follicles at the time of ovulation (Table 2).

A relationship between the follicular size at ovulation, the duration of ovulation and the development of the embryos flushed at 24 h after the ovulation could not be established (Table 3).

Previous investigations of the chronology of porcine embryonic development have been based upon time scales starting at the onset of heat (*Heuser & Streeter* 1929, *Cassar et al.* 1994), insemination (*Hunter* 1974), or hCG-injection (*Hunter et al.* 1993). Considering the data discussed above, the latter parameter is apparently not accurate. The duration of heat and the time of ovulation relative to the onset of heat also vary among sows even within the same breed (*Soede et al.* 1995). Accordingly, in another experiment (*Nissen et al.* 1997) we found the average duration of heat to be 60 ± 13 h (range: 30-89, n=118) and the interval from onset of heat to ovulation to be 42 ± 11 h (range: 17-68, n=118). Thus, the postovulatory age of ova collected at a fixed time interval after the onset of heat would in that case differ up to 51 h, which renders the onset of heat an unprecise measure of ovulation time. *Hunter* (1974) reported that the first cleavage occurs at 14 to 16 h after activation, which, however, was not precisely defined in relation to clinical signs or other events. Our results, obtained by flushing of ova at 24 ± 1 h after the median time of ovulation clearly demonstrated that a difference in the rate of embryonic development exists within the litter of the individual sow.

The relationship between the difference in rate of embryonic development and the sizes of the ovulatory follicles could not be established.

In conclusion, the control of ovulation in sows hCG treatment at day 4 post weaning may affect the follicular growth and the exact timing of ovulation can not be estimated in half of the sows. It is strongly recommended to use ultrasonogra-

phy to monitor the time of ovulation if this parameter is important.

Ova recovered 24 ± 1 h after the median time of ovulation revealed a pronounced diversity (1- to 4-cell stage) within sows. Moreover, administration of hCG before onset of heat, results in decreased follicular size at the time of ovulation.

Acknowledgements

The author thanks Ms. Inger Heinze and Ms. Anna Marie Larsen for technical assistance. The work was supported by The Research Secretariat under the Danish Ministry of Agriculture.

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Sammendrag

Ovulation og embryo udviklingsgrad ved hCG stimulering i søer.

Follikelstørrelse samt ovulationens varighed og tidspunkt blev undersøgt v.h.a. ultralydscanning hos søer i forbindelse med hCG induceret ovulation. Derefter blev embryonernes udviklingsstadium og diversitet vurderet 24 timer efter ovulationen. Krydsningsøer (N=48) blev fravænnet (dag 0), og brunstkontrol

blev udført 2 gange dagligt fra dag 3. Fra dag 4 blev ovarierne ultralydscannet 2 gange dagligt. På dag 4 fik søerne en injektion med 750 ie hCG im og blev insemineret 27 ± 2 og 38 ± 1 timer efter. Fra 38-48 timer efter hCG injektionen blev scanningen udført med 60-90 min. interval. Fireogtyve timer efter ovulationen blev æggelederne på 18 søer skyllet kirurgisk. Af de 48 søer viste 34 brunst 12-36 timer efter hCG behandlingen, og 14 viste brunst før hCG behandlingen. I den første gruppe af søer ovulerede 20 (59%) indenfor intervallet 38 - 48 timer efter hCG injektionen, og follikelstørrelsen umiddelbart før ægløsningen var 7,8 ± 0,6 mm. Af de søer, der viste brunst før hCG behandlingen, ovulerede 7 (50%) indenfor det ovennævnte interval, og den preovulatoriske follikelstørrelse var større (8,3±0,5 mm, $p<0,05$). Ved skylning af 18 søer blev fundet 243 ova fordelt som; 70 (29%) 1-cellestadie, 160 (66%) 2-cellestadie og 13 (5%) 4-cellestadier. En udtalt forskel i udviklingstadium sås såvel indenfor som imellem søer: Fire søer gav 1-4 cellestadier, mens en anden udelukkende gav 2-cellestadier. Det kan konkluderes, at kontrol af ovulationen hos søer med hCG injektion ikke altid er tidsmæssig præcis, og at hCG behandlingen endvidere kan påvirke follikelvæksten. Det anbefales stærkt at benytte ultralyd til scanning af ovulationstidspunktet, hvis denne parameter er af betydning. Embryoner opsamlet 24 ± 1 timer efter ovulationen viste en udtalt diversitet (1- til 4-cellestadier) indenfor og imellem søer. Ingen tydelig relation mellem denne diversity og follikel størrelse ved ovulationen kunne ses ud fra resultaterne.

(Received June 17, 1998; accepted Maj 1, 1999).

Reprints may be obtained from: M. Schmidt, Department of Clinical Studies, Section of Reproduction, The Royal Veterinary and Agricultural University, Dyrlægevej 68, DK-1870 Frederiksberg C, Denmark.