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Effect of injection of a GnRH analogue at the onset of oestrus on LH and FSH release and embryonic mortality in gilts

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Abstract

The effect of i.m. injection of 100 μg Gonadotropin Releasing Hormone (GnRH) analogue at the onset of oestrus on LH and FSH release and number of corpora lutea (CL) and embryos in gilts was investigated. In *Experiment I*, the LH and FSH peaks of 5 GnRH treated gilts were not significantly different from the ones in the previous (control) oestrous period, but both LH and FSH peak tended to be sharper after GnRH injection. In *Experiment II*, GnRH treated and control gilts were slaughtered on Days 4, 11, 13 or 35 of pregnancy. In 15 GnRH treated gilts slaughtered on Days 4, 11 or 13 the number of CL (16.3 ± 2.3; mean ± s.d.) and embryos (15.0 ± 3.0) were significantly increased ($P < 0.05$) when compared with non-treated control gilts (N=15; CL: 14.8 ± 1.5; embryos: 12.1 ± 3.4). On Day 35 the embryonic mortality rate in the GnRH group had increased to 24.7 % and was very low in the control group (11.3 %). Inclusive of the data of the gilts slaughtered on Day 35, the number of CL and embryos in the GnRH group were larger than in the control group (CL: 16.4 ± 2.3 and 15.4 ± 2.0; embryos 14.3 ± 3.3 and 13.0 ± 3.3, respectively), but this was no longer significant. The results of this study indicate that injection of a GnRH analogue at the time of observing first standing oestrus in cyclic gilts tends to induce a sharper LH and FSH peak and tends to increase the number of ovulations and embryos.

Keywords: gilts, GnRH, LH, FSH, embryonic mortality

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Introduction

The time interval between ovulation and mating during oestrus is one of the most important factors with regard to fertility and litter size in pigs (Polge 1969). The optimal time of mating is usually determined in relation to the onset of behaviourial oestrus. This assumes a fixed time interval between the onset of oestrus and the time of ovulation. Several studies (Tilton et al., 1982; Ziecek et al., 1982; Helmond et al., 1986) have shown that this interval may vary considerably, which may result in a less than optimal fertility. In our experimental design we have assumed that Gonadotropin Releasing Hormone (GnRH) can be used to induce a Luteinizing Hormone (LH) peak and subsequent ovulation at a fixed time interval after the onset of behaviourial oestrus. This way the most optimal time of mating can theoretically be determined precisely.

The hypothalamic factor GnRH stimulates the release of both LH and Follicle Stimulating Hormone (FSH) from the anterior pituitary. It has been shown that single or repeated injections of GnRH can induce an LH peak and subsequent ovulation in prepuberal gilts (Chakraborty et al., 1973; Lutz et al., 1985), gilts with a delayed puberty (Edqvist et al., 1978), lactating sows (Cox & Britt, 1982) and anoestrous sows (Armstrong & Britt, 1985). Administration of GnRH at the onset of oestrus may also affect ovulation rate and/or embryonic survival. An intravenous injection of 200 μg GnRH at the onset of puberal oestrus in gilts resulted in an increase in the number of corpora lutea (CL) but did not enhance the number of viable embryos on Day 30 of pregnancy (Archibong et al., 1987). In sows the effect of GnRH seems to be dependent on the condition of the animals. In high-fed sows an intramuscular injection of 50 μg GnRH at the onset of first post-weaning oestrus did not affect the number of CL or viable embryos, but in first-parity low-fed sows GnRH injection resulted in an increased number of viable embryos on Day 25 of pregnancy (Kirkwood et al., 1987). The intramuscular injection of 100 μg GnRH at the onset of first postweaning oestrus in multiparous sows in good condition did not result in a significant increase of litter size (Gooneratne et al., 1989). The studies mentioned above suggest that GnRH may increase the number of CL but not the litter size. A possible explanation is that GnRH affects embryonic development and mortality. In this study, therefore, we investigated the effects of a GnRH analogue injected at the onset of oestrus in cyclic gilts on preovulatory LH and FSH concentrations, and on embryonic mortality and development on Days 4, 11, 13 and 35 of pregnancy.

Materials and methods

Experiment I

Five crossbred gilts (Great Yorkshire × Dutch Landrace) which had shown 3 normal oestrous cycles (18-22 days) were fitted with an indwelling jugular vein catheter (PVC, 1.0 mm internal diameter, 1.5 mm external diameter) 5 days before expected oestrus. Blood samples were collected 3 times daily (9.00, 12.00 and 15.00 h). The gilts were checked for oestrus with a vasectomized boar twice daily (09.00 and

15.00 h). At the onset of standing oestrus (Day 0) 2 ml saline was injected intramuscular (control oestrus). Blood samples were collected at 30 min intervals for the first 4 h following the injection and at 6, 9, 12 and 15 h thereafter. In the next cycle of these 5 gilts, at the time of observing first standing oestrus, 100 μg GnRH analogue (Ovalyse: Upjohn Company, Ede, Netherlands) were injected intramuscular (GnRH oestrus). Blood samples was taken in the same frequency as in the control oestrous period.

Experiment II

Forty-nine crossbred gilts (Great Yorkshire × Dutch Landrace) were checked for oestrus with a vasectomized boar twice daily (09.00 and 15.00 h). Before the second oestrus the gilts were randomly assigned to one of 2 groups. In the GnRH group the gilts were injected intramuscular with 100 μg GnRH analogue at the time of observing first standing oestrus (Day 0). In the control group the gilts were not treated. All gilts were artificially inseminated with fresh diluted semen on Day 1, 24 h after observing first standing oestrus.

Gilts from both groups were slaughtered on Days 4 (N=11), 11 (N=13), 13 (N=11) or 35 (N=14) of pregnancy. These days were chosen to evaluate mortality rate before blastocyst formation (Day 4), before elongation (Day 11), after elongation (Day 13) and at the end of the embryonic stage (Day 35). The reproductive tract of each gilt was removed and transported on ice to the laboratory. The number of CL on each ovary were counted, and the uterus and cervix were separated from the mesometrium.

On Day 4 the oviducts and uterine horns were flushed twice with 10 and 30 ml Dulbecco's phosphate-buffered saline (PBS: Gibco, Paisley, UK), respectively, and the number of embryos were determined. On Day 11 the uterine horns were flushed. The number of blastocysts were determined and their diameters were measured. On Day 13 the uterine horns were opened longitudinally along the antimesometrial border, placed in a dissection tray in Dulbecco's PBS and pinned to the wax base. The blastocysts were detached from the endometrium by a gentle stream of buffer after stretching of the endometrial folds (Bate & King, 1988). In this way filamentous blastocysts were recovered individually. Their numbers were determined after they had been checked for the presence of an embryoblast.

On Day 35 the uterine horns were opened longitudinally and the embryos were removed from the uterus (Van der Lende, 1989). The number of apparently normal and healthy embryos were determined. The weight of the embryos and the extra-embryonic membranes and the length of the embryos and placentae were measured (Van der Lende, 1989).

Hormone analysis

All blood samples were collected in heparinized tubes and centrifuged. The separated plasma was stored at -20 °C until hormone analysis.

Plasma concentrations of LH were measured by a double-antibody radioimmu-

noassay using porcine LH (LER 786-3, potency 0.65 × NIH-LH-S1 units/mg; see Van der Meulen et al., 1990). The sensitivity of the LH (mol. weight 30000 dalton) assay was 0.7 ng/ml or 23.3 pmol/l at the 90 % B/B_0 level. The intra-assay coefficient of variation was 13.8 % and the inter-assay coefficient of variation was 16.8 %.

Plasma FSH concentrations were measured similar to plasma LH, using antiserum to porcine FSH (i531/001: UCB, Brussels, Belgium; working dilution 1:15000) and porcine FSH (i031: UCB; potency 280 × NIH-FSH-P1 units/mg) as a standard and for iodination. The sensitivity of the FSH (mol. weight 34000 dalton) assay was 1.9 ng/ml or 55.9 pmol/l at the 90 % B/B_0 level. The intra-assay coefficient of variation was 8.2 % and the inter-assay coefficient of variation was 11.0 %.

Statistical analysis

Hormone analysis data were transformed to their natural logarithms. Differences in LH and FSH between the control and GnRH oestrous period were tested for significance by Student's paired t-test. Differences in number of CL, embryos and embryonic mortality rate between the control and GnRH group were tested for significance by analysis of variance. The standard deviation of the diameter within litters on Day 11 was tested for significance by analysis of variance with average diameter as covariable (SAS, 1985). All results are expressed as mean ± s.d.

Results

In Experiment I, during both oestrous periods standing oestrus was first detected at the 9.00 h oestrous check. The LH concentration during the 2 pre-oestrous days was the same in both control and GnRH oestrus (0.06 ± 0.02 nmol/l). At the time of observing first standing oestrus LH was increased in control (0.15 ± 0.10 nmol/l) as well as GnRH oestrus (0.14 ± 0.06 nmol/l). In the control oestrus LH was maximal 1 h after observing first standing oestrus (0.16 ± 0.10 nmol/l) and remained around 0.15 nmol/l for another 3 h. In the GnRH oestrus LH showed a further increase up to 2 h after GnRH injection (0.22 ± 0.10 nmol/l) and declined thereafter (Fig. 1).

The FSH concentration during the 2 pre-oestrous days in the control oestrus (0.31 ± 0.15 nmol/l) was higher than in the GnRH oestrus (0.17 ± 0.13 nmol/l) ($P < 0.01$). In the control oestrus FSH was increased at the time of observing first standing oestrus (0.51 ± 0.16 nmol/l) and remained around this level with a maximal value (0.65 ± 0.22 nmol/l) 3 h after injection of saline. In the GnRH oestrus FSH was also increased at the time of observing first standing oestrus (0.35 ± 0.25 nmol/l), increased to 0.59 ± 0.30 nmol/l 2.5 h after GnRH injection, and declined thereafter. The FSH peak occurred 30 min after the LH peak. A second FSH peak occurred on Day 3 in the control oestrus (0.86 ± 0.26 nmol/l) and on Day 2 in the GnRH oestrus (0.78 ± 0.18 nmol/l) (Fig. 1).

Experiment II

At slaughter on Days 4, 11 and 13 in Experiment II, one control and 4 GnRH treated

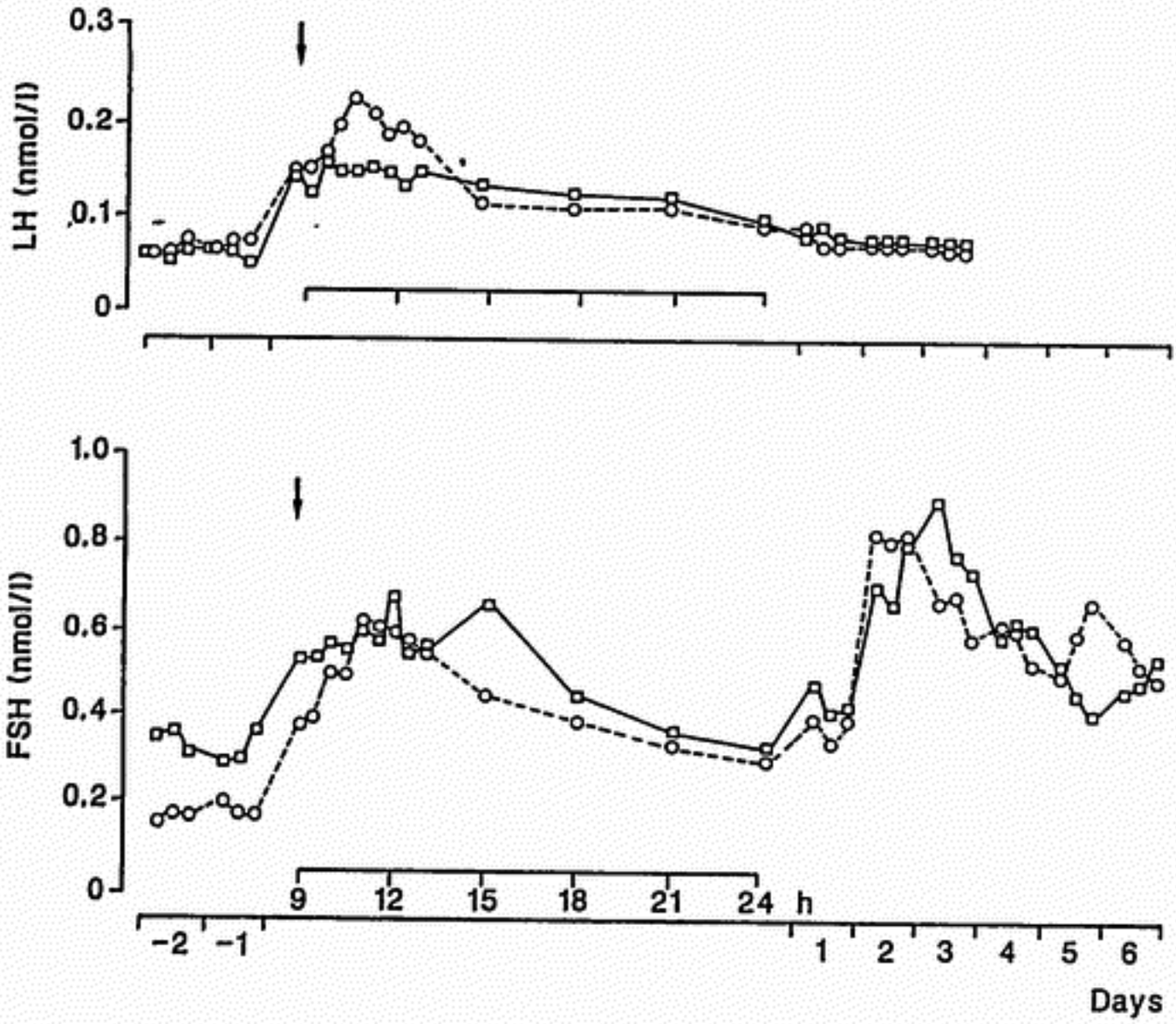

Fig. 1. Plasma concentrations of LH and FSH in 5 gilts during 2 successive oestrous periods. In the first period at the onset of standing oestrus (Day 0,↓) saline (□-□) and in the second period a GnRH analogue (O-O) was injected.

gilts appeared to be non-pregnant and 2 gilts of the Day 35 control group returned to oestrus before slaughter. The pregnancy rate in the control group was 87.5 % and 84.0 % in the GnRH group. The data concerning the CL numbers of the non-pregnant gilts were excluded from analysis. On Day 4 the number of CL in the GnRH group were significantly larger than in the control group ($P < 0.05$), but there was no significant difference in the number of embryos (Table 1). On Days 11 and 13 the number of CL and embryos was larger in the GnRH group than in the control group, but these differences were not significant (Table 1). On Day 35 the opposite was observed; a not significantly larger number of CL and embryos in the control group in comparison with the GnRH group (Table 1).

A significant Day × treatment interaction was observed, which disappeared after omitting the data obtained on Day 35. For the gilts slaughtered on Days 4, 11 and 13 the number of CL and embryos in the GnRH group were larger than in the control group ($P < 0.05$) and the embryonic mortality rate was lower (Table 2). The cell

Table 1. The number of CL and embryos and the embryonic mortality rate of control and GnRH treated gilts slaughtered on Days 4, 11, 13 or 35 of pregnancy.

Group	Day of slaughter	N CL	Number of embryos	Number of rate (%)	Mortality
Control	4	5	15.0 ± 0.7[a]	13.0 ± 2.0	13.5 ± 11.2
GnRH	4	5	17.2 ± 1.9[b]	15.2 ± 3.3	12.3± 10.3
Control	11	5	14.6 ± 2.3	12.4 ± 3.8	16.6 ± 18.3
GnRH	11	5	15.0 ± 1.6	13.6 ± 1.8	9.2 ± 8.9
Control	13	5	14.8 ± 1.3	11.0 ± 4.4	25.8 ± 29.8
GnRH	13	5	16.8 ± 3.0	16.2 ± 3.6	5.8 ± 1.4
Control	35	6	17.0 ± 2.5	15.2 ± 2.1	11.3 ± 9.7
GnRH	35	6	16.7 ± 2.5	12.5 ± 3.5	24.7 ± 20.5

Values with different superscripts are significantly different ($P < 0.05$)

Table 2. The number of CL and embryos and the embryonic mortality rate in the control and GnRH group slaughtered on Days 4, 11 and 13.

Group.	N CL	Number of embryos	Number of rate (%)	Mortality
Control group	15	14.8 ± 1.5[a]	12.1 ± 3.4[a]	18.6 ± 20.4
GnRH group	15	16.3 ± 2.3[b]	15.0 ± 3.0[b]	9.1 ± 8.9

Values with different superscripts are different ($P < 0.05$)

number of Day 4 embryos varied between 1 and 9. The number of one-cell embryos was not significantly different between both groups (control group: 4 one-cell embryos in 3 gilts; GnRH group: 7 one-cell embryos in 3 gilts). On Day 11 the recovered blastocysts were all spherical with a diameter of 4.8 ± 1.1 (control group) and 4.6 ± 0.6 mm (GnRH group). The variation in blastocyst diameter within litters was larger in the control than in the GnRH group (1.1 ± 0.5 versus 0.7 ± 0.1 mm). However, after correction for differences in blastocyst diameter this difference in within-litter variation was not significant ($P=0.068$). On Day 13 most of the recovered blastocysts were filamentous. In the control group in 4 gilts 1 or 2 blastocysts

Table 3. Parameters for embryonic development in the GnRH and control group on Day 35 of pregnancy.

	Control-group	GnRH-group
embryonic weight (g)	3.7 ± 0.4	4.0 ± 0.3
embryonic length (cm)	3.9 ± 0.2	4.0 ± 0.2
placental weight (g)	33.6 ± 5.6	38.1 ± 12.3
placental length (cm)	36.8 ± 25	42.1 ± 8.1
amniotic fluid weight (g)	4.6 ± 0.4	4.9 ± 0.5
allantoic fluid weight (g)	86 ± 47	131 ± 87

were tubular. In the GnRH group in 2 gilts blastocysts were spherical, tubular and filamentous. On Day 35 the weight of the embryos and extra-embryonic membranes and the length of the embryos and placentae were larger in the GnRH treated than in the control gilts, but none of these differences was significant (Table 3).

Discussion

Experiment I

The magnitude of the maximum LH level during control and GnRH oestrus in Experiment I was not different from those reported before (Henricks et al., 1972; Wilfinger et al., 1973a; Parvizi et al., 1976; Brinkley, 1981, Van de Wiel, 1981). In earlier studies a preovulatory FSH peak occurring almost coincidently with the preovulatory LH peak was usually observed in gilts (Van de Wiel, 1981; Vandalem et al., 1979) and sows (Edwards & Foxcroft, 1983), although in some studies this FSH peak was not detected (Brinkley, 1981; Wilfinger et al., 1973b; Rayford et al., 1974).

The maximum LH and FSH levels were not different between the control and GnRH oestrus. Compared with control oestrus, however, both the LH and FSH peak tended to be sharper after injection of a GnRH analogue. Since in the control oestrus the LH peak in all 5 gilts occurred within 4 h after observing first standing oestrus, a sharper LH peak may be the result of endogenous LH release enhanced by GnRH. In anoestrous sows induced to ovulate by pulsatile GnRH administration, the induced LH peak also seems sharper compared with that of sows exhibiting spontaneous oestrus (Armstrong & Britt, 1985). The intramuscular injection of 100 μg GnRH at the onset of first postweaning oestrus in multiparous sows in good condition did also result in sharp LH peaks between 90 and 120 min after injections (Gooneratne et al., 1989). In contrast to our observations the latter study showed a second, probably endogenous, LH peak within 24 hours. Variations in the response of LH to a GnRH injection may be explained by the varying oestradiol concentrations which exist in the time of the GnRH injection.

Experiment II

In Experiment II, the number of CL in GnRH treated gilts was on average one larger than in control gilts. In gilts injected with GnRH at the onset of puberal oestrus (Archibong et al., 1987) and in GnRH treated first-parity low-fed sows (Kirkwood et al., 1987) the number of CL had increased by about 2.5. This increase in number of CL may be due to the ovulation of follicles which would not have ovulated without injection of GnRH, and this in turn may be related to the sharper LH and FSH peak.

On Days 4, 11 and 13 the higher ovulation rate in the GnRH group was accompanied by a decreased embryonic mortality rate and an increased number of embryos. The possible occurrence of a delayed LH peak with respect to the onset of oestrus (Tilton et al., 1982; Ziecik et al., 1982; Helmond et al., 1986) may have been prevented by the injection of the GnRH analogue. In this way potential mortality due to a lack of synchronicity between the time of the preovulatory LH peak and the

onset of oestrus (Tilton et al., 1982; Helmond et al., 1986) may have been reduced. Since the variation in diameter of the Day 11 blastocysts was lower in GnRH treated than in control gilts, the induced sharper preovulatory LH peak may also have affected the duration or pattern of ovulation and as a consequence embryonic survival (Pope et al., 1988).

In this study the number of blastocysts was determined not only before (Day 11) but also just after elongation (Day 13), in order to evaluate a possible effect of this process on mortality rate. The mortality rate in the GnRH group did not change from Day 11 to 13, and was comparable to the mortality rate on Day 4. This indicates that in the GnRH group pre-implantation embryonic mortality occurred before Day 4. In the control group, embryonic mortality increased during the process of elongation.

On Day 35 the number of CL and embryos in the control group exceeded that of GnRH treated gilts. In the Day 35 control group, however, the number of CL was relatively high. The embryonic mortality rate of 11.3 % on Day 35 was low compared with the embryonic mortality rate in the Day 4, 11 and 13 control groups, the embryonic mortality rates in other groups of the same breed on Day 35 at our laboratory and that described earlier for animals which were not treated with GnRH (Flint et al., 1982; Pope & First, 1985; Van der Lende, 1989). On Day 35 the embryonic mortality rate in the GnRH group was 24.7 %. This is higher than Days 4, 11 and 13, but comparable to those described earlier (Flint et al., 1982; Pope & First, 1985; Van der Lende, 1989). The reason for the increase in embryonic mortality rate in the GnRH group between elongation (Day 13) and Day 35 is unknown. The difference in embryonic development on Day 35 between the control and GnRH group is small, and there were no indications that the variation within litters differed between the 2 groups. The difference in placental development may be a reflection of differences in embryonic mortality (Van der Lende, 1989).

Conclusions

The increased number of CL after GnRH injection without a concomitant increase in embryonic mortality rate, may result in an increase in the number of embryos in cyclic gilts treated with GnRH. Such an increase in number of embryos was also observed in low-fed, but not in high-fed, first-parity sows injected with GnRH at the onset of oestrus (Kirkwood et al., 1987). In multiparous sows GnRH injections resulted in slightly, albeit not significantly, increased litter sizes (Gooneratne et al., 1989). Also in gilts treated with GnRH at the onset of puberal oestrus (Archibong et al., 1987) no beneficial effects were observed. Therefore, it seems likely that the potential beneficial effects of GnRH are dependent of the physiological condition and state of the animals. Under our conditions the injection of GnRH analogue at the time of observing first standing oestrus in cyclic gilts results in a sharper LH and FSH peak, and both the number of CL and embryos are slightly increased. Since GnRH did not show any adverse effects on embryonic development, the injection of GnRH could be used by researchers wishing to know the time of conception as precisely as possible and thus the length of time of development of pig embryos.

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