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J Anim Sci 1992. 70:232-242.

Hourly Administration of GnRH to Prepubertal Gilts: Endocrine and Ovulatory Responses from 70 to 190 Days of Age[1]

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ABSTRACT: This study investigated the responsiveness of the pituitary-ovarian axis of prepubertal gilts to hourly injections (i.v.) with GnRH. Six gilts each at 70, 100, 150, and 190 d of age were assigned either to treatment with GnRH or saline. Treatments were given until gilts showed estrus or for 7 d, whichever came first. Hourly pulsing with GnRH resulted in gradually increasing concentrations of estradiol-17β (E_2), a preovulatory surge of LH, and subsequently increased progesterone (P_4) concentrations. The increase in serum P_4 was preceded by ovulation and corpora lutea (CL) formation in two gilts 70 d of age and all older gilts. The interval (h) from start of GnRH treatment to peak E_2 (88 ± 3), peak LH (103 ± 3), and concentrations of $P_4 \geq 1$ ng/mL (144 ± 4) did not differ ($P > .50$) for 18 gilts between 100 and 190 d of age. In two ovulating, 70-d-old gilts, the interval from onset of GnRH treatment to peak E_2 (171 ± 6), peak LH (186 ± 0), and $P_4 \geq 1$ ng/mL (216 ± 4) was lengthened ($P < .001$). Peak concentrations of E_2 (pg/mL) were higher ($P < .01$) at 190 d (48 ± 2) and 150 d (49 ± 2) than at younger ages and lower ($P < .01$) in gilts 70 d of age (31 ± 1) than in gilts 100 d of age (41 ± 2). Peak LH (nanograms/milliliter) was higher ($P < .01$) in gilts 100 d of age (12.7 ± .6) than in older gilts. Concentrations of P_4 were similar ($P > .20$) for all ovulating gilts. The number of CL (12.7 ± .7) did not differ ($P > .20$) for 18 gilts 100 d of age or older but was higher ($P < .01$) than that (4.5 ± 1.1) for two gilts 70 d of age. Corresponding endocrine responses or ovulations were not observed in four 70-d-old gilts treated with GnRH or in gilts given saline. These findings indicate that the functional integration of the pituitary-ovarian axis is completed between 70 and 100 d of age. Hourly treatment with GnRH is an adequate stimulus to induce ovulation in prepubertal gilts as early as 70 d of age. Also, the number of follicles reaching ovulatory competency was similar ($P > .20$) in gilts between 100 and 190 d of age, when GnRH was given on a BW basis.

Key Words: Gilts, GnRH, Estrogens, LH, Progesterone, Ovulation

J. Anim. Sci. 1992. 70:232–242

Introduction

There are two periods of increased gonadotropin release during sexual maturation of the gilt, and both are associated with development of antral follicles. Gonadotropin concentrations are elevated (Diekman et al., 1983; Camous et al., 1985) and the frequency of pulsatile LH release is increased (Foxcroft et al., 1975) coincidentally with tertiary and surface follicle de-

[1]This study was funded by USDA Competitive Grant no. 86-CRCR-1-2179, North Carolina Agric. Res. Service Funds, and the North Carolina Veterinary Competitive Grants Program. The use of trade names in this publication does not imply endorsement by the North Carolina Agric. Res. Service of the products named or criticism of similar ones not mentioned. We greatly appreciate the donation of assay materials by G. D. Niswender, L. E. Reichert, Jr., W. L. Gordon, and O. D. Sherwood and the excellent technical assistance of O. K. Dial and M. J. Hammer.

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Received January 24, 1991.
Accepted July 16, 1991.

velopment during the 3rd mo of life in gilts (Casida, 1935; Oxender et al., 1979; Guthrie et al., 1984). Although increases in gonadotropin secretion have not been observed consistently before onset of puberty (Esbenshade et al., 1982; Diekman and Trout, 1984; Camous et al., 1985), several studies provide evidence that concentrations of LH rise again (Elsaesser and Foxcroft, 1978; Pelletier et al., 1981; Diekman et al., 1983), and nearly hourly pulse frequencies have been observed 3 to 5 d before pubertal estrus (Lutz et al., 1984). In contrast to the events that occur during the peripubertal period, the elevated gonadotropin concentrations during mid-puberty do not support follicular development to the ovulatory stage. The reasons for this failure are poorly understood. However, there is evidence that gonadotropin feedback mechanisms are more sensitive to estrogen in younger gilts (Berardinelli et al., 1984; Schmitz et al., 1985), such that rising estrogen concentrations of follicular origin may not permit adequate gonadotropin release.

The present study was designed to simulate a period of frequent and stable gonadotropin release using hourly administration of GnRH (Wildt et al., 1980, 1981). Hourly treatment with GnRH has been demonstrated previously to be effective in inducing ovulation in the late prepubertal gilt (Lutz et al., 1985) as well as in prepubertal females of other species (Wildt, et al., 1980; Loose and Terasawa, 1985; Pirl and Adams, 1987). To date, however, the relative responsiveness of prepubertal females at various ages has not been investigated in any species. The specific objectives of this study were to contrast the endocrine and ovulatory responses of prepubertal gilts between 70 and 190 d of age to hourly pulsatile administration of GnRH.

Materials and Methods

Animals. Forty-eight Yorkshire × Landrace gilts were obtained from a commercial swine farm. Twelve gilts each were used at 70, 100, 150, and 190 d of age. Gilts at each age were born within 48 h of each other, and none belonged to the same litter. Animals were penned individually at the Laboratory Animal Resources facilities of the College of Veterinary Medicine and kept in air-conditioned rooms at temperatures between 20 and 25°C. The photoperiod was held constant at 12 h dark and 12 h light. Gilts were fed twice daily a commercial diet that met or exceeded the NRC requirements for swine of each age. Water was available ad libitum. The 70- and 100-d-old gilts were fitted with jugular catheters under general anaesthesia 3 d before the start of the experiment. The two older age groups were fitted nonsurgically with jugular catheters, as described by Cox and Britt (1982). Mean BW at the start of the experiment was 25 ± .7, 46 ± 1.9, 82 ± 2.2, and 94 ± 3.4 kg for the gilts of 70, 100, 150, and 190 d of age, respectively.

Experimental Procedures. In a 4 × 2 factorial arrangement, six gilts each, at 70, 100, 150, and 190 d of age, were assigned either to treatment with saline or GnRH (Cystorelin®, Ceva Laboratories, Overland Park, KS). Gilts received either 50 ng of GnRH/kg of BW in 2 mL of saline or 2 mL of saline vehicle (.9% NaCl). The GnRH and saline were administered every 60 min as intermittent injections (i.v.). Treatments were given until gilts showed estrus or for 7 d, whichever came first.

Estrus determinations were conducted four times daily in the absence of a boar to avoid confounding influences on sexual maturation of gilts. Vulvar swelling and reddening were scored numerically each day using the scale 0 = no change in degree of vulvar tumescence or erythema, 1 = small increase, 2 = moderate increase, and 3 = substantial increase in swelling and reddening. Gilts were judged to be in estrus when they assumed a mating stance with characteristic immobility in response to manual back pressure. Ovaries were recovered within 10 d of estrus or cessation of treatment and used to confirm ovulations.

Blood samples were collected every 6 h from d 0 of the trial (d 0 = onset of pulsing) until d 10 to 12. The blood samples were taken immediately before injections of saline or GnRH. A frequent blood sampling period was included for the 70-d-old group on d 6 of the experiment, because five of six gilts did not show signs of estrus after 168 h of treatment with GnRH. During this frequent sampling period, blood samples were collected every 10 min for 1.5 h and then every 15 min for 2 h. The change was necessitated when patency of one catheter was lost temporarily. Blood was allowed to clot at 4°C for 6 h before centrifugation to recover serum. Decanted sera were stored at −20°C until they were assayed.

Hormone Analyses. Concentrations of LH were determined by RIA of duplicate 200-μL aliquots of serum using a procedure described previously (Dial et al., 1983). Sensitivity of the assay, expressed as the lowest weight of hormone different from tubes containing PBS without LH, was 50 pg/tube. Intraassay CV for a low pool (1.9 ng/mL) was 6.1%, for a medium pool (5.5 ng/mL) was 4.6%, and for a high pool (9.5 ng/mL) was 4.0%. Interassay CV was 8.8, 7.6, and 7.9% for low, medium, and high pools, respectively (n = 21 assays).

Serum concentrations of 17β-estradiol (E_2) were determined by using a single antibody, charcoal-dextran RIA of duplicate 200-μL aliquots of serum, as described previously (Cox et al., 1987). Sensitivity of the assay, estimated by adding various amounts of E_2 to serum, was .2 pg/tube. Intraassay CV for a low (28 pg/mL) and a high pool (164 pg/mL) were 4.8 and 5.0%, respectively. Interassay CV were 14.4 and 6.6% for low and high pools, respectively (n = 30 assays).

Progesterone (P_4) concentrations were determined using a single-antibody, charcoal-dextran RIA, as described previously (Almond and Dial, 1990). Serum aliquots of 100 μL were initially diluted with 900 μL of PBS-gelatin. Concentrations of P_4 were measured in duplicate 40-μL aliquots of this dilution after addition of 460 μL of PBS-gelatin. Sensitivity of the assay was 5 pg/tube, based on the statistical discrimination of varying weights of P_4 added to ovariectomized sow serum from serum with no P_4 added. Intraassay CV of a reference serum (21 ng/mL) was 4.8%, and interassay CV was 7.9% (n = 12 assays).

Data Analyses. Concentrations of LH were measured in all samples. Concentrations of E_2 and P_4 were determined in samples taken every 12 h, except the 190-d-old group, for which P_4 concentrations were measured in samples collected at 24-h intervals because a complete set of sera had become unavailable to determine P_4 concentrations every 12 h. Mean LH and E_2 concentrations for individual gilts were defined as the average concentration measured in samples from 6 h after the start of pulsing until 114 h after the preovulatory LH peak. Mean P_4 concentrations represent the average of sample values after the preovulatory LH peak, which were ≤ 1 ng/mL (Armstrong and Britt, 1985).

As described previously (Dial et al., 1984), the preovulatory LH peak was defined as a 2.5-fold increase relative to mean LH; concentrations during a surge remained at least two standard deviation units above mean LH for three consecutive samples taken every 6 h. Baseline concentrations were computed as a mean of all samples measured 12 h in advance and(or) 12 h following the LH surge. The duration of the LH surge included only those samples that were at least two standard deviation units above LH mean concentrations. The interval to the rise in P_4 concentrations was taken from peak LH until the first P_4 measurement whose concentration was at least 1 ng/mL. The characteristics of pulsatile LH release in the 70-d-old gilts were determined according to a procedure described before (Grieger et al., 1986).

All analyses were conducted using the GLM procedure for analysis of variance (PROC GLM; SAS, 1985). Mean LH, E_2, and P_4 concentrations among different ages and treatments were compared by analysis of variance as a 2 × 4 factorial arrangement. The effect of age on estrous, ovulatory, and endocrine responses in gilts pulsed with GnRH was determined by one-way ANOVA, because comparable responses were not observed in saline-treated gilts. In the case of significant differences ($P \leq .05$), means of the various age groups were compared by the lsd procedure on least squares means for preplanned comparisons (LSMEANS; SAS, 1985). The area under the LH surge profile was estimated by trapezoidal approximation in arbitrary units (SAS, 1985).

Depending on the analysis, the data of five gilts were partially or completely omitted from statistical analysis. Loss of catheter patency occurred in a saline-treated, 70-d-old gilt. Pulsatile LH release could not be identified in two GnRH-treated, 70-d-old gilts because the frequent sampling period coincided with the preovulatory LH surge in these gilts. Two 190-d-old, saline-treated gilts attained puberty spontaneously on the 2nd d after the start of the experiment.

Results

Pulsatile administration of saline for 7 d had no apparent effects on endocrine events in prepubertal gilts between 70 and 190 d of age. Hourly pulses of GnRH, in contrast, initiated changes in serum concentrations of E_2, LH, and P_4 characteristic of the preovulatory period. These changes were accompanied by ovulation in two 70-d-old and in all older gilts. There were no differences ($P > .20$) in the time from initiation of pulsing to onset of estrus or in the ovulation rates of GnRH-treated gilts between 100 and 190 d of age. Of the three older age groups, estrus occurred between d 4 and 6 after initiation of GnRH pulsing. The number of corpora lutea (CL) (12.7 ± .7) was similar for the 18 ovulating gilts ($P > .20$). Endocrine and estrous responses were delayed by 3.5 d and ovulation rate was less (4.5 ± 1.1) in two 70-d-old gilts relative to the older gilts that ovulated.

Hormonal Responses. The average concentrations of LH, E_2, and P_4 were affected ($P \leq .05$) by treatment and by prepubertal age (Table 1). Concentrations of LH were higher in GnRH-pulsed gilts between 100 and 190 d of age than in saline-treated females. At 70 d, there were no differences ($P > .40$) in mean LH concentrations between gilts pulsed with GnRH or saline or between gilts that ovulated or failed to ovulate when given GnRH. Mean LH values were 1.8 ± .7 ng/mL and 1.3 ± .2 ng/mL for GnRH-treated ovulating and nonovulating gilts, respectively.

Table 1. Mean hormone concentrations of gilts pulsed with saline or gonadotropin-releasing hormone (GnRH)[a]

Days of age	LH, ng/mL		Estradiol, pg/mL		Progesterone, ng/mL	
	Saline	GnRH	Saline	GnRH	Saline	GnRH
70	1.6 ± .3[b]	1.5 ± .3[bc]	6 ± .4	10 ± 1.6[b]	≤.30	2.5 ± 1.4[be]
100	1.0 ± .1[bc]	2.1 ± .3[be]	9 ± .5	17 ± 1.1[ce]	≤.30	12.5 ± 1.3[e]
150	.6 ± .3[c]	1.2 ± .1[ce]	7 ± .5	19 ± 1.8[cde]	≤.30	12.8 ± 1.8[e]
190	.7 ± .1[c]	1.6 ± .2[bce]	13 ± .9[b]	20 ± 1.3[de]	≤.30	10.5 ± 1.4[e]

[a]Mean ± SEM (n = 6).
[b,c,d]Means with different superscripts differ in columns ($P \leq .05$).
[e]Different from gilts treated with saline ($P \leq .05$).

Serum LH of saline-treated gilts was influenced by age; concentrations were highest at 70 d and decreased to 150 d of age. Concentrations of E_2 and of P_4 were higher in ovulating gilts treated with GnRH than in gilts given saline (Table 1). Concentrations of E_2 increased with age in gilts treated with GnRH, whereas concentrations of P_4 in 20 gilts that ovulated remained similar ($P > .20$). Serum E_2 concentrations of gilts treated with saline were higher at 190 d of age than at the younger ages. Concentrations of P_4 remained at or below the sensitivity of the assay in gilts given saline and in GnRH-treated gilts not ovulating.

Preovulatory LH surges occurred in all GnRH-pulsed gilts at 100, 150, and 190 d of age and in two of six 70-d-old gilts (Table 2). Age influenced responses to treatment with GnRH. The magnitude of LH surges, measured by peak LH and the area under the LH surge, were greater at 100 d than at 150 or 190 d (Table 2). Peak E_2 concentrations preceding the preovulatory LH surge were higher in gilts 190 and 150 d of age than in gilts at the younger ages. Peak E_2 concentrations were decreased further in 70-d-old gilts relative to 100-d-old gilts. In gilts having a preovulatory LH surge, the period of LH suppression coincided with the period of rising serum E_2 (Figures 1 and 2). The pattern of hormone changes observed during the preovulatory period in GnRH-treated gilts that ovulated was characteristic of the periovulatory period of adult female pigs (Figures 1 and 2). The endocrine profile was clearly distinct from the random pattern observed in saline-treated gilts and GnRH-treated gilts that failed to ovulate (Figure 2).

The duration of GnRH treatment until the occurrence of periovulatory changes in LH, E_2, and P_4 was highly uniform among 100-d-old and older gilts (Table 3). The interval from start of pulsing to peak LH did not differ ($P > .50$) between gilts pulsed at 100, 150, and 190 d of age with GnRH. Peak LH release occurred at 103 ± 3 h after the beginning of treatment for 18 gilts between 100 and 190 d of age. Peak LH concentrations in the two 70-d-old gilts that ovulated were measured at 186 h after initiation of pulsing, a delay of approximately 3.5 d relative to older gilts. The two 70-d-old gilts showed asymmetrical surge profiles of shorter duration than older gilts (Figure 2).

The interval from the beginning of GnRH treatment until peak E_2 or until P_4 concentrations rose above 1 ng/mL did not differ ($P > .60$) for gilts between 100 and 190 d of age (Table 3). Peak E_2 concentrations were measured on average at 88 ± 3 h after the start of pulsing for the 18 gilts that ovulated at 100 d of age or older. In these gilts, P_4 concentrations reached ≥ 1.0 ng/mL within 144 ± 4 h after the beginning of treatment. Similar to age-related differences in LH release, the two ovulating, 70-d-old gilts had lengthened

Table 2. Characteristics of luteinizing hormone (LH) and estradiol (E_2) release of gilts treated with gonadotropin-releasing hormone[a]

Days of age	No. of gilts	No. of gilts with LH surge	Peak E_2, pg/mL	Peak LH, ng/mL	Area under LH surge[b]
70	6	2	31 ± .4[c]	10.1 ± 4.3[cd]	95 ± 59[c]
100	6	6	41 ± 1.9[d]	12.7 ± .6[c]	260 ± 8[d]
150	6	6	49 ± 1.5[e]	6.9 ± .9[d]	125 ± 8[c]
190	6	6	48 ± 1.8[e]	7.5 ± 1.1[d]	160 ± 24[c]

[a]Mean ± SEM.
[b]Arbitrary units.
[c,d,e]Means with different superscripts differ in columns ($P \leq .05$).

Table 3. Temporal aspects of estradiol (E_2), luteinizing hormone (LH), and progesterone (P_4) release of gilts treated with gonadotropin-releasing hormone[a]

Days of age	Hours from treatment start to Peak E_2	Peak LH	P_4 rise[b]	Duration of LH surge, h
70[c]	171 ± 6.4[d]	186 ± 0[d]	216 ± 4.2[d]	18 ± 4.2[d]
100	90 ± 4.2	108 ± 5.1	146 ± 5.4	40 ± 2.7
150	87 ± 3.6	99 ± 4.2	138 ± 5.5	38 ± 1.2
190	86 ± 6.3	101 ± 6.8	147 ± 7.8	38 ± 1.2

[a]Mean ± SEM (n = 6, except at 70 d).
[b]P_4 concentrations ≥ 1.0 ng/mL.
[c]Data are from two ovulating gilts.
[d]Different from other values in columns ($P \leq .05$).

intervals from onset of pulsing until the occurrence of periovulatory changes in serum E_2 and P_4 (Table 3). Peak E_2 and P_4 concentrations ≥ 1.0 ng/mL were delayed by 83 h and by 72 h, respectively, relative to older gilts.

The temporal relationships between periovulatory changes in peak E_2, peak LH, and postovulatory changes in P_4 were similar in all ovulating gilts, independent of age. For the 20 ovulating gilts between the ages of 70 and 190 d, the following intervals were observed: peak E_2 to peak LH was 15 ± 1 h, peak E_2 to the initial P_4 rise (≥ 1 ng/mL) was 56 ± 2 h, and peak LH to the initial P_4 rise was 41 ± 2 h.

The patterns of LH release during the time of hourly pulsing of 70-d-old gilts with GnRH or saline are shown in Figure 3. Mean LH concentrations during the frequent sampling period were 1.6 ± .1 ng/mL and 1.2 ± .2 ng/mL for GnRH-treated and saline-treated gilts, respectively, and did not differ ($P > .25$). Basal LH concentrations, however, were higher in GnRH-pulsed gilts, as was LH pulse frequency. Whereas gilts receiving hourly pulses of GnRH responded with synchronous LH release that peaked within 10 min of treatment, LH pulses in saline-treated gilts occurred independently of the times of saline injection.

Ovulatory and Estrous Responses. Hourly infusion of GnRH induced ovulation in all gilts of 100, 150, and 190 d of age, whereas only two 70-d-old gilts ovulated (Table 4). There was no difference in the number of CL in gilts between 100 and 190 d of age ($P > .20$). The 18 gilts at these ages had a mean number of 12.7 ± .7 ovulations (range, 10 to 19). The average number of CL (4.5 ± 1.1) in the two 70-d-old gilts was less than that observed in older gilts. As with estrus, ovulations did not occur in saline-pulsed gilts between 70 and 150 d. The number (mean ± SEM) of visible (≥ 1 mm) surface follicles was nearly identical ($P > .95$) between saline-pulsed, anovulatory gilts (25 ± 7.0) and GnRH-treated, ovulating gilts (25 ± 2.6) at 70 d of age. In contrast, numbers of surface follicles in gilts treated with GnRH at 70 d of age that failed to ovulate were markedly lower (4 ± 2.1).

Table 4. Estrous and ovulatory responses of gilts pulsed with gonadotropin-releasing hormone (GnRH) or saline[a]

Days of age	No. of gilts Treated	In estrus	Ovulating	Hours to estrus	Duration of estrus, h	No. of corpora lutea
GnRH						
70	5	1	2	174[b]	36	4.5 ± 1.1[b]
100	6	5	6	114 ± 3.4[c]	37 ± 2.1	11.3 ± .5[c]
150	6	6	6	104 ± 6.6[c]	44 ± 4.6	14.5 ± 1.5[c]
190	6	6	6	111 ± 7.4[c]	43 ± 3.3	12.2 ± .7[c]
Saline						
70	5	0	0	—	—	—
100	6	0	0	—	—	—
150	6	0	0	—	—	—
190	6	2	2	36 ± 6.0[d]	42 ± 6.0	11.5 ± .5[c]

[a]Mean ± SEM.
[b,c,d]Means with different superscripts differ within columns ($P \leq .05$).

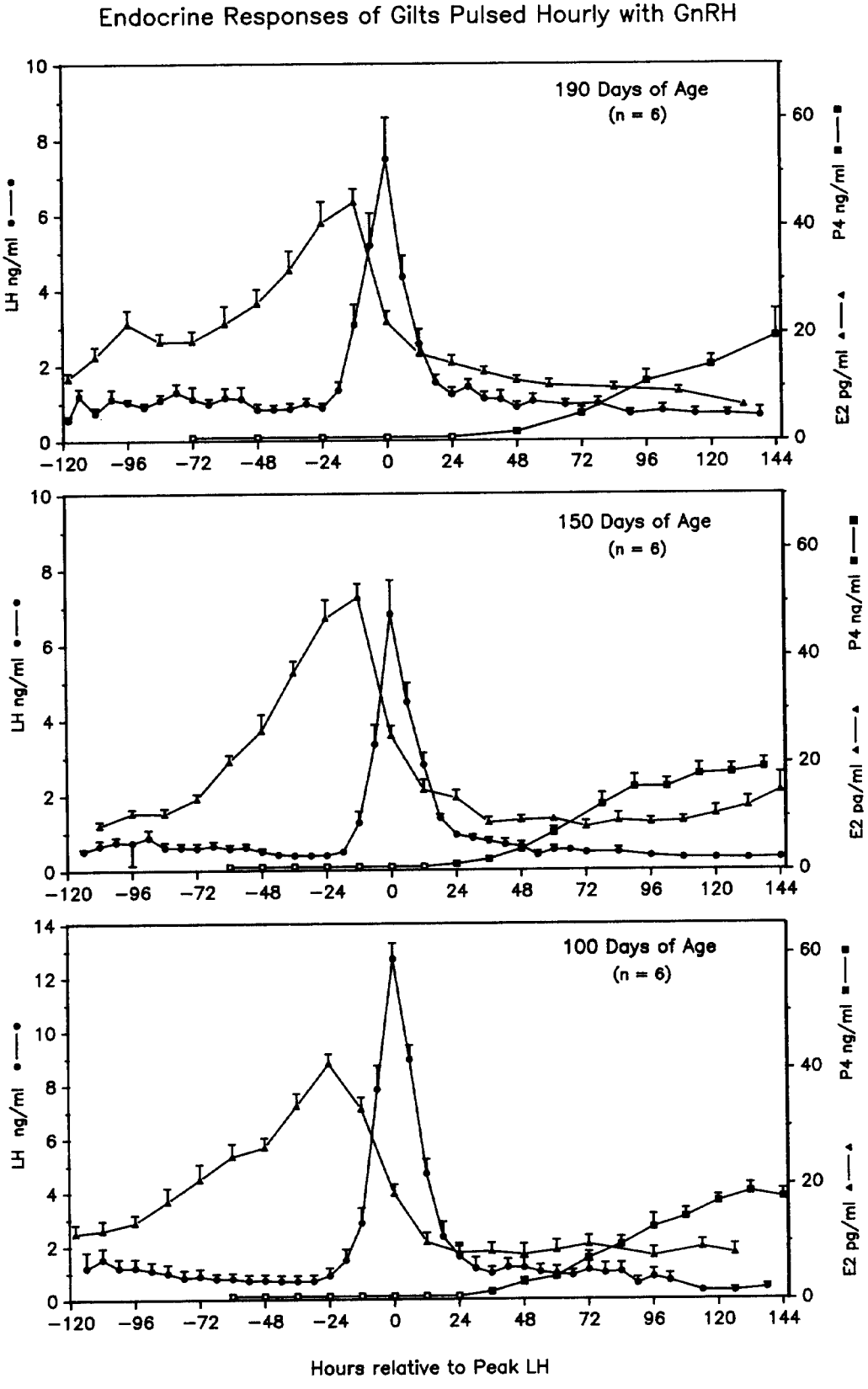

Figure 1. Mean ± SEM for luteinizing hormone (LH; ●), estradiol (E_2; ▲), and progesterone (P_4) responses (□, ≤ .3 ng/mL; ■, > .3 ng/mL) relative to peak LH (Time 0) in six gilts each at 100, 150, and 190 d of age receiving hourly pulses of 50 ng of GnRH/kg of BW. Upper panel, 190-d-old gilts; middle panel, 150-d-old gilts; lower panel, 100-d-old gilts.

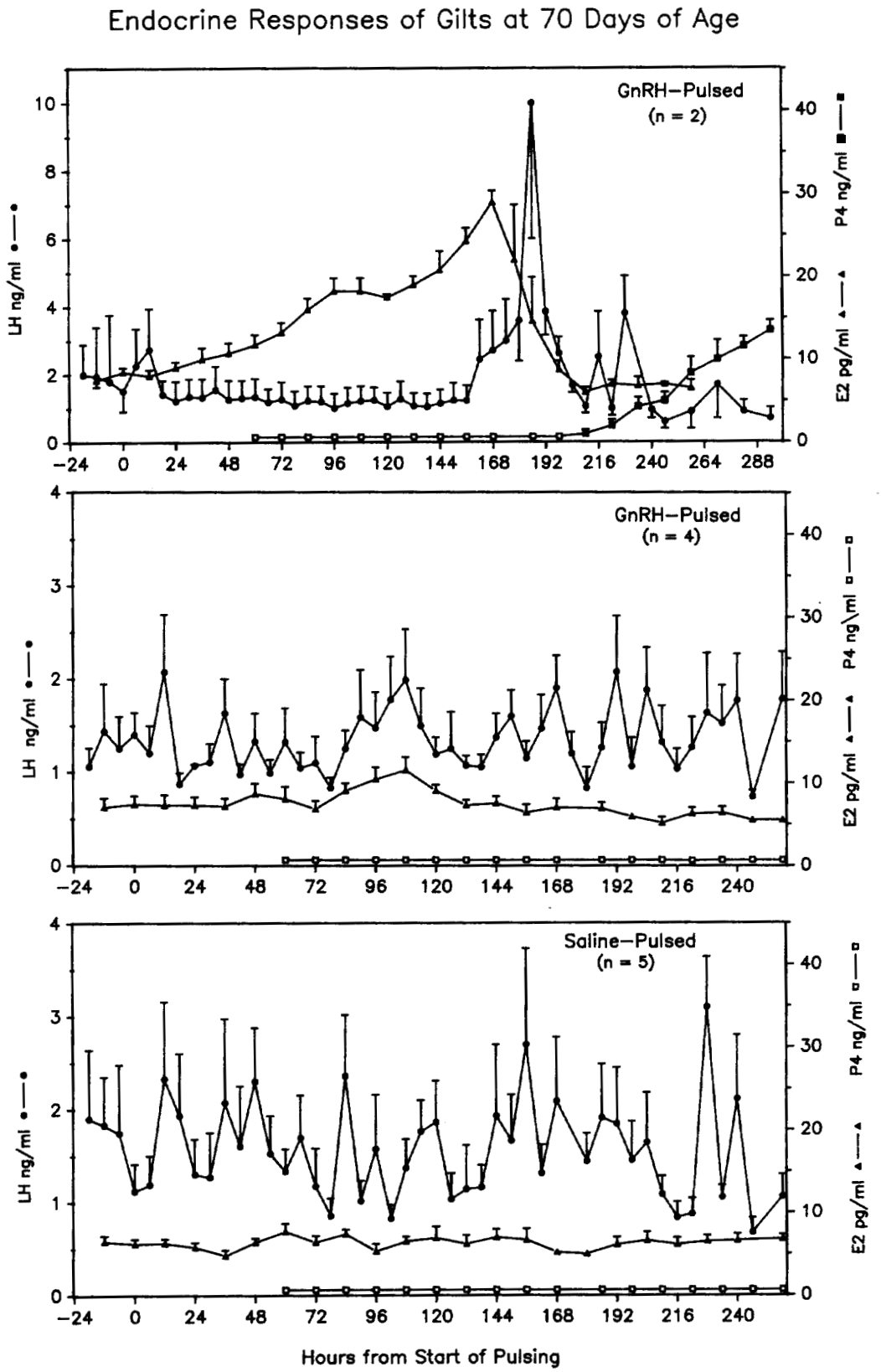

Figure 2. Mean ± SEM for luteinizing hormone (LH; ●), estradiol (E_2; ▲), and progesterone (P_4) responses (□, ≤ .3 ng/mL; ■, > .3 ng/mL) relative to start of hourly pulsing (Time 0) with GnRH (50 ng/kg of BW) or saline in 70-d-old gilts. Upper panel, endocrine responses of two ovulating, GnRH-treated gilts; middle panel, hormone profiles of four nonovulating, GnRH-treated gilts, lower panel, hormone profiles in five saline-treated gilts.

The hourly administration of GnRH induced estrus in five 100-d-old and in all older gilts (Table 4). Only one of six gilts treated hourly with GnRH at 70 d showed estrus. In contrast, all saline-pulsed gilts remained prepubertal except for two gilts at 190 d that attained puberty spontaneously during the first 2 d of the experiment. There was no influence of age on time from first treatment with GnRH to onset of estrus in 17 gilts between 100 to 190 d of age that were observed in heat ($P > .60$). Estrus occurred within 109 ± 4 h (range, 78 to 138 h) after treatments began. Three gilts showed first signs of estrus on d 4, 11 on d 5, and 3 on d 6 of the experiment. Estrus was delayed in one, 70-d-old gilt until d 8. Duration of estrus was similar ($P > .30$) for all 18 gilts between 70 and 190 d of age that showed estrus, lasting for 40 ± 2 h (range, 24 to 60 h).

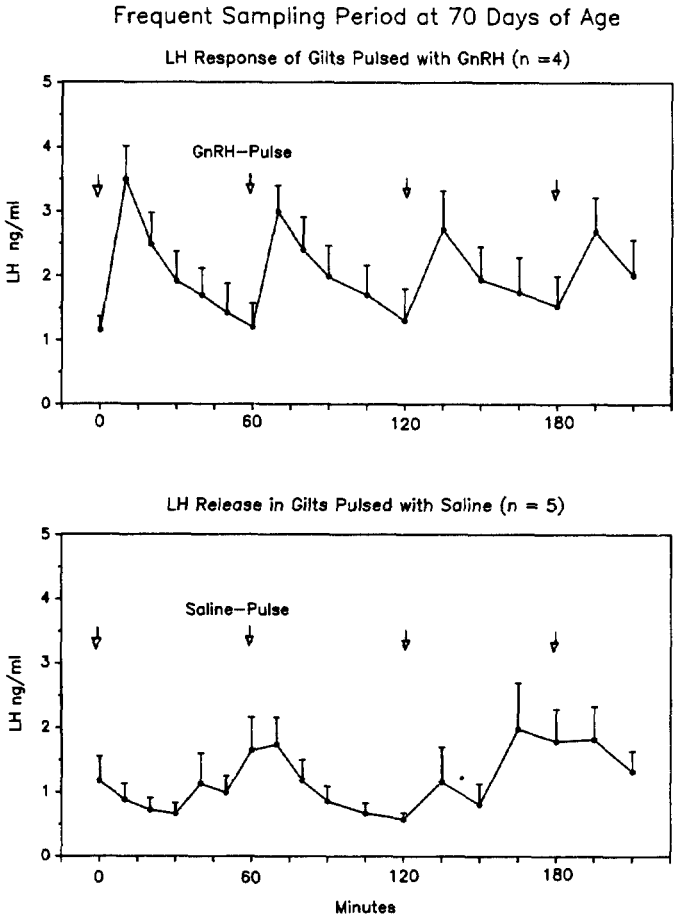

Figure 3. Pulsatile luteinizing hormone (LH, mean ± SEM) release during a frequent sampling period in 10, 70-d-old gilts receiving either hourly pulses of GnRH (50 ng/kg of BW) or saline. Upper panel, pulsatile LH release in four GnRH-pulsed gilts; lower panel, LH release in five saline-treated controls.

Discussion

The findings of this study indicate that between 70 and 100 d of age, gilts acquire the capacity to respond to hourly administration of GnRH with a sequence of endocrine changes culminating in a preovulatory LH surge, ovulation, and estrus. Between 100 d of age and the time of normal onset of puberty, typically between 150 and 190 d of age with this genetic line, gilts showed consistent endocrine, ovulatory, and estrous responses to GnRH, and the responses were highly uniform among the age groups. In contrast, the responses of GnRH-treated, 70-d-old gilts were inconsistent with four of six gilts failing to respond to GnRH stimulation. In the two 70-d-old gilts responding to GnRH, estrous and ovulatory responses were delayed by approximately 3.5 d. Further, peak E_2 concentrations in 70-d-old gilts remained below values observed in older gilts, and ovulation rates were reduced by approximately 60%. Collectively, these findings indicate clear deficiencies in the pituitary-ovarian axis of gilts younger than 100 d that impair or completely prevent those responses observed in older gilts. At 100 d of age and older, the ovary of the prepubertal gilt has matured sufficiently to become fully responsive to a continued gonadotropic stimulus, and hourly pulses of GnRH consistently initiated a sequence of endocrine and behavioral events that was comparable to the sequence occurring during the periovulatory period in the cyclic female. Considering its approximate 21-d estrous cycle length, the pig seems to require approximately 5 to 6 d to respond to increased pulsatile gonadotropin release with estrus and ovulation. The lag between the onset of GnRH pulsing and estrus indicates that the immature pig is like the adult in that following the onset of increased pulsatile LH release, the duration of preovulatory follicular growth is approximately 1 wk or less (Foxcroft and Hunter, 1985).

Chronic hourly treatment with GnRH maintained stable, elevated LH baseline concentrations in gilts as early as 70 d of age (Figure 3). Presumably, although not examined in this study, pulses of GnRH were followed by corresponding releases of LH in older gilts. This may represent the prerequisite gonadotropic environment for the continued growth of antral follicles (Richards et al., 1980; Ryan and Foster, 1980; Kraeling et al., 1986, 1990). Additionally, results of this study indicate that antral follicles must be present at the time of initiation of GnRH pulsing in order for follicles to progress for the first time to the ovulatory stage. The inconsistent responses to pulsatile GnRH administration in 70-d-old gilts may reflect an absence of follicles of a size

capable of responding to GnRH-induced LH release. In another study, we demonstrated that large antral follicles initially become evident between 70 and 90 d of age (Pressing et al., 1990). In previous experiments, treatment with exogenous gonadotropins in advance of this age failedto induce ovulatory responses or was of limited success (Casida, 1935; Majerciak et al., 1969; Oxender et al., 1979). By approximately 100 d of age, tertiary follicles in the prepubertal gilt have become abundant (Erices and Schnurrbusch, 1979; Dyck and Swierstra, 1983; Dufour et al., 1985). At this stage of ovarian development, exogenous gonadotropins, such as pregnant mare's serum gonadotropin (PMSG) with or without hCG, consistently induce precocious ovulation and estrus (Dziuk and Gehlbach, 1966; Majerciak et al., 1969; Kater and Smidt, 1975; Dial et al., 1983). Because tertiary follicles were not observed after repeated injections of PMSG before 70 d of age, it has been suggested that exogenous gonadotropins are ineffective in initiating or promoting antral follicle formation (Oxender et al., 1979). As demonstrated in this study, endogenous gonadotropins released by hourly administration of GnRH to 70-d-old gilts also do not provide the type of trophic stimulus required to initiate follicular development.

The endocrine events initiating the development of follicles to the antral stage remain to be elucidated in the pig. However, follicular development in gilts between 70 and 100 d of age to the antral stage may require initial priming with FSH followed by LH stimulation, a sequence that seems to control follicular development in the adult (Britt et al., 1985; Foxcroft and Hunter, 1985). Earlier studies have shown that FSH concentrations rise to a plateau during the 2nd mo of life, at which time LH concentrations and LH pulse frequency commence a gradual increase (Diekman et al., 1983; Camous et al., 1985). Perhaps, the 70-d-old gilts of this study had not yet experienced FSH-stimulated initiation of follicular development, and thus were unable to respond to the gonadotropin release induced by exogenous GnRH. The ratio of FSH and LH concentrations in the primate is reduced by increasing frequencies of GnRH administration (Wildt et al., 1981). These observations suggest that administering hourly pulses of GnRH to 70-d-old gilts may not provide the appropriate gonadotropic stimulus to initiate follicle development. At 70 d, saline-treated gilts developed small surface follicles ($\leq$ 4 mm) similar in size and number to those observed in the two ovulating gilts receiving GnRH, even though they had much lower frequencies of LH release (95.5 vs 52.5 min between pulses). Apparently, LH pulse frequency is not the only stimulus driving follicular recruitment.

Hourly administration of GnRH has been used by other investigators to study the onset of puberty in the prepubertal gilt and the endocrine physiology of lactational and postweaning anestrus in the sow. Hourly GnRH administration induced ovulation and estrus in all (n = 3) prepubertal gilts at 164 d of age within 6 d of treatment; ovulation rate (8.4) was consistent with but slightly less than the ovulation rate (11.3 to 14.5) of gilts between 100 and 190 d of age in this study (Lutz et al., 1985). The frequency of pulsatile LH release needed to drive follicular development was not elucidated in this study. However, less frequent GnRH administration (i.e., one pulse every 1.5 h) resulted in less consistent ovulatory and estrous responses (Carpenter and Anderson, 1985). Like the prepubertal gilt, both the lactating sow and the sow remaining persistently anestrous following weaning respond to hourly administration of GnRH with estrus and ovulation (Cox and Britt, 1982; Armstrong and Britt, 1985). When lactating sows received one pulse every 2 h, the efficacy of GnRH treatment was only approximately 50% (Cox and Britt, 1982). Collectively, these studies indicate that acyclic female pigs having antral follicles will initiate the series of endocrine events culminating in ovulation and estrus in response to pulsatile gonadotropin stimuli occurring at approximate 1-h intervals. In other mammalian species, the hourly GnRH regimen is similarly effective in inducing ovulation in the prepubertal female (Wildt et al., 1980; Loose and Terasawa, 1985; Pirl and Adams, 1987) and acyclic adult (Knobil et al., 1980; Wright et al., 1984; Johnson, 1987).

In summary, we conclude that sexual maturation during the midpubertal period in the gilt can be characterized as follows: 1) the pituitary is responsive to GnRH well before the appearance of antral follicles and 2) the ovary becomes responsive to gonadotropins between 70 and 100 d of age. Integration of the pituitary-ovarian axis, culminating in pubertal estrus, awaits a central signal, for which exogenous GnRH can substitute well in advance of the normal time of puberty onset.

Implications

The present study demonstrates that hourly pulses of gonadotropin-releasing hormone, administered for 6 d or less, will consistently induce estrus and ovulation in prepubertal gilts as young as 100 d. Although pituitary responsiveness to gonadotropin-releasing hormone is established

before that age, the capacity to ovulate at a physiological rate develops between 70 and 100 d of age. Between 100 and 190 d of age, periovulatory endocrine changes, ovulation rate, and estrous behavior equaled those of gilts that attained puberty spontaneously. Possibly, natural attainment of puberty is also initiated by very frequent gonadotropin-releasing hormone release. If this indeed occurs, increased gonadotropin secretion of short duration would be expected to precede pubertal estrus and ovulation in the gilt.

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