Induction of Luteinizing Hormone Surge by Pulsatile Administration of Gonadotropin-Releasing Hormone Analogue in Cows with Follicular Cysts

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ABSTRACT. LH release in response to pulsatile administration of small amounts of GnRH analogue in cows with follicular cysts was examined. The pulsatile administration of GnRH analogue induced a LH-surge like peak over 10 hr in both normal cows and cows with follicular cysts. The mean peak value of LH in follicular cystic cows did not differ significantly from that of normal cows. All the cows with cysts resumed normal estrous cycles with ovulations within 3 weeks of this treatment. These results suggest that the function of the anterior pituitary for LH release in response to GnRH analogue is not abnormal in cows with follicular cysts, and that cystic cows recover to normal conditions after the pulsatile administration of GnRH analogue. — KEY WORDS: cattle, follicular cyst, LH surge.

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Follicular cysts are a serious cause of reproductive failure in cattle because they occur frequently and prolong the calving interval [5]. It was observed that luteinizing hormone (LH) release in response to an increase in endogenous estradiol-17β was attenuated in cows that formed follicular cysts [6]. These observations led to the hypothesis that the ovarian cysts are caused by abnormalities in the mechanisms of LH release from the hypothalamus-pituitary axis. Some researchers examined LH release from the anterior pituitary in cows with ovarian cysts after a single [1] or a few [8] injection(s) of gonadotropin-releasing hormone (GnRH) or its analogue. Those studies showed that LH release in response to injections of GnRH was higher in cows with follicular cysts than in cows in the luteal phase [8], but almost comparable to that of cows during the late-luteal phase or proestrus [1]. In those studies, however, the LH peaks to GnRH in both normal and cystic cows continued for only 5–6 hr, although the naturally occurring preovulatory LH surge in cows usually lasts for approximately 10 hr [4, 12]. In the present study, we did pulsatile administration of small amounts of GnRH analogue every 20 min for 10 hr in order to induce a LH surge in cows and examined the LH release from the anterior pituitary in cows with follicular cysts.

Four Holstein multiparous cows with ovarian cysts and 4 Holstein multiparous cows with normal estrous cycles were used for this experiment. The four cows with normal estrous cycles were kept at the farm of Yamaguchi University and all of them were non-milking and fed hay and concentrates. The four cows with ovarian cysts were kept at commercial farms near Yamaguchi city and all of them were in lactation and fed hay and concentrates. The cows were examined by rectal palpation and diagnosed as having ovarian cysts if they had follicles equal to or larger than 25 mm in the absence of corpus luteum for at least 10 days. Furthermore, cows were diagnosed as having follicular cysts if the concentrations of progesterone in plasma of the cystic cow was below 1 *n*g/m*l*, as previously reported [11]. One of the cows with ovarian cysts at the commercial farm had been in a cystic condition for approximately 2 months prior to this experiment. The remaining 3 cows with ovarian cysts had been in cystic conditions for 10–13 days prior to this experiment. The day of ovulation of the cows with normal estrous cycles was determined by rectal palpation.

In order to examine the function of LH release from the anterior pituitary in cows with follicular cysts, we decided to use cows in the follicular phase before the onset of LH surge as control animals. In order to induce the follicular phase in normal cows, we injected 30 mg of prostaglandin (PG) F2α (0h) (Panacelan Hi, Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) intramuscularly to normal cows in the mid-luteal phase (10-15 days after the ovulations). We injected the same amount of PGF2α to cows with ovarian cysts since there could be a direct effect of prostaglandins on LH release from the anterior pituitary [13]. At 18 hr after the injection of PGF2α, all the cows were injected intramuscularly with 1 mg of estradiol-17β (Sigma, St. Louis, U.S.A.) dissolved in 3 ml of sesame oil to increase the peripheral concentration of estradiol-17β in all the animals, since the concentration in some of the normal and cystic cows could be lower than those in the follicular phase. It was found that an identical dose of estradiol-17β caused plasma concentrations to increase to 150 *p*g/m*l* within 1 hr of administration and remain above 20 *p*g/m*l* for 24 hr [3] and this treatment enhances sensitivity of the anterior pituitary to GnRH in the ovariectomized cow [7]. At 26 hr after the injection of PGF2α, an intramascular injection of 1 μg of fertirelin acetate (GnRH analogue; Conceral, Takeda Pharmaceutical Co., Ltd., Osaka, Japan) was done and thereafter repeated 29 times every 20 min for 10 hr to induce LH surge. Ten milliliters of blood were collected from the jugular vein with a 21-gauge needle attached to a heparinized disposable plastic pump at 0, 6, 12, 18 and 22 hr, and during the pulsatile administration of GnRH analogue from 26 to 36 hr, blood was collected every 40 min. Plasma was separated immediately after collection by centrifugation at 1,600 × g for 10 min and was kept at $-$ 20°C until assayed for LH and progesterone.

The concentration of LH in plasma was measured by double antibody radioimmunoassay as described previously [10]. The lactoperoxidase method was used to radioiodinate highly purified bovine LH (LER-1072). NIH-LH-B10 was used as the standard, and the sensitivity of the assay was determined to be 50 *p*g/tube. The intra-assay CV was less than 6.8%. LH in all samples was quantified within an assay.

The concentration of progesterone in plasma was measured by an enzyme-immunoassay described previously [9]. Aliquots of plasma were extracted for progesterone assay with 10 volumes of petroleum ether and assays were performed in microtiterplates coated with affinity-purified antirabbit IgG (Kanbegawa Laboratory, Tokyo, Japan) with horse radish peroxidase-labeled progesterone-3(E)-carboxylmethyloxime (Cosmo Bio Co., Ltd., Tokyo, Japan) and anti progesterone-3(E)-carboxylmethyloxime-bovine serum albumin rabbit polyclonal antibody (Cosmo Bio Co., Ltd., Tokyo, Japan). The sensitivity of the assay was less than 4 *p*g/well and cross-reactivities were 100, 12.5, 5, 2, 0.2 and 0.01% for progesterone, 5α-pregnane-3,20-dione, 11α-hydroxyprogesterone, pregnenolone, 20α-hydroxyprogesterone and 17α-hydroxyprogesterone, respectively. The intra-assay coefficient of variation was under 10%. Progesterone in all samples was assayed within an assay.

The differences in LH and progesterone levels between groups were evaluated by Student's *t* test, or Cochran-Cox *t* test when the variance was different.

According to the concentration of progesterone in plasma, all the 4 cows on the commercial farm were diagnosed as having follicular cysts. The changes in concentrations of progesterone in plasma of the normal cows and the cows with follicular cysts are shown in Table 1. The concentrations of progesterone in the normal cows decreased rapidly after the injection of PGF2α and were around or below 1 *n*g/m*l* during the pulsatile administration of GnRH analogue. The concentrations of progesterone in the cows with follicular cysts were below 1 *n*g/m*l* throughout the experimental period. There were significant differences between cows with follicular cysts and normal cows until the time of injection of estradiol-17β ($P<0.05$). Thereafter, the concentrations of progesterone in the normal cows approached those of the cows with follicular cysts, although significant differences were observed between the two groups at 26 and 30 hr after the injection of PGF2α.

The changes in concentrations of LH in plasma of the normal cows and cows with follicular cysts are shown in Table 2. The concentrations of LH in the normal cows increased slightly after the injection of PGF2α and decreased slightly after injection of estradiol-17β. After beginning pulsatile administration of GnRH analogue, the concentration of LH started to increase and showed values significantly higher ($P<0.05$) than that at the time just before the first injection of GnRH analogue from 80 min onwards. The mean concentration of LH reached a peak value at 320 min (range: 240–320 min) after the start of pulsatile administration of GnRH analogue and the mean peak value was 33.6 ± 14.6 *n*g/m*l*. The concentration of LH in the normal cows started to decrease after the peak, even though the pulsatile administration of GnRH analogue was continued. Thus, we demonstrated that the pulsatile administration of GnRH analogue every 20 min for 10 hr can induce a LH surge-like peak over 10 hr in the normal cow.

Table 1. Changes of concentrations of progesterone in plasma in cows with normal estrous cycles and follicular cysts during hormonal treatment with prostaglandin F2α (PGF2α), estradiol-17β (E2) and GnRH analogue (GnRH-A)

Time after PGF2α (hours)	Normal (n=4)	Cystic (n=4)
0	6.56 ± 0.67	0.41 ± 0.14**
6	3.19 ± 0.40	0.26 ± 0.07**
12	1.83 ± 0.17	0.22 ± 0.05***
18 (E2)	1.10 ± 0.04	0.18 ± 0.16*
22	0.93 ± 0.26	0.17 ± 0.03
26(GnRH-A[a)])	0.72 ± 0.19	0.21 ± 0.07*
30	0.91 ± 0.08	0.19 ± 0.04***
36	0.54 ± 0.14	0.17 ± 0.03

Data are expressed as mean ± SEM.
a) GnRH-A was injected every 20 min for 10 hr from 26 hr after the injection of PGF2α.
*P<0.05, **P<0.01, ***P<0.001.

Table 2. Changes of concentrations of LH in plasma in cows with normal estrous cycles (Normal) and follicular cysts (Cystic) during hormonal treatments with prostaglandin F2α (PGF2α), estradiol-17β (E2) and GnRH analogue (GnRH-A)

Time after PGF2α (hours)	Time after start of GnRH-A (min)	Normal (n=4)	Cystic (n=4)
0		0.85 ± 0.06	1.34 ± 0.43
6		1.13 ± 0.12	1.97 ± 0.51
12		1.57 ± 0.42	1.88 ± 0.61
18 (E2)		1.15 ± 0.11	1.53 ± 0.39
22		0.69 ± 0.09	1.13 ± 0.57
26 (GnRH-A[a)])	0	0.81 ± 0.06	1.55 ± 0.80
26.667	40	1.23 ± 0.19	3.14 ± 0.94
27.333	80	2.77 ± 0.59	4.12 ± 0.62
28	120	7.72 ± 2.98	25.84 ± 9.76
28.667	160	9.91 ± 3.55	31.68 ± 8.48
29.333	200	13.46 ± 1.54	29.14 ± 10.16
30	240	17.02 ± 4.27	31.72 ± 7.57
30.667	280	12.53 ± 3.44	26.72 ± 8.51
31.333	320	33.60 ± 14.60	22.58 ± 9.54
32	360	23.14 ± 13.05	12.48 ± 2.14
32.667	400	13.52 ± 5.49	10.60 ± 2.05
33.333	440	10.92 ± 5.03	7.78 ± 2.16
34	480	9.21 ± 4.34	2.90 ± 0.82
34.667	520	3.94 ± 1.32	3.19 ± 0.68
35.333	560	4.11 ± 2.12	2.52 ± 0.60
36	600	2.65 ± 1.25	2.77 ± 0.43

Data are expressed as mean ± SEM.
a) GnRH-A was injected every 20 min for 10 hr from 26 hr after the injection of PGF2α.

The concentration of LH in the cows with follicular cysts, until the start of the injections of GnRH analogue, showed a similar pattern to that observed in the normal cows, and was slightly higher, though not significantly, than that of the normal cows. The concentrations of LH in the cows with follicular cysts increased rapidly and reached a peak value at 240 min (range: 200–240 min) after the first injection of GnRH analogue, and thereafter decreased even though the pulsatile administration of GnRH analogue was continued. The mean LH value of the follicular cystic cows at the peak was 31.7 ± 7.6 *n*g/m*l* and was within the range observed in the normal cows. There were no significant differences between the concentrations of LH in normal cows and cows with follicular cysts at any time point. Thus, the cows with follicular cysts showed a LH surge-like peak similar to the normal cows during the pulsatile administration of GnRH analogue.

One of 4 cows with follicular cysts seemed to have ovulated soon after the pulsatile administration of GnRH analogue, because a corpus luteum with a protrusion was palpated via the rectum a week after the treatment. In the remaining 3 cows luteinization of the cyst wall, judged by rectal palpation, occurred within 2 weeks of the treatment. Thereafter all the 4 cows ovulated within 2 to 3 weeks after the treatment and resumed normal estrous cycles. Thus, all of the cows with follicular cysts were cured by the pulsatile administration of GnRH analogue.

In the present study, we examined the capacity for LH release from the anterior pituitary in the cows with ovarian cysts receiving pulsatile administration of small amounts of GnRH analogue, and the results suggest that the capacity for LH release in cows with follicular cysts is comparable to that of normal cows in the follicular phase. There have been a few previous reports on the release of LH in cows with follicular cysts [1, 8]. In one of those studies [8], LH release in cows with follicular cysts was examined by giving 3 injections of 100 μg of GnRH every 2 hr and the results showed responses in the follicular cystic cows to be approximately 2-fold higher than in cows during the luteal phase. In another study [1], LH release was examined in follicular cystic cows with an injection of 20 μg of GnRH analogue (buserelin), and it was shown that the peak values of LH in the follicular cystic cows were almost comparable to those in cows during the late-luteal phase or proestrus. In those studies, however, the durations of LH peaks in response to GnRH in both normal and cystic cows were only 5–6 hr. This is shorter than the naturally occurring preovulatory LH surge in cows which usually lasts for approximately 10 hr [4, 12]. In our present study, we did pulsatile administration of 1 μg of GnRH analogue every 20 min for 10 hr to induce a LH surge similar to a naturally occurring one and used cows during the follicular phase before an LH surge induced by PGF2α as control animals, in order to clarify in more detail the capacity for LH release in cows with follicular cysts. Our results substantiate the hypothesis that the capacity of the anterior pituitary for LH release in response to GnRH analogue is not abnormal in cows with follicular cysts. Therefore, our results might indirectly indicate abnormality in the hypothalamus or elsewhere. In fact, some researchers have suggested an abnormality in the function of the hypothalamus for GnRH release in response to the rise of estradiol-17β [2, 14]. More studies are needed to conclude whether abnormalities in the hypothalamus are the cause of follicular cysts in cows.

The present study also showed that the pulsatile administration of small amounts of GnRH analogue led the recovery of cystic cows to a normal condition with ovulations. The effectiveness of this method as a treatment for cows with follicular cysts seems to be higher than those previously reported using a single injection of the hormone [5], although the numbers of cows we used is small. Elevated levels of LH over 10 hr induced by the pulsatile administration of GnRH analogue might contribute to the recovery from cystic to normal conditions in all cows. It will be necessary to simplify the method of administration of GnRH analogue using an automatic infusion pump for pulsatile intravenous or subcutaneous administration, in order to apply this method clinically.

In summary, the present study suggests that the function of the anterior pituitary for LH release in response to GnRH analogue is not abnormal in cows with follicular cysts. Furthermore, the pulsatile administration of GnRH analogue induced a LH surge over 10 hr in all the cows and all the follicular cystic cows recovered to a normal condition after this treatment.

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