Effect of FSH on Experimental Induction of Bovine Luteal Hypoplasia

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Summary. It is well known that if ovulation is artificially induced in an immature follicle in the mid-cycle of cows, luteal formation after ovulation is arrested and a hypoplastic corpus luteum is formed. This study was undertaken to investigate if the function of corpus luteum after ovulation is related with the development of ovulating follicle by using FSH. Using 12 cows, 10 or 11 days after spontaneous ovulation, induced ovulation was achieved by administration of 0.8 mg of prostaglandin Fαα analogue (PGFαα-A) followed by the injection of 200 μg of gonadotrophin releasing hormone analogue 16 hr (groups A and B) or 32 hr (groups C and D) after. A dosage of 10 Armour units (AU) of FSH was administered by intramuscular injections, twice at 16 hr interval, starting either -16 hr in group A, 0 hr in groups B and C or 16 hr in group D after PGFαα-A treatment. Luteinization after these treatments was checked by rectal palpation, plasma progesterone levels and macroscopic findings at slaughter. Luteal function in groups A and C were improved, compared with groups B and D. Group C showed almost the same plasma progesterone pattern as that of the normal estrous cycle. However, in groups B and D, the improving effect of FSH on luteal formation was slight or poor. In conclusion, it was confirmed that the cause of luteal hypoplasia is closely related to the degree of follicular maturation at the time of ovulation. KEY WORDS: COW, LUTEAL HYPOPLASIA, INDUCED OVULATION, FSH.

Introduction

Luteal hypoplasia has sometimes been encountered in infertile cows. However, little is known about its etiology at present. In our previous experiment, it seems possible that luteal hypoplasia is prone to occur when ovulation takes place in the early stage of follicular maturation (Ohnami et al., 1986). The present study intends to clarify that stimulation to follicular maturation by the administration of follicle stimulating hormone (FSH) can give some effect on the luteal formation after ovulation, so that the FSH treatment can reduce the occurrence of luteal hypoplasia.
in the mid-luteal stage, 10–11 days after spontaneous ovulation, followed by two intra-muscular injections of 100 µg of gonadotrophin releasing hormone analogue (GnRH-A, Fertirelin acetate, Takeda Chemical Industries, Ltd., Osaka, Japan), each 16 hr (groups A and B) or 32 hr (groups C and D) after injection of PGF$_2$α-A. The interval between GnRH-A injections was 60 min according to Kittok et al. (1973). In our previous experiment (Ohnami et al., 1986), luteal function in cows treated with the above-mentioned procedure of induced ovulation was very poor. Treatment with FSH. The cows were treated with FSH in the manner shown in Table 1. The FSH used was Antrin (Denka Seiyaku Co., Ltd.). Two dosages of 10 Armour units (AU) each, or a total of 20 AU, were injected by the intra-muscular route. When the time of injection with PGF$_2$α-A was regarded as 0 hr, FSH was administered to each group at -6 and 0 hr (group A), 0 and 16 hr (groups B and C), or 16 and 32 hr (group D) after injection of PGF$_2$α-A. The time of injection with PGF$_2$α-A was regarded as 0 hr. FSH was administered to each group at -16 and 0 hr (group A), 0 and 16 hr (groups B and C), or 16 and 32 hr (group D). Groups A, B, C and D consisted of 2, 2, 4 and 4 cows, respectively. Two cows, one from group A and one from group B, were sacrificed 10 days after induced ovulation to examine the condition of luteinization macroscopically. Estrus detection and rectal examination. These examinations were carried out at 8 hr intervals over the period starting from PGF$_2$α-A injection (or first FSH treatment for the cows of group A) to ovulation. In addition, the rectal examination was conducted immediately before induced ovulation and later at intervals ranging from half an hour to 2 hr. After ovulation was induced, the estrous examination was performed twice a day, at 9 a.m. and 5 p.m. The rectal examination was performed concurrently with the estrous examination at 9 a.m. every day.

Estimation of plasma progesterone level. Blood samples were collected daily from eight cows, two cows from each group, for 20 days after PGF$_2$α-A injection, and at other times when necessary. Plasma progesterone levels were estimated by radioimmunoassay (Makino, 1973; Makino et al., 1973), the fractionation and purification by chromatography being skipped.

Results

Table 2 shows the condition of induced ovulation and luteinization in the cows of groups A, B, C and D. Group A. Ovulation with silent estrus took place in two cows, H-4 and N-J, of group A, 16.5 and 33.5 hr respectively after GnRH-A injection, or 25.0 hr on the average. In cow H-4, a small corpus luteum was formed after induced ovulation, showing 1.5 cm in longer diameter 7 days after induced ovulation. It remained this size up to 13 days after induced ovulation, then re-

**Table 1. Schedule of FSH administration in each group**

<table>
<thead>
<tr>
<th>group</th>
<th>Time in hr before (with minus sign preceding) and after PGF$_2$α-A injection</th>
<th>No. of cows used</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-16</td>
<td>FSH$^3$</td>
</tr>
<tr>
<td>B</td>
<td>PGF$_2$α-A</td>
<td>FSH</td>
</tr>
<tr>
<td>C</td>
<td>PGF$_2$α-A</td>
<td>FSH</td>
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<tr>
<td>D</td>
<td>PGF$_2$α-A</td>
<td>FSH</td>
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Remarks: 1) PGF$_2$α-A injection: 0.8 mg of ONO-1052 by the intramuscular route (IM) 10–11 days after ovulation. 2) GnRH-A injection: 100 µg of Fertirelin acetate IM twice at 60 min interval, totaling 200 µg IM. 3) FSH injection: 10 AU of Antrin IM twice, totaling 20 AU IM.
gressed. As a short estrus appeared in this cow 20 days after induced ovulation, the subsequent estrous cycle was almost normal. Cow N-J was sacrificed 10 days after induced ovulation to examine the condition of luteinization macroscopically. Fig. 1 shows the cross sections of the ovaries. The left ovary weighed 5.9 g and the right ovary 5.0 g. At the site of induced ovulation in the left ovary there was a small almost spherical corpus luteum 1.5 cm in longer diameter with a central cavity of 0.4 cm in diameter. The cross section of the corpus luteum was dark orange in color. Moreover, the ovary contained four follicles, 0.8, 0.6, 0.5 and 0.5 cm in diameter, respectively. In a cross section of the right ovary, a small corpus luteum, 0.5 cm in longer diameter, was seen. This was previously formed and regressed after injection with PGF$_{2\alpha}$-A. No follicle greater than 0.5 cm in diameter were seen in the ovary.

Group A in Fig. 3 exhibits plasma progesterone levels in cows H-4 and N-J after induced ovulation. In cow H-4 the level showed a slight increase for 3 days after induced ovulation. Then, it increased rapidly to 6.2 ng/ml 5 days after induced ovulation. After that, it began to decrease, reaching 3.0 ng/ml 8 days after induced ovulation. It then increased to 7.6 ng/ml and remained at a high level until the 13th day, when it began to decrease rapidly. The plasma progesterone level averaged 5.6 ng/ml in cow H-4 over the period from 5 to 13 days after induced ovulation. Cow N-J showed a slow increase for 7 days after induced ovulation with the level rising rapidly to 5.2 ng/ml on the eighth day. In brief, small corpora lutea were formed in these two cows. Judging from the plasma progesterone level, however, luteal function was improved to some extent in these cows by treatment with FSH.

**Group B.** In two cows belonging to group B, H-5, and J-1, ovulation took place 30.5 and 25.5 hr, respectively, or 28.0 hr on the average, after injection with GnRH-A. No estrous behavior was
observed in either of the two cows from injection with GnRH-A to ovulation. Cow H-5 had a small corpus luteum 1.5 cm in longer diameter 10 days after induced ovulation. It manifested short estrus 2 days later, its estrous cycle being reduced remarkably. Cow J-1 was sacrificed 10 days after induced ovulation. The cut surfaces of its' ovaries are shown in Fig. 2. The left ovary weighed 12.0 g and the right ovary 13.3 g. In the right ovary a small, very slender corpus luteum of yellow ocher color was noticed at the exact site where induced ovulation had taken place. Its' cut surface was 2.2 cm in longer diameter. The ovary also contained four follicles,
1.1, 1.0, 0.6 and 0.6 cm in diameter, respectively. The left ovary contained a corpus luteum which had regressed after injection with PGF$_{2\alpha}$-A and two follicles 0.6 cm in diameter.

Group B in Fig. 3 presents the plasma progesterone levels in cows H-5 and J-1 after induced ovulation. In cow H-5, the level showed no increase after induced ovulation, but remained lower than 1.0 ng/ml (0.5 ng/ml on the average) for 12 days after induced ovulation until a short estrus appeared. In cow J-1, the level continued to be low after induced ovulation. It increased to 2.7 ng/ml 8 days after induced ovulation, but began to decrease on the following day, being 0.8 ng/ml on the day it was sacrificed. In short, the FSH injection gave little effect on plasma progesterone levels in group B.

Group C. Four cows in this group, H-4, H-6, S-78 and S-0110, were artificially ovulated 18 to 42 hr, or 26.8 hr on the average, after GnRH-A injection. No estrous behavior was observed in any cow from this group during the period from GnRH-A injection to ovulation. In cow H-6, one follicle from each ovary was ruptured almost simultaneously with GnRH-A injection. Two corpora lutea, 1.5 and 2.0 cm in longer diameter, were seen in either ovary, respectively, 12 days after induced ovulation. In cow H-6, estrus appeared 21 days after induced ovulation and a follicle ruptured the following day. In cow S-78, a corpus luteum 1.6 cm in longer diameter was noticed at the site of ovulation 5 days after induced ovulation. It later developed rather remarkably, being 2.5 cm in longer diameter 10 days after induced ovulation. Silent estrus was found in this cow 22 days after induced ovulation. In cow S-0110, a small corpus luteum 2.2 cm in longer diameter was seen 10 days after induced ovulation and normal estrus 9 days later, when the corpus luteum was reduced to 0.5 cm in longer diameter. In the remaining cow, H-4, a small corpus luteum was formed, being 1.5 cm in longer diameter 12 days after induced ovulation. Normal-like estrus appeared in this cow 18 days after induced ovulation. The corpora lutea formed in cows H-4, H-6 and S-0110 were all small as mentioned above, but did not regress so soon after developed.
Blood samples were collected from two cows, S-78 and S-0110, from group C. Fig. 4 shows the group C plasma progesterone levels after induced ovulation. In cow S-78, the level rose from 2.6 to 4.4 ng/ml within 3-4 days after induced ovulation and continued the high level for 10 days. The level was essentially the same in cow S-0110. It increased to 3.9 ng/ml in 5 days and maintained a high degree (5.6 ng/ml on the average) for 14 days after induced ovulation before beginning to decrease. Thus, the plasma progesterone level in these cows resembled those in the normal estrous cycle. An improved function of corpus luteum by the FSH treatment was observed in group C.

Group D. Four cows, belonging to this group S-G, J-1, H-1 and S-44, ovulated 16-26 hr, or 21.1 hr on the average, after GnRH-A injection. The ovulation was accompanied with silent estrus in all cases. In cow S-G, the corpus luteum, 2.5 cm in longer diameter 7 days after induced ovulation, was very slender like that of the sacrificed cow from group B. This cow showed silent estrus again 21 days after induced ovulation. Also, in cow J-1 the corpus luteum was slender and similar that of cow S-G, reaching a maximum size of 2.5 cm in longer diameter 18 days after induced ovulation. This cow exhibited persistent estrus 27 and 28 days after induced ovulation. The corpus luteum formed in cow H-1 was very slender, 3.2 cm in longer diameter 5 days after induced ovulation. This was reduced to 2.0 cm in longer diameter 13 days after induced ovulation. The subsequent estrus with short duration appeared 10 days later. In the remaining cow from group D, S-44, a slender corpus luteum, 2.0 cm in longer diameter, was formed 7 days after induced ovulation. As it regressed early, the subsequent estrus occurred 4 days later. Thus, the estrous cycle was reduced distinctly, but the estrus was almost normal in nature.

Blood samples were collected from two cows, S-G and J-1, of group D. Fig. 4 presents plasma progesterone levels from group D cows after induced ovulation. This level rose slowly in both cows, reaching 4.7 ng/ml in cow S-G in 14 days and 4.8 ng/ml in cow J-1 13 days after induced ovulation, before falling gradually. In short, all corpora lutea were slender in the four
cows of group D. In cow S-44, the corpus luteum regressed early. Judging from the plasma progesterone levels in cows S-G and J-1, functional activity of the corpora lutea was insufficient. Treatment with FSH, therefore, failed to display the same improving effect on luteal formation in group D as observed in group C.

Discussion

According to Onuma et al. (1969), the follicle stimulating effect of FSH in cows depends on the number of injections rather than on the total dose. In the present experiment, the goal of FSH treatment was not to induce superovulation, but rather to stimulate follicular development after luteal regression induced by PGF$_{2\alpha}$-A. The treatment is successful if only one follicle is developed. In a preliminary experiment, cows were treated with 5 AU of FSH four times at 8 hr intervals, the total dosage of the hormone being 20 AU. They received PGF$_{2\alpha}$-A simultaneously with the first FSH injection. Finally, they were injected with GnRH-A 32 hr after the first FSH injection. As a result, many follicles developed in the ovaries. This treatment was too strong to stimulate single follicle development. Therefore, in the present experiment the number of injections of FSH was reduced. Cows were twice injected with 10 AU of the hormone at a 16 hr interval, the total dosage being 20 AU. This procedure developed only one or two follicles exceeding 1.0 cm in diameter in the ovaries of each cow immediately before ovulation. The number of follicles ruptured was only one in all the cows, except one case in group C in which two follicles ruptured. It was ascertained that this procedure of FSH treatment was sufficient to meet the purpose of the present experiment.

The interval between GnRH-A injection and ovulation was examined in four experimental groups, the average intervals being 25.0, 28.0, 26.8 and 21.1 hr in groups A, B, C and D, respectively. In the previous experiment (Ohnami et al., 1986), the intervals were 37.5 hr in groups A and B, and 20.0 hr in groups C and D without FSH, respectively. Ovulation was induced in the early stage of follicular development after injection with PGF$_{2\alpha}$-A, regardless of FSH treatment.

In the present experiment, the cows of group A had small corpora lutea, but luteal function was improved in them. In the cows of group B, however, luteal function was poor. Of the four cows of group C, three had small corpora lutea, but luteal function was improved in them. In the cows of group D, FSH treatment displayed some effect, although it was minimal. Thus, FSH treatment improved luteal function in groups A, C and D. Among these groups, however, a large difference was observed in the degree of improvement in the luteal function. The degree was the highest in group C and the lowest in group B, being moderate in groups A and D. It is very interesting to note the interval between the first FSH injection and GnRH-A injection and also the interval between PGF$_{2\alpha}$-A injection and GnRH-A injection. Both were relatively long in group C, while relatively short in group B. In groups A and D, however, these intervals were not uniform. These results clearly indicate there is a close relationship with the interval between injections of FSH or PGF$_{2\alpha}$-A and GnRH-A; that is, between the degree of follicular maturation at the time of GnRH-A injection and the function of corpus luteum.

In the other experiment, the present authors induced ovulation in cows having a developing follicle by GnRH-A injection. But, administration of hCG to such cows could not exert any luteotrophic effect on the lutinization process (Ohnami et al., 1987). From these results, it is suggested that whether luteal function would take place satisfactorily or not, might be determined at the time of ovulation. Therefore, it is presumed that luteal hypoplasia is caused when ovulation occurs before granulosa cells and theca interna cells of the follicle are sufficiently sensitized to gonadotrophin and acquired an ability to change themselves to lutein cells.

In conclusion, it was elucidated that luteal hypoplasia was caused that a follicle is immature when ovulation took place.

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References


牛の黄体形成不全の実験的誘発に及ぼす FSH の影響

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