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 $F_{2\alpha}$ analogue ($PGF_{2\alpha}$ -A) followed by the injection of 200 μ g 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 $PGF_{2\alpha}$ -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.

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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.

Materials and Methods

Experimental cows. A total of 10 adult cows, four Japanese Shorthorns, two Jerseys and four Holsteins, were used. Some of them were used more than once, so that a total of 12 experiments could be performed. Their body weights averaged 528 kg, ranging from 360 to 753 kg.

Experimental production of luteal hypoplasia. Luteal hypoplasia was produced by the following method of artificially induced ovulation in the early stage of follicular development. At first, each cow received an intramuscular injection of 0.8 mg prostaglandin $F_{2\alpha}$ analogue ($PGF_{2\alpha}$ -A, ONO-1052, Ono Pharmaceutical Co., Ltd., Osaka,

Table 1. Schedule of FSH administration in each group

group	Time in hr before (with minus sign preceding) and after PGF ₂ α-A injection ¹⁾				No. of cows used
	-16	0	16	32	
A	FSH ³⁾	PGF ₂ α-A FSH	GnRH-A ²⁾		2
B		PGF ₂ α-A FSH	GnRH-A FSH		2
C		PGF ₂ α-A FSH	FSH	GnRH-A	4
D		PGF ₂ α-A	FSH	GnRH-A FSH	4

Remarks: 1) PGF₂α-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.

Japan) in the mid-luteal stage, 10-11 days after spontaneous ovulation, followed by two intramuscular 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 PGF₂α-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 intramuscular route. When the time of injection with PGF₂α-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 PGF₂α-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 PGF₂α-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 2. Conditions of induced ovulation and luteinization in cows injected with FSH before induced ovulation

Group	Cow	Estrus ¹⁾	Induced ovulation				Subsequent estrus	
			Ovary	hr after PGF ₂ α -A injection	hr after GnRH-A injection	Corpus ²⁾ luteum formed	Type	Days after induced ovulation
A	H-4	—	Right	32.5	16.5	1.5	Short estrus	20
	N-J ³⁾	—	Left	49.5	33.5	1.5 ⁴⁾	—	—
B	H-5	—	Left	46.5	30.5	1.6	Short estrus	12
	J-1 ³⁾	—	Right	41.5	25.5	2.2 ⁴⁾	—	—
C	S-78	—	Left	74.0	42.0	2.5	Silent estrus	22
	S-0110	—	Right	50.0	18.0	2.0	Normal estrus	19
	H-6 ⁵⁾	—	Left	60.0	28.0	1.5	Normal estrus	21
			Right			2.0		
	H-4 ⁵⁾	—	Left	51.0	19.0	1.5	Normal like estrus	18
D	S-G	—	Left	57.0	25.0	2.5	Silent estrus	20
	J-1 ⁶⁾	—	Left	49.5	17.5	2.5	Persistent estrus	27-28
	H-1 ⁵⁾	—	Right	58.0	26.0	3.2	Short estrus	23
	S-44 ⁵⁾	—	Right	48.0	16.0	2.0	Normal estrus	11

Remarks: 1) Time interval between PGF₂ α -A injection and ovulation.

2) Maximum longer diameter (cm).

3) Sacrificed 10 days after induced ovulation.

4) The longer diameter of the cut surface of the corpus luteum observed at slaughter.

5) No blood samples collected.

6) Retarded regression of corpus luteum formed.

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 PGF₂ α -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 ovu-

lation. 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

Fig. 1. Bilateral ovaries of cow N-J from group A 10 days after induced ovulation (1). Cut surfaces of a hypoplastic corpus luteum. (2) Follicle 0.8 cm in diameter. (3) Cut surfaces of a previously formed corpus luteum which regressed after $\text{PGF}_2\alpha$ -A injection.

Fig. 2. Bilateral ovaries of cow J-1 from group B 10 days after induced ovulation. (4) Cut surfaces of a previously formed corpus luteum which regressed after $\text{PGF}_2\alpha$ -A injection. (5) Cut surfaces of a hypoplastic corpus luteum.

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 ochre 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,

Fig. 3. Plasma progesterone patterns in cows injected with FSH before induced ovulation (groups A and B).

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 $\text{PGF}_2\alpha$ -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.

Fig. 4. Plasma progesterone patterns in cows injected with FSH before induced ovulation (groups C and D).

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 $\text{PGF}_2\alpha\text{-A}$. 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 $\text{PGF}_2\alpha\text{-A}$ 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 $\text{PGF}_2\alpha\text{-A}$, 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 $\text{PGF}_2\alpha\text{-A}$ 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 $\text{PGF}_2\alpha\text{-A}$ 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 luteinization 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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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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発育途上の卵胞に排卵を誘起すると黄体形成不全が発生することから、その成因は排卵時の卵胞の成熟状態と関連していることが推察された。そこで、この点を確かめるために FSH を使用して、卵胞の成熟度を促進させたときの排卵後の黄体形成状態を調べた。排卵誘起は黄体期の成牛に対し PGF₂α analogue 注射後16時間 (AおよびB群), 32時間 (CおよびD群) に GnRH analogue を注射して行った。FSH は 20 AU を 2 回に分け、それぞれ 10 AU ずつ注射し、その注射時期は PGF₂α analogue 注射時を 0 時として、A群: -16および0時, BおよびC群: 0および16時, D群: 16および32時であった。A群の誘起排卵後に形成された黄体は小型であっ

た。しかし、血中 progesterone (P) 値は排卵後上昇し黄体形成は改善された。B群の形成黄体も小型であったが、血中P値は誘起排卵後も低値で推移し、黄体形成は不良のままであった。一方、C群の形成黄体も小型のものが多かった。しかし、血中P値の消長は正常性周期のパターンに類似し、C群の黄体形成はかなり改善された。D群には細長い黄体が形成され、誘起排卵後の血中P値の上昇も極めて緩慢であったが、軽度ながら FSH 注射の効果が認められた。以上の結果から、黄体形成の改善がC群、A群およびD群の順に認められ、黄体形成不全の成因が排卵時の卵胞の成熟状態と密接に関連していることが確かめられた。