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Luteinizing Hormone Releasing Hormone and Placental Gonadotrophins for Theraphy of Ovarian Quiescence in Cattle

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Abstract

This study was conducted to develop an effective theraphy on ovarian quiescence in cattle. In ovarian quiescent cattle, luteinizing hormone releasing hormone analogue (LH-RH-A) induced ovulation indirectly about 36 hr after injection by releasing luteinizing hormone (LH). Human chorionic gonadotrophin (hCG) induced ovulation directly about 36 hr after injection without LH release. Pregnant mare serum gonadotrophin (PMSG) stimulated development, maturation and active estradiol-17β (E2) secretion of follicle(s), so that the actively secreted E2 triggered ovulatory LH surge about 35 hr after injection. Consequently, PMSG induced ovulation about 72 hr after injection. After induced ovulation, hypoplastic and short-lived (Type I) corpus luteum (CL) developed (i.e. induced CL) on LH-RH-A treatment. Type I and well-developed but slightly short-lived (Type II) CL developed on hCG treatment, and Type I, Type II and well-developed and normal life span (Type III) CL did on PMSG treatment. The ovarian cyclic activity started at lower rates on each treatment with LH-RH-A, hCG and PMSG. For initiating the ovarian cyclic activity it was recognized that induced CL was functional enough to increase plasma progesterone level and that a follicle matured with active E_2 secretion in accordance with regression of the CL. Therefore, to promote the ovarian cyclic activity PMSG was supplementally dosed at a low level in 6 days after LH-RH-A treatment. The PMSG treatment was expected to stimulate maturation and active E_2 secretion of a follicle developing along with regression of the induced CL. This treatment induced the ovarian cyclic activity at a high rate.

Discipline: Animal industry
Additional key words: hCG, inactive ovary, LH-RH, PMSG

Introduction

Anestrus due to ovarian quiescence (inactive ovary) frequently takes place in grazing and group feeding heifers just as postpartum cows[4,6,7]. The disorder has been considered as a result of insufficient secretion of gonadotrophins from the pituitary[1,4,6]. Gonadotrophins and luteinizing hormone releasing hormone (LH-RH; synonym for gonadotrophin releasing hormone) theraphy has been recommended[1,2,4,6,9,18,19]. However, efficacy of these treatments has not been always satisfactory[2,4,6].

The simultaneous injection with pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (hCG)[19] and an injection with LH-RH analogue (LH-RH-A) alone were not so effective. The former treatment induced the ovarian cyclic activity in 54% and the latter did in 31%, though both the treatments induced ovulation in most cases[16]. More effective theraphy is able to be developed by elucidating following points: characterization of hormones' action and reactions to the hormones, and necessary and sufficient incidents for

initiating the ovarian cyclic activity.

In this study, changes of ovarian structure were examined by palpation *per rectum*, and peripheral plasma luteinizing hormone (LH), estradiol-17ß (E_2) and progesterone (P) level were determined by radioimmunoassay after injection with LH-RH-A, hCG, and PMSG in ovarian quiescent cattle. A theraphy which was expected to be effective was tested regarding its efficacy.

The cattle used in this study were mostly Holstein heifers and most of the cattle had small- or middle-size follicle(s) by palpation. Details of experimental animals and methods employed are referred to original papers[10-16].

LH-RH analogue

Following injection with 100-400 μg LH-RH-A, plasma LH increased quickly to a peak (nealy 70 ng/m*l*) about 2 hr after the treatment in all the cases (Fig. 1).

Ovulation was induced about 36 hr after the treatment in most cases (80%), as in 2 of 3, 2 of 3 and all 4 cases which received a single injection of 100 μg, double injections of 100 μg at 1 hr interval and a single injection of 200-400 μg, respectively. Plasma E_2 level did not increase from the treatment to the induced ovulation. Figs. 2 and 3 show changes in ovaries and plasma P and E_2 levels before and after the LH-RH-A treatment.

A corpus luteum (CL) developed after the induced ovulation (induced CL) in almost all cases (88%), but it was less than 18 mm in maximum diameter

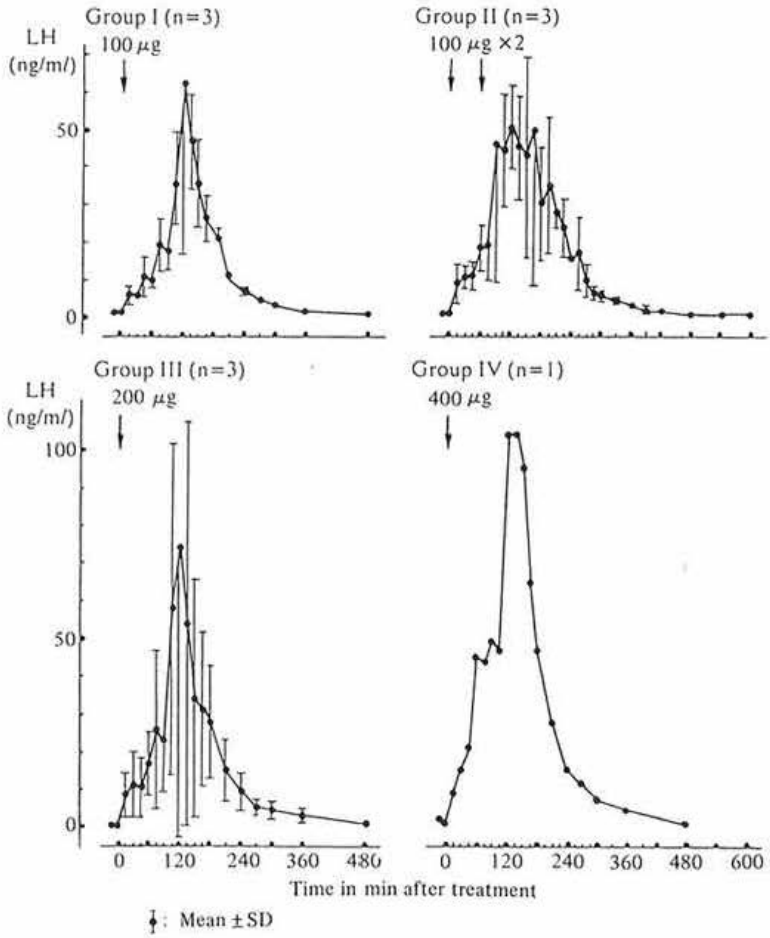


Fig. 1. Mean serum LH level following LH-RH-A injection in ovarian quiescent heifers in experimental groups I, II, III and IV

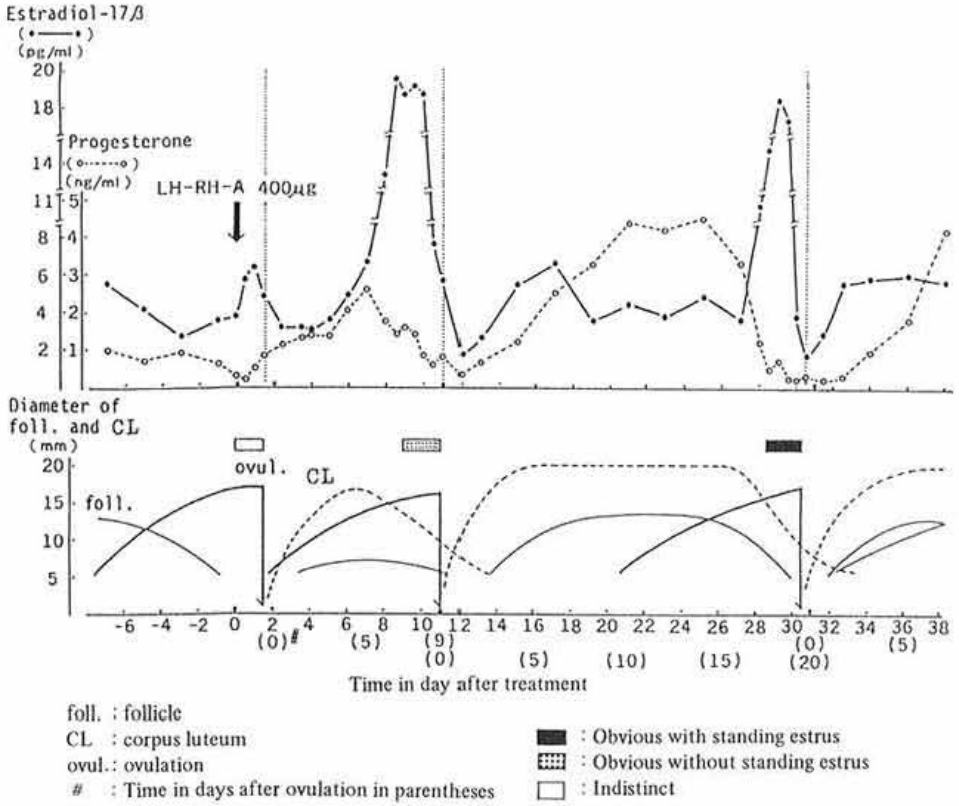


Fig. 2. Ovarian changes and plasma progesterone and estradiol-17β levels in heifer No. 527 with quiescent ovaries following LH-RH-A treatment

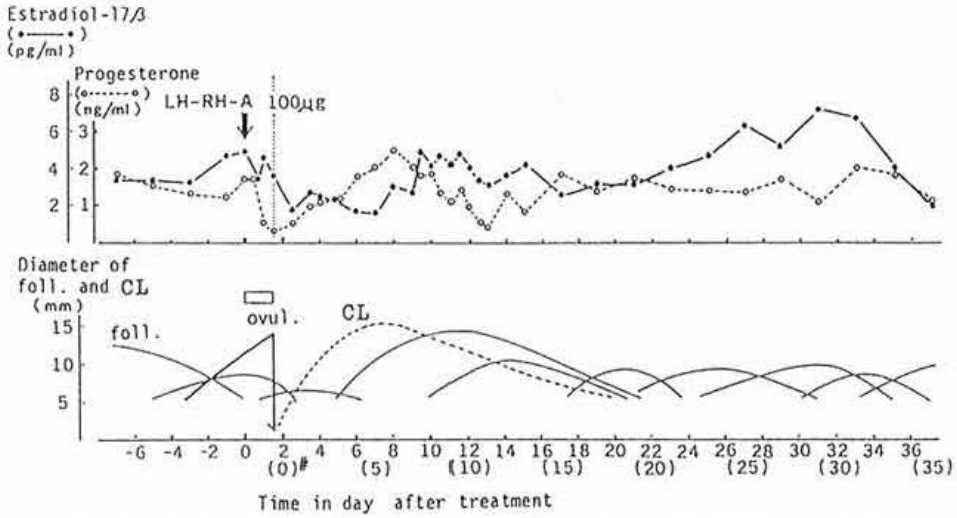


Fig. 3. Ovarian changes and plasma progesterone and estradiol-17β levels in heifer No. 525* with quiescent ovaries following LH-RH-A treatment
* On 2nd treatment in 21 days after 1st treatment.
See the legends of Fig. 2.

Table 1. Changes in ovaries and plasma progesterone and estradiol-17β levels in ovarian quiescent heifers following LH-RH-A[a] treatment

Experimental groups and heifer no.	Induced ovulation	Induced CL[b]	Development of foll.[c] with regression of induced CL	P[d] rise with development of induced CL	E_2[e] rise with follicle growth[f]	2nd ovulation and onset of ovarian cyclic activity
I-525	−	−	−	−	−	−
II-523	−	−	−	−	−	−
I-522	+	−	−	−	−	−
II-522*	+	+	+	−	−	−
III-522**	+	+	+	−	−	−
III-525**	+	+	+	−	+	−
I-525*	+	+	+	+	−	−
II-524	+	+	+	+	+	+
III-523*	+	+	+	+	+	+
IV-527	+	+	+	+	+	+

a): LH-RH analogue (Fertirelin acetate). b): Induced CL is the CL developed after induced ovulation. c): Follicle. d): Progesterone. e): Estradiol-17β. f): Growth of follicle coinciding with regression of induced CL.
*On 2nd and **3rd treatment in 21 days after 1st treatment and in 41 days after 2nd treatment, respectively.
−: Negative. +: Positive.

and began to degenerate about 6 days after the ovulation. Namely, the CL was subnormal in size or hypoplastic and short-lived (Type I). Plasma P level increased slightly showing a small peak (about 2.5 ng/ml) along with development of the CL in majority (57%).

Before and during regression of the induced CL, development of a follicle was confirmed in each case. But the increase of plasma E_2 level in accordance with the follicular development was demonstrated only in about a half of the cases (50%).

In almost all the cases (75%) in which the E_2 increase occurred, the follicles ovulated (second ovulation) and the ovarian cyclic activity was resumed. However, in the other cases the follicle atrophied and the ovaries became quiescent again. Table 1 indicates changes in ovaries and plasma P and E_2 levels and onset ovarian cyclic activity following the LH-RH-A treatment.

In the cases that initiated the ovarian cyclic activity on the treatment, the following events occurred: ovulation was induced, the induced CL developed, plasma P level increased in accordance with development of the CL, a follicle developed and matured with obvious E_2 rise along with regression of the CL, and the follicle ovulated

Following points were identified: LH-RH-A induced ovulation about 36 hr after the treatment through the release of LH from the pituitary. The induced CL was always Type I. To initiate the ovarian cyclic activity on the treatment, the induced CL was to be functional enough to elevate plasma P level, and along with the regression of the CL a follicle developed and matured with active E_2 secretion.

hCG

Following injection with 750-6,000 IU hCG, ovulation was induced about 36 hr after the treatment in almost all the cases (92%), as in 2 of 3 and all 8 cases which received a single injection of 750 IU and 1,500-6,000 IU, respectively. Neither E_2 increased nor ovulatory LH surge occurred after the treatment. Fig. 4 shows changes in ovaries and plasma P and E_2 levels before and after the hCG treatment.

CL were formed after the ovulation in all the cases and classified into two types. One was Type I and held a majority (60%). The other was Type II; i.e. well-developed but slightly short-lived CL which started regression about 11 days after ovulation and held a minority (40%). Plasma P level increased in accordance with development of the CL in all the cases, reaching a peak with about 3.2 ng/ml in that of Type I and 8.5 ng/ml in that of Type II.

Althouth a follicle developed before and during regression of the induced CL in each case, plasma

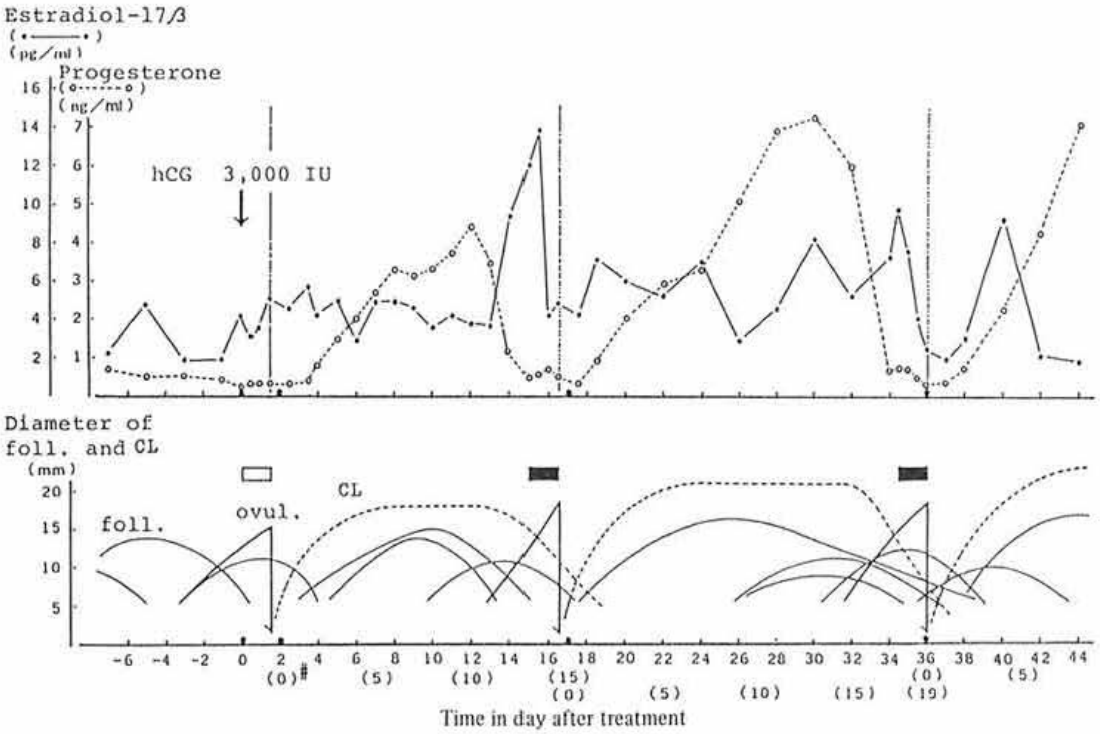


Fig. 4. Ovarian changes and plasma progesterone and estradiol-17β levels in a heifer with quiescent ovaries following hCG treatment
For abbreviations and symbols, see the legends of Fig. 2.

E_2 level increased only in one-third (30%) of the cases reaching a peak (about 13 pg/ml) in accordance with development and maturation of the follicle. In all the cases in which the increase of E_2 took place, the follicle ovulated and the ovarian cyclic activity started. However, in cases with no increase of E_2, the follicle atrophied and the ovaries became quiescent.

The ovarian cyclic activity was resumed when a chain of the following events occurred after the treatment: induced ovulation, development of CL with an increase of plasma P level, maturation of a follicle with an increase of E_2 along with regression of the CL, and second ovulation.

Following points were clarified: Ovulation was induced about 36 hr after the treatment without ovulatory LH surge by direct action of hCG. Two types of CL, i.e. Type I and Type II, were developed after the induced ovulation. To initiate the ovarian cyclic activity, the induced CL was functional enough to elevate P level in plasma and a follicle developed and matured with an obvious E_2 increase in plasma according to regression of the CL.

PMSG

Injection with 500–4,000 IU PMSG induced ovulation in 2–4 (mostly 3–4) days after the treatment in almost all the cases (90%), as in 4 of 6 and all 14 cases which received a single injection of 500 and 1,000–4,000 IU, respectively. Plasma E_2 level increased to a high level about 2 days after the treatment. Plasma LH level revealed the ovulatory LH surge. The surge started about 35 hr and reached a high level (nearly 32 ng/ml) about 39 hr after the treatment. Fig. 5 shows changes in ovaries and plasma P and E_2 levels after the PMSG treatment, and Fig. 6 shows changes of plasma LH level following the treatment.

Three types of CL were found in the induced CL; the majority was Type I (61%), Type II was in a small number (11%), and the well-developed CL

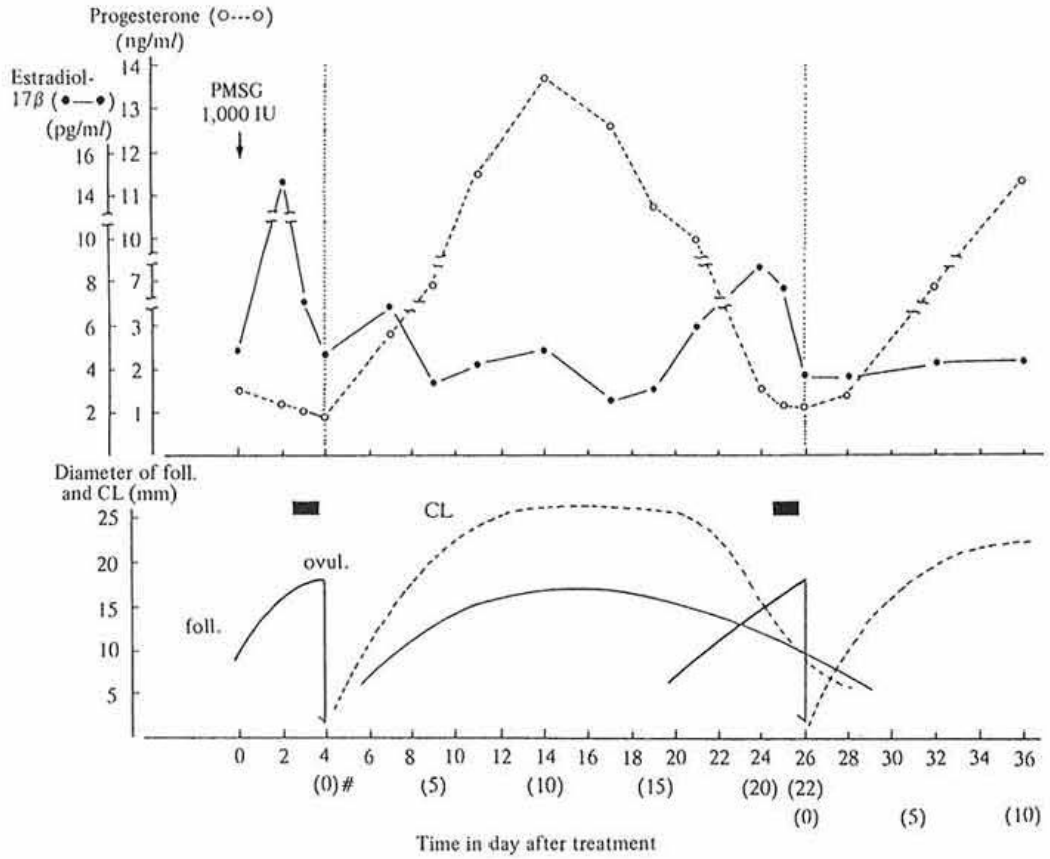


Fig. 5. Ovarian changes and plasma estradiol-17β and progesterone levels in a heifer with quiescent ovaries following PMSG treatment
For abbreviations and symbols, see the legends of Fig. 2.

with a normal life span (Type III) held a minority (30%). Plasma P level increased in company with development of the induced CL.

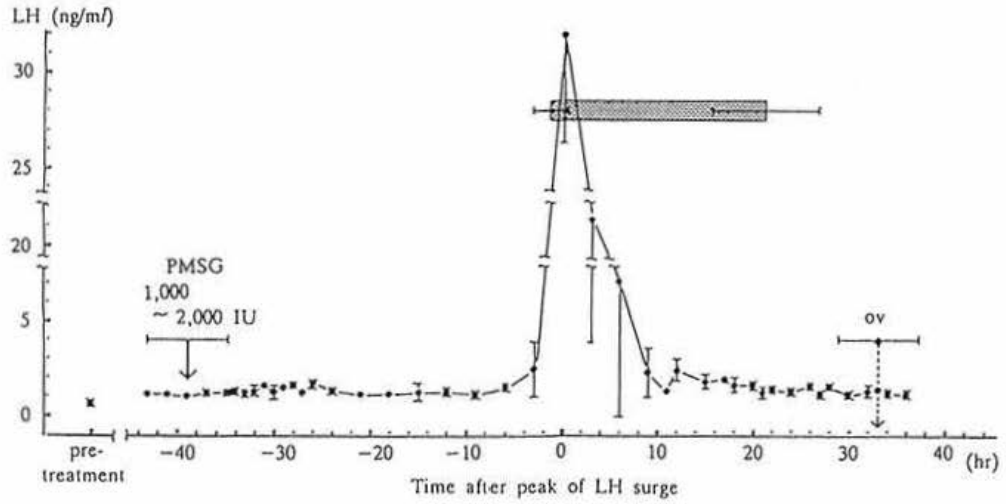
Following regression of the induced CL, the second ovulation came out and the ovarian cyclic activity started in minority (33%), in which the E_2 level increased prior to the second ovulation. In the other cases the ovaries became quiescent again.

Several or more than 10 follicles developed and reached maximum size about 8–10 days after the treatment, in some cases which received 1,000 IU and in most cases which received 2,000–4,000 IU, being accompanied by an increase of E_2 level.

Following points were clarified: PMSG stimulated growth and maturation of follicle(s) to secrete E_2 actively. The actively secreted E_2 triggered the ovulatory LH surge about 35 hr after the treatment under a low plasma P level. Ovulation occurred consequently about 72 hr after the treatment. Three types of CL, i.e. Type I, Type II and Type III, were developed after the induced ovulation. In the cases in which the ovarian cyclic activity started, the plasma E_2 level increased before the second ovulation. Many follicles developed after the treatment with 1,000 or more IU.

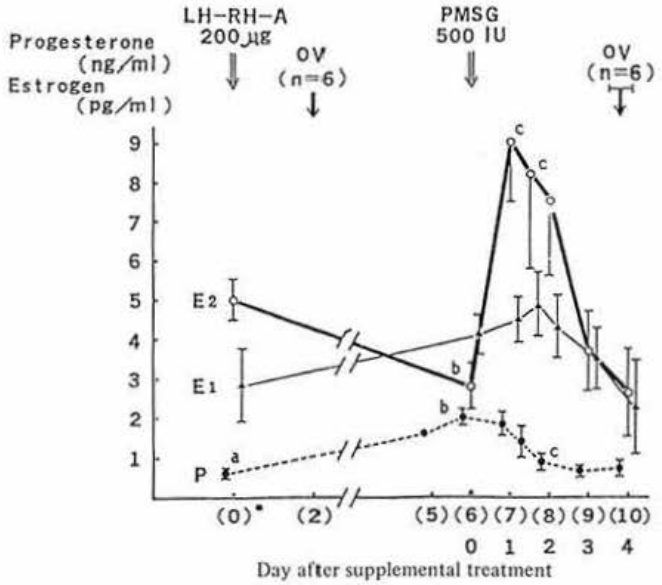
Effective hormone theraphy

Ovarian response to treatment with LH-RH-A was more uniform than that with hCG and PMSG. To initiate the ovarian cyclic activity on the treatments, a follicle developed and matured with active E_2 secretion according to regression of the induced CL. PMSG stimulated development and maturation



: Mean ± SD, ▨: Estrus, ov: Ovulation.

Fig. 6. Changes of blood LH level following PMSG treatment in ovarian quiescent cattle (n=4)



LH-RH-A: LH-RH analogue (Fertirelin acetate)
OV: Ovulation
E_2: Estradiol-17β
E_1: Estrone
P: Progesterone
: Mean ± SE
* in parentheses: Day after LH-RH-A treatment
a–c: Significant difference was found between a and b with $p<0.01$ and between b and c with $p<0.05$.

Fig. 7. Changes in the mean plasma progesterone and estrogen levels after supplemental treatment with PMSG in Group I heifers (n=6)

Table 2. Occurrence of estrus and ovulation, onset of ovarian cyclic activity, and fertility following supplemental treatment with PMSG and $PGF_{2\alpha}$-A

Group	Supplemental treatment[a]		No. of heifers	Estrus[c]		Ovulation		Onset of ovarian cyclic activity[e] (%)	Conception rate[f] (%)
	PMSG (IU)	$PGF_{2\alpha}$-A[b] (μg)		No. of heifers (%)	Time[d] (day)	No. of heifers (%)	Time (day)		
I	500	-	13	5 (38.5)	$3.2 \pm 0.8^{g,h)}$	13 (100)[h]	$4.2 \pm 0.7^{h,j)}$	13 (100)[h]	10/12 (83.3)
II	500	500	20	9 (45.0)	$2.0 \pm 0^{i)}$	18 (90.0)[h]	$3.3 \pm 0.8^{i)}$	18 (90.0)[h]	10/17 (58.8)
III	-	500	12	4 (33.3)	$2.0 \pm 0^{i)}$	5 (41.7)[i]	$3.4 \pm 0.5^{k)}$	5 (41.7)[i]	4/4 (100)

a): In six days after treatment with 200 μg of LH-RH analogue (Fertirelin acetate).
b): Prostaglandin $F_{2\alpha}$ analogue (ONO-1052).
c): Standing estrus.
d): Days after supplemental treatment (0 is the day of supplemental treatment).
e): No. of heifers.
f): No. of heifers conceived/no. of heifers inseminated on succeeding ovulation to supplemental treatment.
g): Mean ± SD.
h-k): Significant difference was found between h and i with $P < 0.01$ and between j and k with $P < 0.05$ (vertical line).

of follicles to promote active E_2 secretion (Fig. 5). Based on these findings, a low dosage of PMSG was chosen to reinforce the treatment with LH-RH-A. The supplemental treatment was expected to stimulate maturation and active E_2 secretion of the follicle and to promote consequential ovulation and the ovarian cyclic activity. The supplemental treatment was done around the time when the induced CL started to regress and a follicle developed.

The supplemental treatments with 500 IU PMSG alone (Group I), simultaneous 500 IU PMSG and 500 μg Prostaglandin $F_{2\alpha}$ analogue (ONO-1052; $PGF_{2\alpha}$-A: Group II), and 500 μg $PGF_{2\alpha}$-A alone (Group III) were put into practice in the heifers, in which ovulation was induced within 2 days and induced CL developed after the treatment with 200 μg LH-RH-A. $PGF_{2\alpha}$-A was applied to promote regression of the induced CL[17].

The results obtained were as follows: Plasma P level decreased to about 1 ng/ml in 2 days after the supplemental treatment in Group I. The decrease occurred on the next day after the supplemental treatment in Groups II and III. Plasma E_2 level increased to a peak (about 9 pg/ml) in 1-1.5 days after the supplemental treatment in PMSG-treated groups (Groups I and II). However, such an obvious increase was not observed in Group III. Fig. 7 shows changes in the mean plasma P and estrogen levels after the supplemental treatment with PMSG in the Group I heifers.

Ovulation took place in almost all the cases of PMSG-treated groups (100 and 90% in Groups I and II, respectively), and these ovulation rates were significantly higher ($P < 0.01$) than that (42%) of non-PMSG-treated group (Group III). The ovarian cyclic activity was resumed in all the cases, in which the ovulation occurred after the supplemental treatments. However, the ovaries became quiescent again in the cases in which the ovulation did not come out. Table 2 indicates occurrence of estrus and ovulation, onset of ovarian cyclic activity, and fertility following the supplemental treatments.

Estrus appeared in 33-45% of the heifers following the supplemental treatments with no significant difference among the groups. Conception rates in relation to the consequential ovulation following the supplemental treatments were 59-100% without any significant difference among the groups.

Following points were identified: The supplemental treatment with PMSG stimulated the development and the maturation of follicle to secrete E_2 actively. And the treatment consequently promoted the second ovulation and the initiation of the ovarian cyclic activity. It was thus indicated that the supplemental treatment with PMSG after an LH-RH-A treatment was effective theraphy on ovarian quiescence in cattle.

Conclusion

The ovarian cyclic activity was resumed in a higher rate (90-100%) by supplemental injection of PMSG

after LH-RH-A treatment than by treatment with 100–400 μg LH-RH-A alone (43–47%), 750–6,000 IU hCG alone (30%), 500–4,000 IU PMSG alone (33%), and simultaneous treatment with 1,000 IU PMSG and 1,000 MU (= ca. 3,000 IU) hCG (54%), even when the rates were calculated only for the cases in which ovulation was induced and resultant CL developed after the treatments. Therefore, the supplemental injection with PMSG after LH-RH-A treatment was recognized to bring about satisfactory results in theraphy on ovarian quiescence in cattle.

Nutritional deficiencies bringing in loss of body weight[4,5,8], sucking[3,4] and increase of corticoids in blood[4] reduce frequency of the pulsatile LH release to lower blood LH level and cause consequential suppression of ovarian function. So it is suspected that such agents as nutritional and metabolic disorders, chronic debilitating diseases, and environmental stresses should affect endocrine condition. When animals are affected severely by the agents, the endocrine condition must be suppressed far from normal. In such cases hormone theraphies must be unavailable. Therefore, hormone theraphies should be applied after restoration of the nutritional, healthy, environmental and concomitant endocrine condition by eliminating the agents. It has to be taken into consideration that hormone theraphies triggered the ovarian cyclic activity only in the animals which restore the endocrine condition to nearly or almost normal one.

References

1) Arthur, G. H., Noakes, D. E. & Pearson, H. (1989): Veterinary reproduction and obstetrics (Theriogenology). Bailliere Tindall, London, 341–383.
2) Asdell, S. A. (1949): Hormones and the treatment of sterility in dairy cattle: a review. *J. Dairy Sci.*, **32**, 45–59.
3) Garcia-Winder, M. et al. (1984): Effect of suckling and ovariectomy on the control of luteinizing hormone secretion during the postpartum period in beef cows. *Biol. Reprod.*, **31**, 771–778.
4) Hopkins, S. M. (1986): Bovine anestrus. *In* Current theraphy in theriogenology 2 — diagnosis, treatment and prevention of reproductive diseases in small and large animals. ed. Morrow, D. A., W. B. Saunders Company, Philadelphia, 247–250.
5) Imakawa, K., Kittok, R. J. & Kinder, J. E. (1984): Luteinizing hormone secretion after withdrawal of exogenous progesterone in heifers fed three levels of dietary energy. *J. Anim. Sci.*, **58**, 151–158.
6) Laing, J. A. (1970): Fertility and infertility in the domestic animals — aetiology, diagnosis and treatment. Bailliere Tindall and Cassell, London, 355–428.
7) Roberts, S. J. (1986): Veterinary obstetrics and genital diseases (Theriogenology). David and Charles, Vermont, 434–580.
8) Rutter, L. M. & Randel, R. D. (1984); Postpartum nutrient intake and body condition: effect on pituitary function and onset of estrus in beef cattle. *J. Anim. Sci.*, **58**, 265–274.
9) Salisbury, G. W., VanDemark, N. L. & Lodge, J. R. (1978): Physiology of reproduction and artificial insemination of cattle. W. H. Freeman and Company, San Francisco, 647–679.
10) Kamomae, H. et al. (1985): Ovarian changes and plasma progesterone and estradiol-17β levels in ovarian quiescent heifers after treatment with LH-RH analogue. *Jpn. J. Anim. Reprod.*, **31**, 48–56 [In Japanese with English summary].
11) Kamomae, H. et al. (1986): Ovarian changes and plasma progesterone and estradiol-17β levels in ovarian quiescent heifers after treatment with hCG. *Jpn. J. Anim. Reprod.*, **32**, 13–23 [In Japanese with English summary].
12) Kamomae, H. et al. (1988): Effects of LH-RH analogue on LH release and ovarian function in ovarian quiescent heifers. *Jpn. J. Vet. Sci.*, **50**, 613–621.
13) Kamomae, H. et al. (1988): The effects of PMSG on ovarian function in ovarian quiescent heifers. *Jpn. J. Vet. Sci.*, **50**, 1222–1231.
14) Kamomae, H. et al. (1989): Blood LH level and induced ovulation after hCG and PMSG treatment in ovarian quiescent cattle. *Jpn. J. Vet. Sci.*, **51**, 467–473.
15) Kamomae, H. et al. (1990): Activation of quiescent ovaries by administration of PMSG after LH-RH analogue treatment in heifers. *Theriogenology*, **34**, 975–988.
16) Kaneda, Y. et al. (1979): Responses of quiescent ovaries to gonadotropins and an LH-RH analogue in grazing heifers. *Jpn. J. Anim. Reprod.*, **25**, 89–93 [In Japanese with English summary].
17) Kaneda, Y. et al. (1981): Synchronization of estrus in the cow by intramuscular injection with prostaglandin $F_{2\alpha}$ or its analogue (ONO-1052). *Jpn. J. Anim. Reprod.*, **27**, 93–100 [In Japanese with English summary].
18) Venzke, W. G. (1949): Relation of hormones to lowered fertility in cattle. *J. Amer. Vet. Med. Ass.*, **115**, 347–352.
19) Yamauchi, M. et al. (1958): Studies on the treatment of ovarian dysfunction in cattle with equine gonadotrophin and human chorionic gonadotrophin. *Jpn. J. Anim. Reprod.*, **3**, 117–120 [In Japanese].

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