Blood LH Level and Induced Ovulation after hCG and PMSG Treatment in Ovarian Quiescent Cattle

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ABSTRACT. Ovarian quiescent cattle bearing follicle with palpable size were treated with single intramuscular injection of 750–6,000 IU of human chorionic gonadotrophin (hCG) in 13 cases and 1,000–2,000 IU of pregnant mare serum gonadotrophin (PMSG) in 5 cases. Changes of blood luteinizing hormone (LH) level, estrus and ovulation after the treatments were examined. After the hCG treatment LH level became slightly high from 0.2–0.6 ng/ml of pre-treatment to 0.3–1.9 ng/ml of post-treatment and maintained the level up to ovulation without the ovulatory LH surge. Ovulation was induced about 36 hr after the treatment in 12 cases. The ovulations were all silent ovulations. After the PMSG treatment LH level became slightly high from 0.6 ng/ml of pre-treatment to 1.3 ng/ml of post-treatment and the level lasted until the ovulatory LH surge. The ovulatory LH surge occurred about 39 hr after the PMSG treatment in 4 cases with a peak of about 32 ng/ml. Ovulation was induced about 74 hr after the treatment in all 5 cases. Four cases showed estrus but one in which the LH surge could not be confirmed did silent estrus preceding the induced ovulations. It was demonstrated that hCG induced ovulation without the LH surge but PMSG induced the ovulatory LH surge and the subsequent ovulation in ovarian quiescent cattle.—**KEY WORDS:** cattle, hCG, LH, ovarian quiescence, PMSG.

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Human chorionic gonadotrophin (hCG) and pregnant mare serum gonadotrophin (PMSG) are commonly used in treatment for ovarian disorders, improving the fertility, and induction of superovulation in cattle [5, 11, 15]. The actions of these hormones had been investigated mainly in laboratory small animals [10, 14, 16, 17, 20, 21]. Several reports have been published to demonstrate peculiarities of action of and reaction to the hormones in cattle [1–4, 12, 19], but effects on follicular growth, ovulation, corpus luteum (CL) formation and CL function are not well understood or known. The authors have been examining effects of

the gonadotrophins and an analogue of luteinizing hormone-releasing hormone (LH-RH-A) on follicular growth, ovulation and CL formation in ovarian quiescent heifers [6–9]. Those results indicate that hCG acted on follicle to ovulate directly, but PMSG acted on follicle to secrete estrogen actively so that the ovulatory LH surge and ovulation occur subsequently.

In this report, change of blood luteinizing hormone (LH) level and ovulation following hCG and PMSG treatment were examined so as to clarify the action of the hormones inducing ovulation in ovarian quiescent cattle.

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MATERIALS AND METHODS

Animals and treatments: Eleven ovarian

quiescent cattle bearing follicle(s) with palpable size were treated with hCG (Experiment A) or PMSG (Experiment B). Ovarian quiescence were diagnosed by repeated rectal palpations and clinical examinations of ovaries and other genital organs at 1–2 days interval for at least last 9 days.

Experiment A consisted of Experiments A-1 and A-2. In Experiment A-1, 5 Holstein heifers received a single intramuscular (IM) injection of 750–3,000 IU of hCG. When the treatment did not induce the normal ovarian cyclic activity, 2nd and 3rd treatment were conducted 25–26 and 59 days after the preceding treatment with 2–4 times dosage. A total of 11 cases were attained in the 5 heifers and divided into 4 treatment groups. Every 3 cases of Group I, II and III received 750, 1,500 and 3,000 IU of hCG, respectively. Two cases of Group IV did 6,000 IU of hCG. Mean age and body weight of the cases were 18.2 ± 2.7 (SD) months and 337.1 ± 21.8 kg. In Experiment A-2, 2 Japanese Black received a single IM injection of 3,000 IU of hCG. The mean age and body weight were 9.5 ± 4.9 years and 291.0 ± 96.2 kg. The hCG preparation used was PUBEROGEN (Sankyo Co., Ltd., Tokyo).

In Experiment B, 4 Japanese Black received a single IM injection of 1,000 IU of PMSG. One of them received a 2nd single IM injection of 2,000 IU of PMSG on 153 days after the 1st treatment. Mean age and body weight of the cases were 8.8 ± 4.4 years and 288.8 ± 70.9 kg. The PMSG preparation used was PEAMEX (Sankyo Co., Ltd., Tokyo).

Blood sampling: Blood samples were collected via jugular venipuncture 10 min and just before the treatment and then at 15–60 min intervals for 8 hr after the treatment in Experiment A-1, and 30, 10 min and just before the treatment and then at 1 hr interval until ovulation for 33–36 hr in Experiment A-2. In Experiment B blood

samples were collected in the same way as Experiment A just before the treatment and then 2–12 hr intervals until ovulation for 72–84 hr. Serum or plasma were separated and stored at -20°C until analyzed.

Estrus and ovulation: Standing estrus and other estrous signs were observed at 6–12 hr intervals. Occurrence of ovulation was examined by rectal palpation at 3–6 hr intervals.

Animal feeding: Five heifers used in Experiment A-1 were kept in free stall. They were fed with the concentrates and hay in the ratio of 110, 120 and 105% to dry matter (DM), digestible crude protein (DCP) and total digestible nutrients (TDN) of 0.8 kg daily gain (DG) level of the Japanese Feeding Standard for Dairy Cattle (1987), respectively. The 2 cattle submitted to Experiment A-2 and the 2 cases of Experiment B were grazed in daytime and fed supplementally with 0.5 kg of the concentrates in a day. Other 3 cases of Experiment B were kept in stanchion stall and fed with the concentrates and hay cube in the ratio of 55, 165 and 65% to DM, DCP and TDN of 0.2 kg DG level of the Japanese Feeding Standard for Beef Cattle (1987), respectively.

LH assay: Blood LH was quantified by double antibody radioimmunoassay method described by Niswender *et al.* [13] with some modification [9]. Anti-bovine LH serum (B 225) was generously supplied by Dr. G. D. Niswender. Highly purified bovine LH, i. e. LER-1072-2 and LER-1716-2 were kindly provided by Dr. L. E. Reichert and by Dr. T. Johke severally, and the former was used in Experiment A-1 and the latter was done in Experiments A-2 and B. The sensitivity, intra-assay and inter-assay coefficient of variations were 0.10 ng/ml, 13.0% and 20.9%, respectively.

Statistical analysis: Data were analyzed by Student's *t*-test and difference was considered significant when the probability (P) was

Table 1. Blood LH level, estrus and ovulation following hCG treatment in ovarian quiescent cattle (Experiment A)

Experiment	Group	hCG injected (IU)	No. of cattle	Pre-treatment LH level ¹⁾ (ng/ml)	Post-treatment LH level ²⁾ (ng/ml)	Highest LH value ³⁾ (ng/ml)	Peak of LH surge (ng/ml)	Occurrence of estrus	Ovulation after treatment (hr)
A-1	I	750	3	0.14±0.12 ^{a)}	0.15±0.15 ^{b)}	0.66±0.33 ^{m)}	NO	NO	39.0±4.2 (n=2)
	II	1,500	3	0.17±0.06	0.24±0.22 ^{c,d)}	0.72±0.61	NO	NO	38.0±3.5 (n=3)
	III	3,000	3	0.12±0.08	0.39±0.46 ^{c,e,g)}	1.83±0.67	NO	NO	38.0±3.5 (n=3)
	IV	6,000	2	0.27±0.16	0.59±0.35 ^{c,f,h)}	1.90±0.07 ⁿ⁾	NO	NO	36.0±0 (n=2)
Overall mean				0.17±0.11 ¹⁾	0.32±0.35 ²⁾	1.22±0.75	NO	NO	37.8±2.9 (n=10)
A-2		3,000	2	0.62±0.20 ^{k)}	1.90±0.92 ^{l)}	2.34±1.43	NO	NO	34.5±2.1 (n=2)

1) Mean value of 10 and 0 min or 30, 10 and 0 min before the treatment.

2) Mean value for 8 hr after the treatment.

3) Highest value during 8 hr after the treatment.

a) Mean ± SD.

NO: No occurred.

b-n) Significant differences were detected between b and c, d and f, g and h, and k and l with $P<0.01$, and between d and e, i and j, and m and n with $P<0.05$, respectively.

<0.05.

RESULTS

Change of LH level, estrous signs and ovulation after hCG treatment were shown in Table 1. In Experiment A-1, LH level changed in low levels for 8 hr after the treatment without the ovulatory LH surge, though the level became slightly elevated. Mean highest LH value during 8 hr were 0.66, 0.72, 1.83 and 1.90 ng/ml in Groups I, II, III and IV, respectively. The slight elevation of LH was dose responsive and the mean highest value of LH also seemed to become higher as the dosage increased. Ovulation was induced in 10 cases except 1 of Group I, and mean time course of ovulation were 36.0–39.0 hr in every group. Estrous signs were unnoticeable at the ovulation, i. e. silent ovulation.

In Experiment A-2, LH level continued low up to ovulation, though slight increase of LH occurred and lasted after the treatment. Ovulation was induced mean 34.5 hr after the treatment. Estrous signs were obscure and silent ovulation occurred.

The results obtained in Experiment A were shown in Fig. 1, conclusively. After hCG treatment blood LH levels lasted low up to the induced ovulation without the ovulatory LH surge.

Change of blood LH level, estrus and ovulation following PMSG treatment were shown in Table 2. In all 5 cases LH level lasted low for 30–42 hr after the PMSG treatment, though the level became slightly elevated. And then the ovulatory LH surge occurred in 4 cases. The surge showed a high peak with 31.88 ng/ml at 39 hr after the treatment as means. About the 4 cases, change of LH, estrus and ovulation were shown in Fig. 2 as the time when the LH level showed a peak was reckoned to 0. Estrus started 1.5 hr before the LH peak and lasted for 22.5 hr as means. Ovulation occurred mean 33.0 hr after the peak of LH. In 1 case (No.145) LH level reached 3.05 and 3.10 ng/ml at 36 hr and 48 hr after the treatment. But the level decreased to low with 1.45 ng/ml at 54 hr and then lasted low (1.0–1.6 ng/ml). This case showed ovulation 84 hr after the treatment with silent estrus.

Fig. 1. Change of blood LH level following hCG treatment in ovarian quiescent cattle. $\bar{x}$: Mean $\pm$ SD, ov: Ovulation.

Table 2. Blood LH level, estrus and ovulation following PMSG treatment in ovarian quiescent cattle (Experiment B)

Cattle No.	PMSG injected (IU)	Pre-treatment LH level ¹⁾ (ng/ml)	Post-treatment LH level ²⁾ (ng/ml)	Ovulatory LH surge		Ovulation after peak of LH surge (hr)	Time from treatment to	
				Time from treatment to peak (hr)	Peak value (ng/ml)		Estrus (hr)	Ovulation (hr)
137	1,000	0.60	1.25 $\pm$ 0.20 ^{a)}	36	35.00	36	36	72
140	1,000	0.45	1.15 $\pm$ 0.19	34	23.50	27	42	72
145	1,000	0.60	1.16 $\pm$ 0.25	ND	ND	ND	NO	84
146	1,000	0.65	1.54 $\pm$ 0.17	36	35.00	36	36	72
137*	2,000	0.65	1.35 $\pm$ 0.17	39	34.00	33	36	72
Overall mean		0.59 $\pm$ 0.08 ^{b)}	1.27 $\pm$ 0.23 ^{c)}	39.0 $\pm$ 4.2	31.88 $\pm$ 5.60 ^{d)}	33.0 $\pm$ 4.2	37.5 $\pm$ 3.0	74.4 $\pm$ 5.4

1) The value just before the treatment.

2) Mean value from the treatment to LH surge.

a) Mean $\pm$ SD.

ND: Not determined.

NO: No occurred.

* Treated again 153 days after the preceding PMSG treatment.

b, c, d) Significant difference ($P < 0.01$) was detected between b and c, and c and d, respectively.

DISCUSSION

hCG induced ovulation without the ovulatory LH surge about 36 hr after treatment in ovarian quiescent cattle was demonstrated. It is generally accepted that LH is

the main ovulatory hormone [15, 18]. LH-RH-A caused LH release which reached peak 2 hr after treatment in ovarian quiescent heifer [9]. Both LH-RH-A and hCG induced ovulation about 36 hr after the treatments [7, 8]. These understanding and

Fig. 2. Change of blood LH level following PMSG treatment in ovarian quiescent cattle (Nos. 137, 140, 146, 137*; $n=4$). $\bar{x} \pm SD$; $\blacksquare$: Estrus, ov: Ovulation.

findings indicate that if hCG caused ovulation by provoking the ovulatory LH surge, the LH surge should occur within several hr after the treatment same as after the LH-RH-A. But the LH surge did not occur after the hCG treatment. Further, as previously reported [8], blood estradiol-17 β (E₂) level did not show obvious increase from treatment to ovulation. These facts indicate that hCG acts directly on follicle to induce ovulation. Dobson and Fitzpatrick [1] showed that hCG caused ovulation without inducing the endogenous ovulatory LH surge about 22 hr after treatment in cycling cows. Sasamoto *et al.* [16] also reported that hCG induced ovulation within 18 hr after treatment but the ovulatory LH surge did not occur during the period in diestrus rat. Present result agreed with those ones.

LH level became slightly higher after hCG treatment in overall mean of Experiment A-1, and in Experiment A-2. Post-treatment LH increase became conspicuous as the dosage increased. Two possibilities are suspected for the reason; one is hCG cross-reacts to bovine LH in the assay and

another is hCG stimulates the LH release in low level. As to the former Dobson and Fitzpatrick [1] noticed hCG did not appreciably cross-react to bovine LH. In our assay hCG cross-reacted very slightly as 1 IU of hCG corresponded to 0.01–0.06 ng of bovine LH in range of 0.1–100 IU of hCG (unpublished data). When hCG treated is all absorbed into blood flow, the blood hCG concentration is calculated as much as 0.26 IU/ml after treatment of 6,000 IU. Under the such low concentration of blood hCG the cross-reaction of hCG to LH could be negligible. So, the slight increase of LH after the treatment might come from hCG stimulating the LH release in low level. Supplementally, both the pre- and the post-treatment LH levels were different between Experiments A-1 and A-2. These differences might come from the different two lot of bovine LH were utilized in the LH assays of Experiment A-1 and Experiment A-2.

This experiment demonstrated that the ovulatory LH surge was induced about 39 hr after PMSG treatment in ovarian quiescent cattle. Blood E₂ level reached a peak on 2nd

day after PMSG treatment [6] and the increased estrogen (E) triggered the ovulatory LH surge under the low concentration of progesterone (P) [11, 15]. These facts indicate that PMSG make follicle grow and secrete E actively so that increased E trigger the LH surge and ovulation occurred subsequently. Echternkamp [2] reported that PMSG caused E₂ peak and subsequent ovulatory LH surge about 60 hr after the treatment in postpartum ovarian inactive beef cows. In perpubertal female rat, peaks of E₂ and ovulatory gonadotrophin surge occurred 42–54 hr and 52–61 hr after PMSG treatment, respectively, and ovulation took place 66 hr or within 72 hr after the treatment [10, 14, 17, 20, 21]. Present result agrees with those results. In present result the LH surge occurred earlier than in postpartum ovarian inactive beef cows [2] as described above. The earlier occurrence of LH surge may come from that in the cattle used follicles were already palpable and the follicles responded to PMSG and secreted E₂ actively immediately after the treatment so that E₂ level reached the high in a short time.

LH level became slightly higher after the PMSG treatment and lasted the level until the ovulatory LH surge. Wettemann *et al.* [19] pointed out the similar increase after PMSG treatment in cows. They indicated that PMSG cross-reacted to LH in their radioimmunoassay and blood PMSG concentration 12 hr after treatment with 2,000 IU was calculated to correspond to 3 ng/ml of LH. They considered the slight increase of LH after PMSG to come from the cross-reaction. The authors also noticed that PMSG cross-reacted to LH and 0.01, 0.1, 1, 10, 100 and 1,000 IU of PMSG corresponded to about 0.07, 0.4, 1, 2, 7, and 90 ng of bovine LH, respectively, in this assay (unpublished data). When injected PMSG all absorbed into the blood flow, the blood PMSG concentration was calculated to

0.04–0.07 IU/ml and considered to correspond to about 0.3–0.5 ng/ml of LH. This cross-reaction of PMSG to LH may attribute to the increase of LH, but the increase was not fully explained only by the cross-reaction because some disparity is present between the post-treatment LH increase and the cross-reaction value. Therefore it is suspected that PMSG might stimulate the LH release in low level.

In 1 case the ovulatory LH surge could not be certified after the PMSG treatment. But LH level increased to about 3.1 ng/ml at 36 and 48 hr after the treatment. The LH surge should be occurred between 36 and 48 hr after the treatment in this case.

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要 約

卵巢静止の牛における hCG および PMSG 処置後の血中 LH の推移と誘起排卵：加茂前秀夫*・金田義宏¹⁾・百目鬼郁男²⁾・中原達夫³⁾（農林水産省家畜衛生試験場，¹⁾岩手大学農学部，²⁾農林水産省畜産試験場，³⁾東京農業大学総合研究所，*現在，農林水産省畜産試験場）——卵巢静止の牛13例に人絨毛性性腺刺激ホルモン（hGC）750～6,000 IU，5例に妊馬血清性性腺刺激ホルモン（PMSG）1,000～2,000 IU をそれぞれ筋肉内に1回注射し，末梢血液中の黄体形成ホルモン（LH）の推移および発情と排卵の状況を調べた。hCG 処置後，血中 LH は処置時の値（0.2～0.6ng/ml）より幾分増加（0.3～1.9ng/ml）して排卵まで推移したが，排卵性 LH 放出はみられなかった。排卵は処置後36時間前後に12例で誘起されたが，いずれも無発情排卵であった。PMSG 処置後，血中 LH は処置時の値（0.6ng/ml）より幾分増加（1.3ng/ml）して推移した後，4例において39時間前後に明瞭な排卵性 LH 放出（32ng/ml 前後）を示した。排卵は処置後74時間前後に全例で誘起された。誘起排卵に先だって4例で発情が発現したが，排卵性 LH 放出が確認できなかった1例は鈍性発情であった。これらのことから，卵巢静止の牛において，hCG は排卵性 LH 放出を惹起することなく排卵を起こすのに対し，PMSG は排卵性 LH 放出を惹起することにより排卵を起こすことが明らかになった。