

# Luteotropic Effect of Pregnant Mare Serum Gonadotropin in Cattle

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**ABSTRACT.** Pregnant mare serum gonadotropin (PMSG) is a long acting luteotropic with follitropin activity. Cattle were used to examine whether a smaller dose of PMSG has substantial luteotropic effect without excessive follitropic effects. Eleven Japanese Black heifers were randomly assigned to two groups. Animals were administered 500 IU PMSG on the day of ovulation (Day 0) to promote the formation of corpus luteum (group A; N=5) or Day 7 to stimulate the luteal function (group B; N=6). Four of them were given injections of saline on Days 0 and 7 of the preceding estrous cycle for control. All animals were examined by palpation per rectum every other morning and bled every day for steroids analyses. The length of estrous cycle was shortened by the treatment in group A compared with the previous cycle, whereas it was extended in group B ( $P<0.05$ ). Progesterone secretion was not enhanced in group A, but it was significantly elevated and sustained on higher levels in group B ( $P<0.05$ ) as compared with the control. Although estradiol-17 $\beta$  concentrations were significantly increased in both PMSG-treated groups ( $P<0.05$ ), no excessive follicular development was observed. It is concluded that 500 IU PMSG administered on Day 7 enhances luteal function without excessive follicular development, whereas the administration on Day 0 has an adverse effect on luteal function.—**KEY WORDS:** estradiol-17 $\beta$ , heifer, luteotropic effect, PMSG, progesterone.

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Maternal pregnancy recognition in cattle is caused by the paracrine signal from the intrauterine embryo, which prevents pulsatile release of PGF<sub>2 $\alpha$</sub>  and insures maintenance of the functional corpus luteum [1]. Therefore, exogenous stimulations to the corpus luteum and/or uterus may supplement insufficient endogenous signals and aid the survival of embryos. So that, various kinds of hormones were hence employed directly or indirectly to make the intra-uterine environment better for embryo survival, and consequently to improve the pregnancy rate of cattle [4, 13, 19, 22, 23, 25, 27, 32, 33].

Pregnant mare serum gonadotropin (PMSG) is a heterodimeric glycoprotein hormone produced in the endometrial cup of the equine placenta. The amino acid sequence of the hormone specific  $\beta$  subunit of PMSG is identical with the subunit of equine luteinizing hormone [2, 31]. Therefore, PMSG fundamentally has luteotropic activity [5]. PMSG has the longest half life in bovine circulation among the natural gonadotropins [21], because its molecule bears highly sialylated oligosaccharides [5, 6], which make PMSG resistant to metabolism and filtration in the liver and kidney, respectively [20, 29]. A long lasting effect is desirable to maintain a responsive luteal function for a longer period. Therefore, PMSG has the clinical potential of stimulating luteal function. Nevertheless, PMSG, because of its inherent follitropin activity [5], has been widely employed in the induction of multiple ovulation in cattle [8], but has never been exploited for its luteotropic effect in the field.

PMSG was selected for use in this experiment because of its luteotropic activity and durability. However, there was concern about the strong follitropic effect of PMSG. The objective of this experiment, therefore, was to

examine whether a smaller than usually recommended dose of PMSG administered to cattle on specific days of the estrous cycle could still maintain substantial luteotropic effects with diminishing follitropic effect.

## MATERIALS AND METHODS

Eleven regularly cyclic Japanese Black heifers aged 20 to 35 months and weighed 430 to 470 kg, under normal management in a herd at the National Institute of Animal Industry, were divided into two groups. Five animals of group A and 6 animals of group B were administered a single intramuscular injection of 500 IU PMSG (PEAMEX; Sankyo Co., Ltd., Tokyo, Japan) constituted in 1 ml of physiological saline on the day of ovulation (Day 0) and on Day 7, respectively. Four animals, one of group A and three of group B were given intramuscular injections of 1 ml physiological saline on Days 0 and 7 of the preceding estrous cycle to the PMSG treatment (Control).

The behavior of animals was observed in a paddock twice daily — once in the morning and once in the evening. The condition of the internal genitalia was also examined at least every other morning by palpation per rectum.

Blood samples for progesterone (P<sub>4</sub>) and estradiol-17 $\beta$  (E<sub>2</sub>) assays were collected daily by jugular venipuncture in all the heifers throughout the treated estrous cycle. Plasma was immediately separated by centrifugation at 1,800  $\times$  g for 1 hr at 4°C and stored at –35°C until steroid analysis was performed.

Plasma P<sub>4</sub> was extracted twice with diethyl ether and analyzed by validated radioimmunoassay [9]. E<sub>2</sub> from 5 ml

of plasma was derived by the solid-phase extraction method with a small reversed-phase cartridge containing octadecylsilane bonded-phase packing (SEP-PAK C<sub>18</sub> original cartridge; Millipore Corp. Waters Chromatography Div., Milford, MA 01757) [15]. Briefly, the cartridge was washed with 10 ml of distilled water and 3 ml of 40 (V/V)% methanol following the plasma application. E<sub>2</sub> adsorbed to the packing was eluted with 3 ml of 75 (V/V)% methanol. The elution solvent was evaporated at 60°C in a nitrogen gas atmosphere. The recovery of E<sub>2</sub> by this method was more than 94%. The extracted E<sub>2</sub> was analyzed by validated radioimmunoassay using two fifths of the extract [10].

Data were analyzed statistically by the F-test for variance and Student's *t*-test for the mean.

## RESULTS

Lengths of consecutive estrous cycles before and after PMSG administration are summarized in Table 1. In group A, the average number of days of the estrous cycle following PMSG treatment was shortened by 2 days compared with the previous cycle. The mean length of the next cycle of group A became shorter than that of the treated cycle because two animals showed irregularly short cycles of 7 and 9 days. The estrous cycles of all heifers in group A subsequently restored regularity. On the contrary, in group B, the estrous cycles of all heifers treated with PMSG were extended for 1 to 4 days compared to the previous cycle ( $P < 0.05$ ). Regularity in the estrous cycle subsequently returned.

Changes in the length of the major axis of the corpus luteum observed by palpation per rectum during the treated estrous cycle are outlined in Fig. 1. It is seen that group A had the highest growth rate of the corpus luteum. The corpus luteum in all animals of group A also felt softer than that of the others in the luteinizing stage. The mean length of the corpus luteum of group A was approximately 2 mm longer than that of the control at its maximum. The corpus luteum of group A, nevertheless, had the shortest life. The corpus luteum of group B was slightly enlarged following PMSG administration. The functional luteal stage was extended for 2.5 days in the

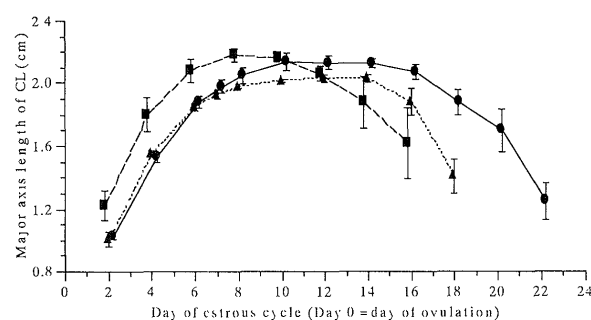


Fig. 1. Changes in the mean ( $\pm$ SEM) length of the major axis of the corpus luteum as observed by palpation per rectum during the treated estrous cycle. A single injection of 500 IU PMSG was made on Day 0 in Group A (■; N=5) or on Day 7 in group B (●; N=6). Controls (▲; N=4) were given physiological saline on Days 0 and 7.

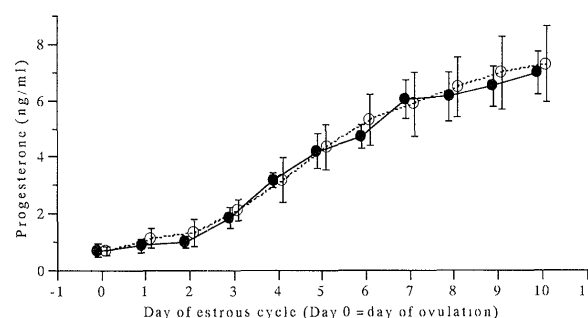


Fig. 2. Changes in the mean ( $\pm$ SEM) plasma progesterone concentrations in heifers administered 500 IU of PMSG (●) or saline (○) on the day of ovulation.

mean (Days  $15.5 \pm 1.0$  to  $18.0 \pm 2.2$ ), and the onset of luteolysis and estrus were consequently retarded for that period of time in group B. One (N=4) or two (N=1) follicular developments were noted in group A by palpation. The follicular size of group A attained 12 to 18 mm in diameter 6 to 8 days after PMSG administration. Faint symptoms of estrus were observed in some animals of group A in response to the development of follicles, which were, however, not ovulated. On the other hand, the follicles of group B reached maximum size (12 to 16 mm) 1 to 2 days after PMSG administration. No other symptom was observed in group B.

Peripheral plasma P<sub>4</sub> profile of group A was quite the same as the profile of the control in the formative period of a corpus luteum (Fig. 2). Similarly, there were no differences between both groups for P<sub>4</sub> during the functional luteal stage (data not shown). The P<sub>4</sub> profile of group B from Days 0 to 14 of the treated estrous cycle is shown in Fig. 3 together with the profile of the control. The increasing rate of P<sub>4</sub> concentration was significantly elevated by PMSG injection ( $P < 0.05$ ). The highest level attained 2 days after injection was 9.1 ng/ml in the mean of six heifers, approximately 2 ng/ml higher than the mean level of the control on the same day. Thereafter, the

Table 1. Lengths of consecutive estrous cycles of heifers treated with PMSG or saline

Group	n	Previous cycle	Treated cycle	Following cycle	
				First	Second
A <sup>a)</sup>	5	21.4 $\pm$ 1.0 <sup>d)</sup>	18.8 $\pm$ 1.0	16.0 $\pm$ 3.3	20.2 $\pm$ 1.0
B <sup>b)</sup>	6	20.8 $\pm$ 0.5	23.3 $\pm$ 0.6 <sup>e)</sup>	20.7 $\pm$ 0.6	21.5 $\pm$ 0.4
Control <sup>c)</sup>	4	21.0 $\pm$ 0.7	20.3 $\pm$ 0.6	—	—

a) PMSG (500 IU) was given on the day of ovulation (Day 0).

b) PMSG (500 IU) was given 7 days after ovulation (Day 7).

c) Saline was injected on Days 0 and 7.

d) Interovulatory interval (Days $\pm$ SEM).

e) Significantly extended from the previous cycle ( $P < 0.05$ ).

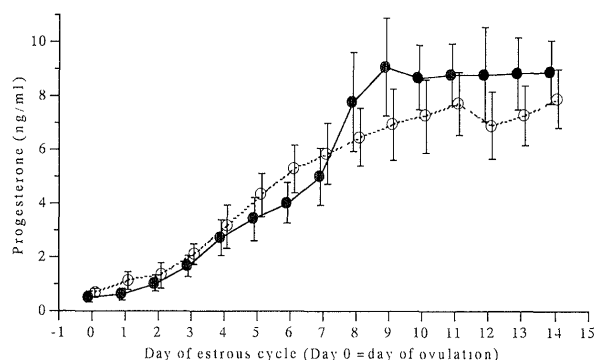


Fig. 3. Mean ( $\pm$ SEM) plasma progesterone concentrations in heifers administered PMSG (●) on Day 7 or saline (○) on Days 0 and 7 as control.

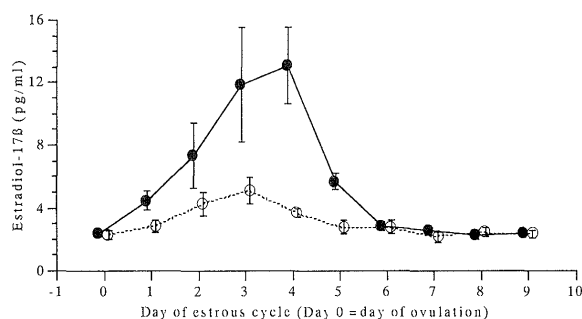


Fig. 4. Changes in the mean ( $\pm$ SEM) plasma estradiol-17 $\beta$  concentrations in heifers treated with PMSG (●) or saline (○) on the day of ovulation.

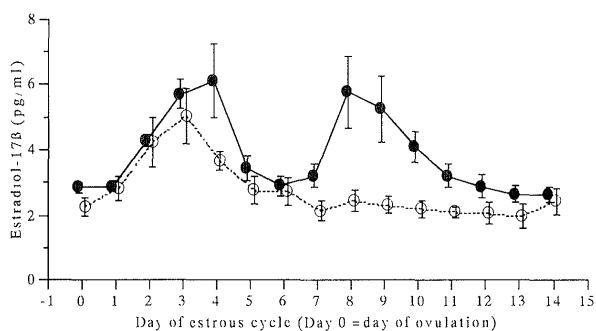


Fig. 5. Changes in the mean ( $\pm$ SEM) plasma estradiol-17 $\beta$  concentrations in heifers treated with PMSG (●) 7 days after ovulation or saline (○) on the day of ovulation and 7 days after ovulation.

higher level was sustained until the regression of the corpus luteum. The mean  $P_4$  concentration (8.7 ng/ml) of the treated group during Days 8 to 14 was significantly higher than the level (7.2 ng/ml) of the control ( $p < 0.05$ ). Furthermore, the variance within the treated group was significantly wider than that of the control ( $p < 0.05$ ).

$E_2$  profile of group A following PMSG treatment is shown in Fig. 4 with the profile of the control. Concentration of  $E_2$  in peripheral blood increased rapidly after

PMSG treatment and in some case ran up over the level of normal estrus on Day 3 or 4. Nevertheless, the concentration was reduced to the same level as the control by Day 6 in all heifers and thereafter changed similarly to the control. The variances of  $E_2$  concentrations in the treated group were wider than those of the control on Days 3 and 4 ( $P < 0.05$ ). The mean concentrations on Days 4 and 5 were more elevated for group A in comparison with those of the control ( $P < 0.05$ ). The  $E_2$  profile of group B from Days 0 to 14 is shown in Fig. 5 with the profile of the control. The change of  $E_2$  in group B was similar to that of the control until PMSG administration. There was a small peak of 3 to 9 pg/ml between Days 2 to 4 in both groups. The  $E_2$  concentration of group B attained the highest level (5.8 pg/ml) on the day (Day 8) following PMSG administration, and then gradually declined. However, the mean concentrations of group B were significantly higher than those of the control during Days 8 to 11 ( $P < 0.05$ ), and the variances in  $E_2$  concentration on Days 8 and 9 of group B were larger than those of the control ( $P < 0.05$ ).

#### DISCUSSION

Equine LH receptors weakly bind PMSG [26] and equine luteal tissue does not have specific receptors for PMSG [30], so that PMSG does not act to stimulate secretion of  $P_4$  from the cyclic luteal cell of a horse [18]. In contrast, PMSG binds to the LH receptors in other species equally as well as hCG and LH [5, 18]. Both PMSG and equine LH competitively suppressed the binding of bovine LH to the LH receptors of the bovine luteal cells in our preliminary experiment. Since the LH receptors of cattle can not distinguish LH from PMSG, PMSG acts as LH in cattle.

In the present study, Day 0 administration of PMSG was not effective in stimulating  $P_4$  secretion. This finding is similar to previous researches which demonstrated that hCG administration on the day of estrus or on the day of insemination was neither effective in stimulating  $P_4$  secretion nor in improving the conception rate in cattle [24, 27, 32]. On the contrary, PMSG administration on Day 0 intensively enhanced  $E_2$  secretion. Plasma  $E_2$  concentration reached a level almost equal to the peak during estrus. In the superovulation of cattle using PMSG, its residue induces excessive follicular development continuously after ovulation, which affects the delicate balance of  $P_4$  and  $E_2$  and reduces the viability of embryos in the oviduct [7]. Follicular development and the increase in the estrogen production are observed during the early luteinizing stage under normal conditions in cattle, because the immature corpus luteum is incapable of completely suppressing follicular growth. This was also observed in the control of this study. PMSG accelerated the ordinary follicular development and estrogen production in this experiment. Furthermore, the administration of PMSG on Day 0 caused a short estrous cycle in some animals although the reason is not clear. These results indicate that PMSG should not be administered by the

time that a corpus luteum is functional enough to suppress follicular development.

The administration of PMSG on Day 7 appeared to induce further growth of the corpus luteum and raised plasma  $P_4$  concentration in this study. These effects are similar to those reported previously showing a weight gain in the corpus luteum [12] and increases in the plasma  $P_4$  level after administration of hCG [3, 12–14, 16, 24, 33]. Furthermore, the length of estrous cycle was extended by PMSG injection on Day 7. The extension of the estrous cycle was also reported in the case of hCG treatment [3, 16, 27]. The result of this study indicates that PMSG has the same potential as hCG to stimulate the corpus luteum in cattle.

Five hundred IU of PMSG did not induce ovulation in any heifers. This finding is consistent with the fact that PMSG administered for superovulation rarely induces ovulation directly in spite of its high dosage. On the other hand, it was reported that hCG administered 7 days after estrus increases the total  $P_4$  secretion by inducing ovulation of the large follicles present at that time and the development of accessory corpora lutea [24, 27]. Sirrois and Fortune [28] showed that a large follicle is present within 6 to 8 days after estrus in normally cyclic heifers. Fricke *et al.* [12] indicated that the large follicle present 6 days after estrus is capable of forming a functional corpus luteum following ovulation in response to hCG. Rajamahendran and Sianangama [24] suggested that hCG administration 7 days after insemination is the most efficacious for producing accessory corpora lutea, and hence reduces the incidence of early embryonic mortality in cattle [24, 27]. According to these reports, it is seemed that the effect of hCG administration on the plasma  $P_4$  concentration in cattle depends on the growth of the accessory corpora lutea. However, in our previous experiment [17], the administration of porcine LH induced ovulation in cattle, but plasma  $P_4$  concentration was not elevated, except an ephemeral increase before ovulation, within the first several days following the administration. This [17] indicates that subsequent increase in bovine plasma  $P_4$  concentration was affected by the dose of porcine LH. These findings indicate that plasma  $P_4$  concentration does not increase without direct stimulation to the corpus luteum irrespective of the presence of the accessory corpora lutea in its early luteinizing stage. Fricke *et al.* [12] also suggested that the initial increase in plasma  $P_4$  concentrations resulted from the effects of hCG on the function of the corpus luteum that was present at the time of hCG administration. The reason for this is that hCG does not enhance  $P_4$  secretion of accessory corpora lutea in early luteinizing stage [12, 24, 32]. These reports suggested that long lasting stimulation is more important than the induction of accessory corpora lutea in order to promote the luteal function. In this experiment, PMSG administered on Day 7 maintained higher  $P_4$  levels until the regression of the corpus luteum without inducing the development of accessory corpora lutea. This finding indicates that the effect of PMSG administration to  $P_4$

secretion is a consequence of direct stimulation to the corpus luteum already present at that time. It also suggests that PMSG is active longer than hCG in the direct stimulation of the corpus luteum.

In the present study, PMSG administered on Day 7 also induced follicular development and estrogen production. As mentioned above [28], a large follicle exists within 6 to 8 days after estrus in normally cyclic heifers. PMSG stimulates the estrogen production of the follicle [11]. Plasma  $E_2$  concentration reached the peak 3 or 4 days after treatment on Day 0, whereas the peak was attained 1 or 2 days following PMSG administration on Day 7. Its level was similar to the untreated peak during the early luteinizing stage. Further development of the follicle was probably suppressed by the functional corpus luteum. It is suggested that 500 IU of PMSG administered on Day 7 did not have a bad influence on cattle through its follitropin activity because no other symptoms were observed.

In this study, significantly wide variations in both  $P_4$  and  $E_2$  concentrations were observed after PMSG administration as compared with the control. This indicates that the dose of PMSG might be insufficient for some animals and further research needs to be conducted to determine the appropriate dosage. Notwithstanding this, it is suggested from the results of this experiment that the administration of PMSG on Day 7 of pregnancy or on the day of embryo transfer could be utilized as a stimulant to the luteal function in cattle.

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