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## OVARIAN RESPONSE AFTER FOUR CONTINUOUS ADMINISTRATIONS OF GONADOTROPHIN IN BEEF CATTLE\*

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After 4 repeated treatments of gonadotrophin, 8 beef cattle showed an average of 12.1, 10.1, 12.1 and 8.4 corpora lutea at the 1st, 2nd, 3rd and 4th treatments, respectively. However, different dosages of gonadotrophin ranging from 1,600 to 3,500 IU of pregnant mare's serum gonadotrophin (PMSG), were used. The average number of corpora lutea decreased from 12.4, 8.4, 9.8 and 6.0 during the 1st, 2nd, 3rd and 4th treatments respectively, when the same dose of PMSG (2,000 IU) was used. However, there was no significant difference in the number of corpora lutea among the 4 treatments observed statistically ( $P > 0.05$ ). In one case, there was an increase in the number of corpora lutea after the PMSG was changed to a follicle stimulating hormone (FSH). There was a significant difference in the average number of corpora lutea when the interval between administrations was less than 60 days or more than 70 ( $P < 0.001$ ). The number of follicles and corpora lutea were analysed statistically. There was no significant difference in the rank correlation between the follicle and the corpus luteum in each treatment.

### INTRODUCTION

In the course of embryo transfer techniques for cattle, reliable superovulation is one of the most important procedures. However, many researchers have reported wide individual variations in ovarian response<sup>1,2,4,6,8,10</sup> as well as ineffectiveness of repeated treatments of gonadotrophin.<sup>3,5,9,11</sup>

In the previous paper,<sup>5</sup> we reported on the results of an analysis relating to corpora lutea and 2 continuous administrations of PMSG in beef cattle.

In this paper, we report on the ovarian responses after 4 continuous administrations of gonadotrophin in 8 beef cattle.

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\* A part of this study was released at the 88th Meeting of the Japanese Society of Veterinary Science on March 29, 1980 in Tokyo.

## MATERIALS AND METHODS

Six Simmentals and 2 Limousins were used in this study. All 8 cattle were heifers at the beginning of the experiment, however, 3 cattle had calves between the 2nd and 3rd treatments of gonadotrophin.

The PMSG and prostaglandin  $F_2\alpha$  treatment for superovulation were the same as those described in the previous paper.<sup>5)</sup> On Day 6, following estrus (estrus as Day 0) and 48 hours of fasting, the animals were anesthetized, and the corpora lutea of each were examined via midventral laparotomies. The number of corpora lutea and follicles (more than 1.0 cm in diameter) in both ovaries was then recorded.

After the initial PMSG treatment, most of the animals were allowed at least two or more estruses, which occurred spontaneously. Each animal was given a 2nd, 3rd and 4th PMSG treatment between Days 9 and 14 in precisely the same manner as in the initial treatment, except for 1 Simmental heifer. This animal received 40 mg of FSH (5 mg  $\times$  2 times per day) for the 4th treatment instead of PMSG.

The interval between each treatment for the 8 animals was 58-249 days except in the 3 cases when the delivery of a calf occurred during the experiment. One Limousin and 2 Simmental heifers gave birth to calves after the 2nd treatment: the interval between the 2nd and 3rd treatments for each was 555, 565 and 590 days, respectively.

The statistical analysis was carried out at Hokkaido University Computing Center.

## RESULTS AND DISCUSSION

Tables 1-8 show the ovarian responses of the 8 animals after gonadotrophin and prostaglandin  $F_2\alpha$  treatments. In case Nos. 1-3, decreases or poor responses for the 2nd, 3rd and 4th treatments (tabs. 1-3) were not observed. In case No. 4, however,

TABLE 1 *Ovarian response of case No. 1*<sup>\*1</sup>

Treatment	PMSG			NUMBER		
	Dose	Date <sup>*2</sup>	Interval	Corpora lutea	Follicles <sup>*3</sup>	Total
1st	2,000 <sup>IU</sup>	13 <sup>Day</sup>	— <sup>days</sup>	7	6	13
2nd	2,500	13	71	22	20	42
3rd	2,000	13	92	15	2	17
4th	2,000	13	77	12	5	17
Total	8,500	52	240	56	33	89
Average	2,125	13.0	80.0	14.0	8.3	22.3

Notes: <sup>\*1</sup> Simmental heifer

<sup>\*2</sup> Administration date from estrus (estrus as Day 0)

<sup>\*3</sup> More than 1.0 cm in diameter

TABLE 2 Ovarian response of case No. 2\*

Treatment	PMSG			NUMBER		
	Dose	Date	Interval	Corpora lutea	Follicles	Total
1st	2,000 <sup>IU</sup>	12 <sup>Day</sup>	— days	15	6	21
2nd	1,600	10	60	7	4	20
3rd	2,000	9	249	11	9	20
4th	2,000	13	97	6	5	11
Total	7,600	44	406	39	24	63
Average	1,900	11.0	135.3	9.8	6.0	15.8

Note: \* Simmental heifer

TABLE 3 Ovarian response of case No. 3\*

Treatment	PMSG			NUMBER		
	Dose	Date	Interval	Corpora lutea	Follicles	Total
1st	2,000 <sup>IU</sup>	11 <sup>Day</sup>	— days	14	9	23
2nd	2,000	12	148	14	9	23
3rd	2,000	13	95	10	3	13
4th	3,000	12	137	11	3	14
Total	9,000	48	380	49	24	73
Average	2,250	12.0	126.7	12.3	6.0	18.3

Note: \* Simmental heifer

TABLE 4 Ovarian response of case No. 4\*

Treatment	PMSG			NUMBER		
	Dose	Date	Interval	Corpora lutea	Follicles	Total
1st	2,000 <sup>IU</sup>	11 <sup>Day</sup>	— days	3	0	3
2nd	2,000	12	61	1	0	1
3rd	2,500	12	82	11	2	13
4th	3,500	12	62	2	0	2
Total	10,000	47	205	17	2	19
Average	2,500	11.8	68.3	4.3	0.5	4.8

Note: \* Limousin heifer

TABLE 5 *Ovarian response of case No. 5*<sup>\*1</sup>

Treatment	PMSG			NUMBER		
	Dose	Date	Interval	Corpora lutea	Follicles	Total
1st	2,000 <sup>IU</sup>	14 <sup>Day</sup>	— <sup>days</sup>	22	8	30
2nd	2,000	14	97	5	9	14
3rd	2,000	13	63	3	7	10
4th	FSH 40 <sup>mg*2</sup>	14	66	15	8	23
Total	—	55	226	45	32	77
Average	—	13.8	75.3	11.3	8.0	19.3

Note: \*<sup>1</sup> Simmental heifer  
\*<sup>2</sup> 5 mg×2 times per day

TABLE 6 *Ovarian response of case No. 6*<sup>\*</sup>

Treatment	PMSG			NUMBER			REMARKS
	Dose	Date	Interval	Corpora lutea	Follicles	Total	
1st	2,000 <sup>IU</sup>	10 <sup>Day</sup>	— <sup>days</sup>	14	0	14	
2nd	2,000	9	86	10	15	25	
3rd	2,000	12	555	1	0	1	Delivery
4th	3,000	11	85	8	0	8	
Total	9,000	42	—	33	15	48	
Average	2,250	10.5	—	8.3	3.8	12.0	

Note: \* Simmental

TABLE 7 *Ovarian response of case No. 7*<sup>\*</sup>

Treatment	PMSG			NUMBER			REMARKS
	Dose	Date	Interval	Corpora lutea	Follicles	Total	
1st	2,000 <sup>IU</sup>	10 <sup>Day</sup>	— <sup>days</sup>	12	13	25	
2nd	2,500	8	72	10	10	20	
3rd	2,500	14	565	27	6	33	Delivery
4th	2,500	10	58	13	16	29	
Total	9,500	42	—	62	45	107	
Average	2,375	10.5	—	15.5	11.3	26.8	

Note: \* Simmental

TABLE 8 Ovarian response of case No. 8\*

Treatment	PMSG			NUMBER			REMARKS
	Dose	Date	Interval	Corpora lutea	Follicles	Total	
1st	1,600 <sup>IU</sup>	10 <sup>Day</sup>	— <sup>days</sup>	10	4	14	
2nd	2,000	9	160	12	8	20	
3rd	2,000	10	590	19	6	25	Delivery
4th	2,000	13	82	0	0	0	
Total	7,600	42	—	41	18	59	
Average	1,900	10.5	—	10.3	4.5	14.8	

Note: \* Limousin

poor responses were observed except in the 3rd treatment. In case No. 5, reduction of corpora lutea after the 2nd and 3rd treatments of PMSG was observed.

We suggested the significant individual variations of ovarian response occur from one animal to the other and/or from one treatment to the other.

There was an increased number of corpora lutea after the 4th treatment when the gonadotrophin was changed from PMSG to FSH. A reduced number of corpora lutea suggested that antibodies to PMSG were formed after repeated administrations. We are presently investigating whether PMSG and FSH may be used alternately when a reduced number of corpora lutea is observed. In recent experiments we have obtained reasonable results from practical superovulation by using PMSG for the first two treatments and FSH for the latter two when it is necessary to avoid a reduction of ovulation. In this way, the problem of ineffectiveness of repeated administrations of gonadotrophin is reduced.

Three animals (cases Nos. 6-8) delivered calves between the 2nd and 3rd treatments of PMSG, and there was no tendency toward ovarian response after calving or during the 4 continuous treatments observed.

Table 9 shows the average number of corpora lutea for each treatment in each

TABLE 9 Average number of corpora lutea

PMSG DOSE	PMSG TREATMENTS				TOTAL	AVERAGE
	1st	2nd	3rd	4th		
1,600~3,000 <sup>IU</sup>	12.1 (N=8)*	10.1 (N=8)	12.1 (N=8)	8.4 (N=8)	42.7	10.7
2,000 <sup>IU</sup> only	12.4 (N=7)	8.4 (N=5)	9.8 (N=6)	6.0 (N=3)	36.6	9.2

Note: ( )\* Number examined

(P<0.05)

animal and selected cases of the same dose of PMSG (2,000 IU). There were no changes in the average number of corpora lutea during the 4 continuous treatments using different doses of PMSG ranging from 1,600 to 3,500 IU. However, the number of corpora lutea decreased to an average of 12.4, 8.4, 9.8 and 6.0 during 4 continuous treatments when the same dose of PMSG (2,000 IU) was used. There was no significant difference in the average number of corpora lutea among 4 continuous treatments ( $P > 0.05$ ).

In the previous paper,<sup>5)</sup> we observed the expected reduction of corpora lutea after the 2nd treatment of PMSG; however, a dose increase of 500 IU or more of PMSG for the 2nd PMSG treatment had a significant effect.

The relation between the administration intervals and the average number of corpora lutea is shown in table 10. The number of corpora lutea increased significantly at intervals of more than 70 days as compared to intervals of less than 60 days.

TABLE 10 *Administration intervals and number of corpora lutea*

INTERVALS	NUMBER EXAMINED	AVERAGE NUMBER OF CORPORA LUTEA
60 days or less	6	6.8
70 days or more	18	10.3

( $P < 0.001$ )

In the previous paper,<sup>5)</sup> we also observed that an interval of more than 70 days after the 1st treatment was effective. The results of this study showed the same tendency in administration intervals. In general, immunological antibodies in animals are reduced or disappear completely after certain periods. NAKAHARA et al. ('61) reported that anti-PMSG was negative 100 days after PMSG injection. The repeated treatment of beef cattle with PMSG in our previous study<sup>5)</sup> and in the present one indicates that an interval of 70 days or more is most effective.

As to ovarian responses, the number of follicles (1.0 cm or larger in diameter) and corpora lutea was statistically analysed by means of KENDALL's rank correlation rule. The number of corpora lutea and follicles did not change after the 1st, 2nd and 3rd treatments were analyzed. However, there were significant differences after the 4th treatment and in the total number observed from the 1st to 4th treatments ( $P < 0.01$ ).

The repeated treatment of beef cattle with PMSG in this study indicated that significant individual variations exist among animals and treatments and that an interval of 70 days or more between treatments is effective. There was no strong correlation between the number of corpora lutea and follicles.

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