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ANALYSIS RELATING TO CORPORA LUTEA AND TWO CONTINUOUS ADMINISTRATIONS OF PMSG IN BEEF CATTLE

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One hundred and eight beef cattle were divided into 2 groups of 54 cattle each. The 1st group was administered 2,000 IU of pregnant mare's serum gonadotrophin (PMSG) for the first treatment, and the same dose of PMSG was applied for the second treatment 54-257 days later. The 2nd group of 54 cattle received 1,600-2,500 IU of PMSG for the first treatment and a dose increase ranging from 100 to 1,000 IU for the second treatment 36-206 days later. Both groups received the PMSG on Days 9-14 (estrus as Day 0), and 2 days after the administration of PMSG, 25 mg of prostaglandin $F_2\alpha$ was injected intramuscularly. Two days after the prostaglandin $F_2\alpha$ treatment, estrus detection was performed and the cattle were inseminated artificially. The animals were operated at midline under general anaesthesia at 5-7 days after estrus, and a number of corpora lutea were observed in situ. In the 54 cattle of the 1st group, the average number of corpora lutea was 15.6 after the first PMSG treatment, and 11.3 after the second. In the 2nd group, 13 cattle received a dose increase of 100-400 IU of PMSG for the second treatment, which resulted in the average number of 12.0 corpora lutea after the first PMSG treatment, and 8.3 after the second. Furthermore, 31 cattle received a dose increase of 500-750 IU of PMSG for the second treatment, resulting in an average number of 6.6 corpora lutea after the first treatment, and 8.5 after the second. Finally, 10 cattle received a dose increase of 1,000 IU of PMSG for the second treatment, which resulted in an average number of 6.5 corpora lutea after the first treatment, and 10.7 after the second.

INTRODUCTION

The transfer of fertilized embryos from one animal to another has long been considered a useful research tool among researchers in the field of animal reproduction. In recent years, the application of embryo transfer techniques to cattle has aroused interest in several countries. There are several technical difficulties of procedure in embryo transfers. Among these difficulties, reliable superovulation has been regarded as one of the most important factor. Many researchers have reported wide variations in individual ovarian responses and in the amount of ovulation in cattle after injections with

a standard dose of PMSG (ROWSON, '51 ; GORDON et al., '62 ; HAFEZ et al., '63 ;SCANLON et al., '68 ; MAULEON et al., '70 ; GORDON, '75). However, if PMSG administration was repeated in the following estrus cycles, it was ineffective. These results were in accord with the findings of WILLETT et al. ('53) and other cattle researchers (HAFEZ et al., '65 ; SAUMANDE & CHUPIN, '77). It has been suggested that gonadotrophins similar to PMSG simulated antigens which induced the formation of antibodies when injected into animals (NAKAHARA et al., '64 ; SCHAMS et al., '78). This reduction in ovulation after PMSG treatments is an important factor in embryo transter when repeated treatments are needed.

In this paper we report the results of an analysis relating to corpora lutea and 2 continuous administrations of PMSG in beef cattle.

MATERIALS AND METHODS

One hundred and eight cattle consisting of 46 Simmental, 25 Charolais, 20 Limousin and 17 other beef breeds were used. Ninety-two out of 108 cattle were heifers, and the remaining 16 were cows. The 108 beef cattle were used as shown in table 1 ; all demonstrated normal reproductive tracts as determined by rectal examination.

For the first PMSG treatment, each animal was given a single intramuscular injection of 1,600–2,500 IU of PMSG at Days 9–14 after estrus (estrus as Day 0), which was followed 48 hours later with intramuscular injections of 25 mg of prostaglandin $F_2\alpha$ or its analogue. The animals were then inseminated 2–3 times with frozen semen at 12 hour intervals during estrus (fig. 1). As shown in table 1, 4 different brands of PMSG from 4 companies, which were divided into 2 groups, were used in this study. The 1st group consisted of the same dose of PMSG (2,000 IU) for the first and second treatment, while the 2nd consisted of dose increases ranging from 100–1,000 IU of PMSG between the first (1,600–2,500 IU) and second (2,000–3,000 IU) treatments (tab. 2).

On Days 5–7, following estrus and 48 hours fasting, the animals were anaesthetized,

TABLE 1 *PMSG used in this study*

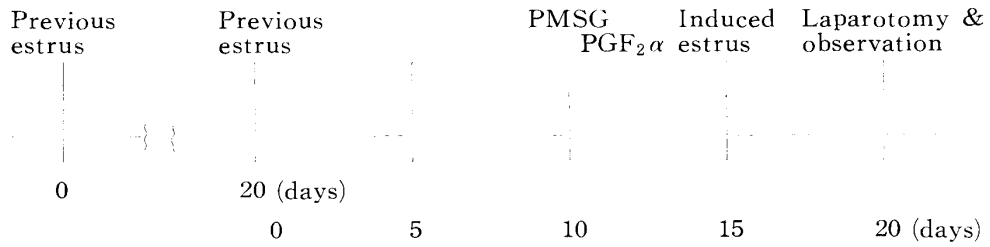
PMSG	TREATMENT		REMARKS*
	First	Second	
A	65	41	34
B	36	55	30
C	5	2	—
D	2	10	—
Total	108	108	—

* Same combination of PMSG between first and second treatments

TABLE 2 *PMSG doses and interval between first and second treatments*

GROUP	NUMBER OF ANIMALS EXAMINED	PMSG TREATMENTS		INTERVALS
		First	Second	
I	54	2,000 IU	2,000 IU	54–257 days
II	54	1,600 ≀ 2,500	2,000 ≀ 3,000	36–206
Total	108	1,600 ≀ 2,500	2,000 ≀ 3,000	36–257

FIGURE 1 *Procedure for PMSG treatment*



and the corpora lutea of each were examined via midventral laparotomies. The number of corpora lutea in the two 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 second PMSG treatment between Days 9 and 14 in precisely the same manner as in the initial treatment.

RESULTS

Out of the 108 cattle, 98 of the animals which received 2,000 IU of PMSG for the first treatment were selected for statistical analysis (fig. 2). The average number of corpora lutea differed significantly between the Simmental and the Limousin breeds ($P < 0.05$).

As shown in table 3, the same batches and doses of PMSG were selected for both the first and second treatments. The number of corpora lutea after the second treatment was lower than that of the first treatment in all breeds.

Table 4 shows the average number of corpora lutea among the 4 sub-groups of PMSG dosages. In sub-groups 1 and 2, the same dose, or an increased dose of PMSG for the second treatment, revealed a decreased number of corpora lutea, however, in sub-groups 3 and 4, an increase of 500–1,000 IU of PMSG for the 2nd treatment showed

FIGURE 2 *Comparison of the number of corpora lutea for all breeds after the first treatment of PMSG*[*1]

NS	*	NS	Simmental N=44 CL=10.2
NS	NS	Charolais N=22 CL=12.0	
NS	Limousin N=17 CL=15.4		
Others N=15 CL=12.5			

*1 Ninety-eight of the 108 cattle selected to receive 2,000 IU of PMSG for the first treatment were used for the analysis.
NS Not significant
* Significant ($P<0.05$)
CL Average number of corpora lutea

TABLE 3 *Average number of corpora lutea between first and second PMSG treatments for all breeds*[*1]

BREEDS	PARITY	NUMBER OF ANIMALS EXAMINED	AVERAGE NUMBER OF CORPORA LUTEA		
			First treatment	Second treatment	Difference
Simmental	Heifers	11	13.1	8.9	−4.2
Charolais	Heifers	7	16.1	9.0	−7.1
Limousin	Heifers	5	23.4	19.5	−3.9
Others[*2]	Heifers	6	16.2	14.0	−2.2
Subtotal	Heifers	29	17.2	12.9	−4.3
Mixed[*3]	Cows	5	13.8	11.2	−2.6
Total	Heifers and cows	34	16.5	12.5	−4.0

*1 Out of the 108 animals, the 34 which had the same dose and source of PMSG for both the first and second treatments were selected for this table.
*2 Including 2 Maine-Anjou, 2 Blonde d'Aquitaine, 1 Murray Grey and 1 Chianina breeds
*3 Including 2 Charolais, 2 Simmental and 1 Limousin breeds

TABLE 4 *Number of corpora lutea and animals between first and second treatments*

GROUP	SUB-GROUP	INCREASED DOSE OF PMSG FOR SECOND TREATMENT	NUMBER OF ANIMALS EXAMINED	AVERAGE NUMBER OF CORPORA LUTEA			NUMBER OF ANIMALS FOR SECOND TREATMENT		
				First treatment	Second treatment	Difference after second treatment (%)	Increased CL	Same CL	Decreased CL
I	1	0 IU	54	15.6	11.3	−4.3 (42.0)	13	1	40
	2	100-400	13	12.0	8.3	−3.7 (40.9)	3	0	10
II	3	500	30	6.8	8.5	+1.7 (55.6)	17	1	12
	4	750-1,000	11	6.5	10.7	+4.2 (48.7)	8	1	2
Total			108	10.2	9.7	−0.5 (48.7)	41	3	64

CL: Corpora lutea

TABLE 5 *Relation between interval and number of corpora lutea*

INTERVAL BETWEEN FIRST AND SECOND TREATMENTS	NUMBER OF ANIMALS EXAMINED*	AVERAGE NUMBER OF CORPORA LUTEA				NUMBER OF ANIMALS AT SECOND TREATMENT		
		First treatment	Second treatment	Difference	Second/First	Increased CL	Same CL	Decreased CL
54- 66 days	9	14.5	8.1	−6.4	55.9%	1	0	8
70- 79	9	15.4	17.6	+1.2	114.3	3	0	6
80- 87	11	17.1	12.5	−4.6	73.1	4	0	7
90- 98	7	17.4	10.1	−7.3	58.0	1	1	5
102-257	18	14.3	9.7	−4.6	67.8	3	0	15
Total	54	15.6	11.3	−4.3	72.4	12	1	41

* Out of the 108 animals, the 54 which received the same dose of PMSG (2,000 IU) for both the first and second treatments were selected for this table.

FIGURE 3 *Statistical analysis between PMSG dose and number of corpora lutea*

*	*	NS	I (0 IU) N=54 42.0%
*	NS	II (100–400 IU) N=13 40.9%	
NS	II (500 IU) N=30 55.6%		
III (750–1,000 IU) N=11 66.2%			

* Significant ($P<0.05$)
NS Not significant
% Increase of corpora lutea after second treatment
IU Increased dose of PMSG for second treatment

TABLE 6 *Statistical analysis*[*1] *of corpora lutea between first and second PMSG treatments of Simmental cattle*

GROUP	BREEDS	PMSG DOSE FOR SECOND TREATMENT	INTERVAL	NUMBER OF ANIMALS EXAMINED	RESULTS
I	Simmental	Same	54- 79 days	6	*
II	Simmental	Same	80–174	14	NS
III	Simmental	Increased	36–156	26	NS

*1 χ^2 test on corpora lutea between first and second treatments
* Significant ($P<0.05$)
NS Not significant

an increased number of corpora lutea. Figure 3 provides an evidence that a dosage of between 400 and 500 IU of PMSG had an effect on the proportion of corpora lutea.

The relation between the administration intervals and the average number of corpora lutea is shown in table 5. The number of corpora lutea increased slightly at 70–79 days, however, there was no significant difference between this interval and the average number of corpora lutea.

The Simmental breed was selected in order to examine the difference in the number of corpora lutea between the first and second treatments by the χ^2 test. Table 6 shows that there was a significant difference between the first and second treatments when

the interval between the two treatments was 54–79 days ($P<0.05$), however, there was no significant difference when the interval was 80–174 days, nor was there any difference when the dose was increased during the 36–156 days interval.

Discussion

The ability of PMSG to induce multiple ovulations has led to the wide scale use of the hormone for stimulating superovulation for the purpose of embryo transfer in cattle. A reduced ovulation rate in cattle given repeated PMSG administrations has been recorded by several researchers (Hafez et al., '65; Saumande & Chupin, '77). It has been suggested that PMSG simulates antigens which induce the formation of antibodies, however, Newcomb et al. ('79) have suggested that antibodies to PMSG are unlikely to be formed when normal levels of PMSG are administered to cattle, and that it is also unlikely that the high steroid levels which appear after the first PMSG treatment impaire follicle development.

In this study we observed the expected reduction of corpora lutea at the second treatment of PMSG, however, a dose increase of 500 IU or more of PMSG did not affect their reduction. To explain the reduction of ovarian response to repeated PMSG treatments, some investigators have put forth the possibility that antibodies to PMSG are formed (Nakahara et al., '64; Schams et al., '78). The possibility also exists that the reduced response to repeated treatments is attributable to individual immunity to either the FSH or to the LH moieties of PMSG, since PMSG contains both FSH and LH biological activities.

Newcomb et al. ('79) have reported the significant effect of PMSG doses (1,000 and 2,000 IU) after the second treatment, although they did not show evidence that the ovarian response was affected by the PMSG batch used. In this study, there was a significant difference when the PMSG dose was increased by 500 IU or more, however, our standard PMSG dose was approximately 2,000 IU, and there was no significant difference when the dose was increased by 100–400 IU.

Reduction of the ovarian response of cattle to repeated superovulation treatments has been recorded by several authors (Hafez et al., '65; Saumande & Chupin, '77). It is well known that the dosage and the interval between the two PMSG treatments are important factors. In general, immunological antibodies in animals are reduced by a titer or disappear completely after certain periods. Nakahara et al. ('61) have reported that anti-PMSG was negative 100 days after the PMSG injection. It has been suggested that the injection of exogenous gonadotrophin may result in the temporary loss of specific hormone receptors. The repeated treatment of beef cattle with PMSG in this study indicated that a dose increase of 500 IU or more for the second PMSG treatment had a significant effect, and that an interval of 70 days or more from the first treatment was effective. There was no increase in the number of corpora lutea when 100–

400 IU of PMSG were injected and less than 70 days had expired before the second PMSG treatment.

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