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PROSTAGLANDINS AND SUPEROVULATION IN THE BOVINE

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SUMMARY

Prostaglandin (PG) synchronisation has allowed the use of pregnant mares serum gonadotrophin (PMSG) at different stages of the oestrous cycle. It has been shown that treatment with PMSG after the 8th day of the cycle results in higher mean ovulation rates, a higher number of animals having a large number of fertilised, recoverable eggs compared with treatment given earlier in the cycle. The combination of PMSG treatment with PG synchronisation diminishes the degree of synchrony of ovulation that is obtained with PG treatment alone. Recent work has explored the way in which PG treatment may be used to control the number of ovulations following PMSG treatment on the 15th day of the cycle. The possible methods of using PMSG and PG treatments in combination to control superovulation in the cow is discussed.

INTRODUCTION

Superovulation in the bovine has been induced by the use of pregnant mares serum gonadotrophin (PMSG) given on the 15th or 16th day of the oestrous cycle (AVERY *et al.*, 1962 ; BROCK and ROWSON, 1952 ; GORDON *et al.*, 1962 ; HAFEZ *et al.*, 1963 ; HAFEZ *et al.*, 1965 ; HAMMOND and BHATTACHARYA, 1944 ; LAMOND, 1970 ; LASTER, 1973 ; ROWSON, 1951). This technique of superovulation leads a variable number of ovulations and also a variable occurrence of oestrus and of silent oestrus following PMSG treatment (GORDON *et al.*, 1962). The recent use of prostaglandin PG $F_{2\alpha}$ and its analogues have greatly facilitated the degree to which the oestrous cycle of the cow can be synchronized (TERVIT *et al.*, 1973 ; LAURDALE *et al.*, 1974 ;

LOUIS *et al.*, 1973 ; LAMOND *et al.*, 1973). Preliminary report on the use of PMSG and PG in combination have been reported by TERVIT *et al.* (1973) and ELSDEN *et al.* (1974).

MATERIALS AND METHODS

The experiments were conducted over a period of two years, using two breeds of cattle (*Hereford/Friesian* and *Angus cross* heifers). The animals were housed during the duration of the experimental period (usually about one week) whether the experiments were conducted in summer or winter. In summer, the animals grazed at pasture and in winter were fed straw supplemented with a barley concentrated diet. Vasectomized bulls fitted with chin-ball mating harnesses were used to detect oestrus, and the animals were observed at least once a day. The animals were either mated to entire bulls or artificially inseminated during the oestrus following treatment.

Pregnant mares serum gonadotrophin (Folligon, Intervet Co. Ltd.) was used to induce superovulation ; it was made up in sterile diluent at a concentration of 500 IU/ml. Two prostaglandin $F_{2\alpha}$ analogues were used to synchronize oestrus in the experiments : ICI 79,939 was injected intramuscularly at a dose of 800 μg and 200 μg 48 hours and 72 hours after PMSG ; ICI 80,996 was injected at a dose of 500 μg 48 hours after PMSG.

Laparoscopy was performed during oestrus and the subsequent day to determine the number of preovulatory follicles, number of corpora lutea and the occurrence of ovulation. The same technique was also used to determine pregnancy between the 40-70th days of gestation. Laparoscopy was performed essentially according to the method described by WISHART (1972) except that the animals were only off food and water for a maximum of 8-18 hours. A mid-ventral laparotomy was performed under fluothane/oxygen anaesthesia following thiopentone induction 3-4 days after oestrus for egg recovery and the determination of ovarian morphology. The animals were off food and water for only 18 hours prior to anaesthesia.

Samples of blood for hormone analysis were taken from the jugular vein into evacuated tubes containing disodium ethylene diamine tetra-acetate or sodium heparin. They were stored in ice, the plasma separated by centrifugation at 4° and stored at — 20° until assayed. Serum luteinizing hormone LH concentration was assayed against an antiserum raised in rabbits and using NIH bovine-LH as a standard (CHESSWORTH and TAIT, 1974). The level of sensitivity of the assay for LH was 0.2 ng LH/ml plasma.

RESULTS

Injection of PMSG and PG during the luteal phase of the cycle

Ovulatory and oestrous response.

Thirty-five heifers were injected with 1500-2000 IU PMSG at different stages of the oestrous cycle followed by 800 μg and 200 μg of ICI 79,939 48 hours and 72 hours later. Twenty-eight of the animals came into oestrus following this treatment: the number of animals showing oestrus on the 3rd, 4th and 5th days following the initial PG treatment was 3, 18 and 5 respectively. Twenty-nine animals had ovulated and the details of the response are given in table 1.

A similar pattern of ovulatory response was observed from a group of animals treated at Cambridge. The mean ovulatory response for animals treated with lower PMSG between D4-7 was 7.43 ± 1.4 (32) compared with 12.3 ± 1.8 (58) for those treated between D8-12 ($P < 0.05$) : for those treated between D13-16 the mean value was 6.94 ± 2.7 (12).

TABLE I

Effect of day of treatment with PMSG on number of ovulations and of unovulated follicles (> 14 mm)

Day of treatment	No. of animals	No. of corpora lutea	No. of animals with unovulated follicle > 14 mm
D5-7	11	5.0 ± 0,96*	9
D8-11	9	10.3 ± 1.10	6
D12-14	9	8.9 ± 0.95	6

* $P < 0.05$.

Further analysis of this latter data indicated that a significantly greater number of animals had an ovulation rate of > 8 or of > 10 when they were treated from D8-12 of the oestrous cycle compared to treatment earlier in the cycle (table 2).

TABLE 2

Variation in distribution of ovulations, eggs recovered and fertile eggs recovered with day of treatment with PMSG

	Day of PMSG treatment		χ^2 value
	4-7	8-12	
Total no. of animals	32	58	
No. with > 8 ovulations	10	43	13.95***
No. with > 10 ovulations	8	36	10.22**
No. with > 6 eggs recovered	11	35	4.58*
No. with > 8 eggs recovered	5	28	8.11**
No. with > 10 eggs recovered	4	19	3.59 NS
No. with > 6 fertile eggs recovered	2	20	7.44**
No. with > 8 fertile eggs recovered	2	15	3.98*

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$.

Egg recovery and fertilisation of eggs.

It was found that a higher number of animals treated between the 8-12th days of the cycle had > 8 of > 10 eggs recovered after operation compared to those

animals treated earlier (table 2). The number of animals that had more than 6 or more than 8 fertile eggs recovered was also greater in those treated on the 8-12th days of the oestrous cycle.

Timing of LH release.

Previous observation indicated that the peak level of luteinizing hormone occurred from 64-72 hours after injection of the prostaglandin analogue ICI 79,939 the mean value being 68.0 ± 1.1 (8) hours (unpublished data). The preliminary data available from animals injected with PMSG and PG during the luteal phase of the cycle indicated that the peakof LH secretion occurs from 41-54 hours after PG treatment (mean 47.6 ± 2.0 hours (5)) : the range of LH values was from 2.2-49.0 ng/ml. The earlier peak of LH after PMSG treatment is probably indicative of the earlier onset of oestrus that occurs in these animals.

Injection of PMSG during the follicular phase of the oestrous cycle

Very few animals were treated in the late luteal and in the follicular phase of the oestrous cycle in the previous experiments since the prostaglandin analogue is only effective in synchronizing oestrus when given between the 4th and the 16th days of the cycle. There was a suggestion however that the ovulatory response might be slightly lower during the follicular phase of the cycle than that seen on D8-12 but that the variation might also be greater. An attempt was made to examine whether it would be possible to control ovulation when PMSG was given on D15 or D16 of the oestrous cycle.

Ovulatory response.

A total of 64 HF cows were treated with PMSG on D15 or on D16 of the oestrous cycle, and the number of preovulatory size follicles was determined by laparoscopy during the ensuing oestrus. In addition, 42 of the animals were given 500 μg of the prostaglandin analogue ICI 80,996 48 hours later. The results of this trial are set out in table 3. There was no difference between the two treatments in the number

TABLE 3

Effect of different treatments on distribution of follicle numbers

	PMSG	PMSG + PG	χ^2 value
Total number of animals	22	42	
No. with > 2 follicles	15	31	NS
No. with 2 follicles.....	2	16	4.48*
No. with 2 or 3 follicles	5	21	3.39*

* $P < 0.05$.

of animals that superovulated. There were however a greater number of animals having only 2, or 2 or 3 follicles when PMSG and PG treatment was combined indicating that there had been an alteration in the ovarian response.

Oestrous response.

There was a very high incidence of oestrus following PMSG treatment and the pattern of the occurrence of oestrous behaviour is set out in table 4. It was found that the combination of prostaglandin and PMSG treatment caused a better grouping of the oestrous behaviour : 34/42 animals showed oestrus 4-5 days after PMSG and PG treatment compared with 8/21 treated with PMSG alone.

TABLE 4

Effect of treatment on the pattern of oestrous behaviour

	PMSG	PMSG + PG	χ^2 value
Total number of animals	22	42	
No. in oestrus.....	21	40	NS
3 days after PMSG	5	5	NS
4 days after PMSG	8	25	2.24
5 days after PMSG	0	9	10.82***

*** P < 0.001.

Timing of ovulation.

It had been found that ovulation occurred between 79-91 hours after the injection of ICI 79,939 (unpublished data) and that the analogue 80,996 also caused ovulation at a similar time following treatment. In the present study it was found that the first ovulation had occurred in some animals as early as 52 hours after PG (*i. e.* 102 hours after PMSG) but in others no ovulation had occurred as late as 110 hours after PG (table 5). The two animals in the 100 + hour period were possibly

TABLE 5

Incidence of first ovulation after PMSG and PG treatment

	Hours after PG injection (1)						
	40+	50+	60+	70+	80+	90+	100+
Animals with 1 or > 1 ovulation	0	4	3	4	2	4	1
Animals not ovulating	2	6	7	9	2		1

(1) PMSG injected 48 hours prior to PG injection.

not typical in that they had a delayed oestrus following PG treatment. Between 50-80 hours after PG about 50 p. 100 of the animals had one or more ovulation and, although only a few observations were made later than 90 hours, it would appear that ovulation had started in most animals by that time. It was not possible in this study to say when ovulation had been completed in these animals.

DISCUSSION

The availability of prostaglandin and of prostaglandin analogues have made it possible to vary experimentally the length of the oestrous cycle of the cow. The use of PMSG and PG in combination during the luteal phase of the oestrous cycle indicate some of the factors responsible for the ovarian response following superovulatory treatment. The main finding from the present study was the differing response of the ovarian follicle population during the different stages of the oestrous cycle. In two separate experiments it was found that the highest ovulatory response was obtained from a greater number of animals during the 8-12th days of the oestrous cycle. The number of animals with a high number of eggs recovered and of fertile eggs recovered was also significantly greater during this period. ELSDEN *et al.* (1974) reported that a combined PMSG and PG used during the mid-luteal phase of the cycle was more effective in terms of oestrous and ovulatory response compared to PMSG given on the 16th day of the cycle. There are apparently two waves of follicular growth in the cow occurring at the beginning of the oestrous cycle and at the 12-14th day of the cycle (RAJAKOSKI, 1960 ; MARIANA and NYUGEN HUY, 1973). The latter authors also demonstrated that PMSG treatment on D6 and on D16 of the cycle did not alter the proportion of the different types of follicles seen within the ovary (MARIANA and NYUGEN HUY, 1973). HAY and MOOR (1973) showed that PMSG injected during the luteal phase of the sheep stimulated the growth and the production of oestrogen from all follicles. The response could be due to the action of PMSG on the medium-sized follicles in the ovary. Other factors may be acting for HENRICKS *et al.* (1973) found that PMSG had a luteotrophic action on the corpus luteum and that the degree of superovulatory responses is related to the area common to the plasma progesterone/oestrogen curves. This finding would suggest that the elevated progesterone level during the mid-luteal phase and its interaction with the increasing follicular oestrogen production may in part be responsible for the increased ovulation response during the 8-12th days of the cycle.

The combination of PMSG and PG treatment during the follicular phase of the oestrous cycle gave a better synchronization of oestrous than when PMSG is used alone. ELSDEN *et al.* (1974) found that the number of animals showing oestrus was greater when PMSG and PG was injected during the luteal phase compared to PMSG given alone during the follicular phase of the cycle. Although PG is only effective in causing luteolysis when given between D4 and D16 (TERVIT *et al.*, 1973) its use later in the oestrous cycle will limit the degree of luteotrophic action of PMSG (HENRICKS *et al.*, 1973) and thus lead to a better synchronization of oestrus. The enhanced oestrous response and behaviour may be responsible for the more controlled ovulation rate that was seen in these experiments.

Most experiments reported to date have used an interval of 2 days between the use of PMSG and PG with the exception of TERVIT *et al.* (1973) who comment that an interval of 1 or 2 days gave essentially similar superovulatory responses after PMSG treatment. If the superovulatory response does in fact depend upon the interaction of the progesterone/oestrogen balance following treatment then further studies with a variable interval between PMSG and PG would need to be conducted to elucidate this mechanism.

This paper has shown that the combined use of PMSG and PG has greatly enhanced the research into the control of superovulation. The time of the cycle during which their use is most effective in causing superovulation has been demonstrated, and therefore should aid the research on superovulation and synchronisation of the donor animal in studies concerned with egg transfer techniques. The use of PG with PMSG during the follicular phase of the oestrous cycle appears to be a way of controlling multi-ovulation in the cow and thus possibly a step towards controlled multiple pregnancy in the cow.

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RÉSUMÉ

PROSTAGLANDINES ET SUPEROVULATION CHEZ LA VACHE

La synchronisation des œstrus avec les prostaglandines a permis l'utilisation de PMSG à différents moments du cycle œstrien chez la Vache. Le traitement avec PMSG après le 8e jour du cycle donne des taux moyens d'ovulation plus élevés qu'avec un traitement donné plus tôt dans le cycle, un nombre plus important d'animaux ayant un grand nombre d'œufs fécondés et récupérés. La combinaison PMSG + PG diminue le degré de synchronisation des ovulations par rapport à celui obtenu avec les prostaglandines seules. Dans un travail récent, il a été étudié la façon avec laquelle le traitement avec prostaglandines doit être utilisé pour contrôler le nombre d'ovulations après traitement PMSG le 15e jour du cycle. Les méthodes possibles d'utilisation des traitements PMSG et prostaglandines ensemble pour contrôler la superovulation chez la Vache sont discutées.

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