

PLASMA PROGESTERONE LEVELS IN LACTATING EWES AFTER HORMONE-INDUCED OVULATION DURING THE NON-BREEDING SEASON

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OPSOMMING: PLASMA PROGESTERONPEILE BY LAKTERENDE OOIE NA OVULASIE AS GEVOLG VAN HORMOON-TOEDIENING GEDURENDE DIE NIE-TEELSEISOEN

Bronstigheid, ovulasie en periferere plasma-progesteronkonsentrasies is bepaal by 58 lakterende Suid-Afrikaanse Vleismerino-ooie wat verskillend met progestogeen, dragtige merrieserum (DMS), prostaglandien en gonadotrofiëse vrystellingshormoon (GnVH) behandel is. Die ooie is by 2 post partum-intervalle gedurende die nie-teelseisoen behandel. Ovulasie wat by 46 ooie plaasgevind het, het geen betekenisvolle variasie tussen die 6 hormoonbehandelings getoon nie. Slegs 7 ooie wat beide progestogeen en DMS ontvang het, is bronstig gevind. By hierdie behandeling het die plasmaprogesteroonpeile ooreengestem met dié wat vir spontane estruskringlope by nie-lakterende ooie gerapporteer is. Ooie wat progestogeen tussen 2 gespaseerde inspuitings van DMS ontvang het, het 'n normale duurt, maar 'n verlaagde piekkonsentrasie (1,40 ng/ml) van progesteronproduksie getoon. 'n Hoe persentasie van die ooie wat met DMS alleen (60%) of GnVH alleen (70%) behandel is, het subnormale piekkonsentrasies en verkorte periodes van verhoogde plasmaprogesteroon gehad. Hierdie subnormale progesteronproduksie is nie teegewerk deur inspuiting van DMS tweemaal per dag vir 16 dae na GnVH nie. Prostaglandien het klaarblyklik geen luteolitiese effek gehad by sekere ooie waar funksionale corpora lutea a.g.v. DMS toediening verkry is nie. Daar was geen beduidende verskille wat betref bronstigheid, ovulasie of progesteronproduksie tussen ooie wat of 22 of 35 dae na partus behandel is nie.

SUMMARY:

Oestrus, ovulation and peripheral plasma progesterone concentrations were recorded in 58 lactating South African Mutton Merino ewes treated variously with progestagen, pregnant mare serum gonadotrophin (PMSG), prostaglandin and gonadotrophin releasing hormone (GnRH) at 2 post partum intervals (22 or 35 days post partum) during the non-breeding season.

Ovulation was induced in 46% ewes, with no significant variation between 6 different hormone treatments. Only 7 ewes (78%), all treated with progestagen and PMSG, were detected in oestrus.

Plasma progesterone levels in ewes treated with progestagen and PMS were similar to those reported for spontaneous oestrous cycles in non-lactating ewes. Ewes treated with progestagen between 2 spaced injections of PMSG showed a normal duration of progesteron production, but reduced peak concentration, viz. (1,40 ng/ml). A high proportion of ewes treated with PMSG alone (60%) or GnRH alone (70%) showed subnormal peak progesterone concentrations and shortened periods of elevated plasma progesterone. This subnormal progesterone production was not counteracted by twice-daily injections of PMSG for 16 days after GnRH injection. Prostaglandin appeared to have no luteolytic effect in some ewes in which functional corpora lutea had been induced by PMSG injection.

There were no significant differences in oestrus, ovulation or progesterone production between ewes treated 22 and 35 days post partum.

When ewes lamb in spring they usually commence sexual activity at a later stage post-partum than where lambing occurs in autumn (van Niekerk & Mulder, 1965) and attempts to induce rebreeding in lactating ewes shortly after parturition during the non-breeding season have generally been unsuccessful. Ovulation and oestrus may be induced by treatment with progestagen and pregnant mare serum gonadotrophin (PMSG), but impaired fertilization (Dawe & Fletcher, 1976) and embryo survival (Cognie, Hernandez-Barreto & Saumande, 1975) result in poor conception rates until 7 or 8 weeks post partum.

Although the causes of early post partum reproductive failure have not been clearly defined, several instances of hormonal imbalance have been reported. Luteinizing hormone (LH) release after treatment with progestagen and PMSG is lower in lactating than in non-lactating ewes (Pelletier, 1976). Prolactin is released in response to the suckling stimulus (Lamming, Moseley & McNeilly, 1972), and this may inhibit LH releasing hormone (Luw,

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Lishman, Botha, Arangie, Poultney & Gunter, 1976). Progesterone production, too, may be impaired, though the evidence is based on visually abnormal corpora lutea (Restall, Kearins, Hurdegen & Carberry, 1978) rather than on measured progesterone production. Cognie *et al.* 1975) reported that progesterone levels in pregnant ewes were higher 10-14 days after ovulation in lactating than in non-lactating ewes, but progesterone production following ovulation induced by early post partum treatment with progestagen and PMSG has otherwise not been noted.

The experiment reported here was designed to evaluate luteal function in lactating ewes following ovulation induced by various treatments with progestagen, PMSG, prostaglandin and gonadotrophin releasing hormone at 2 intervals after parturition. Particular attention was focussed on the possibility that PMSG treatment might induce inadequate luteal function such as has been observed where GnRH was used in attempts to initiate breeding (Haresign, Foster, Haynes, Crighton & Lamming, 1975; Haresign & Lamming, 1978).

Material and Methods

Animals

Sixty lactating South African Mutton Merino ewes of mixed ages were selected on the basis of time of lambing. Thirty ewes lambed between 11 and 19 September and 30 between 27 September and 3 October 1978, so that they were on average 35 days and 22 days post partum respectively when ovulation was induced by hormone treatment on 18 October. The ewes continued to suckle their lambs and were maintained as a single flock throughout the duration of the experiment.

Treatments

Ewes in each time-of-lambing group were allotted at random to 6 treatment groups each of 5 ewes:

Group (i):

PROG / PMSG – Intravaginal sponges impregnated with 60 mg of synthetic progestagen (“Repromap”, Upjohn) were inserted for an 8-day period beginning on 10 October. A single intramuscular injection of 600 iu PMSG was administered when the sponges were withdrawn on 18 October.

Group (ii):

PMSG / PROG / PMSG – “Repromap” sponges were inserted for an 8-day period beginning on 10 October. Two intramuscular injections each of 600 iu PMSG were administered, the first when sponges were inserted and the second when sponges were withdrawn on 18 October.

Group (iii):

PMSG / PG / PMSG – A single intramuscular injection of 600 iu PMSG was administered on 10 October. On 18 October, a single intramuscular injection of 125 µg of synthetic prostaglandin (“Estrumate”, I.C.I.) was administered, followed immediately by a second intramuscular injection of 600 iu PMSG.

Group (iv):

PMSG – A single intramuscular injection of 600 iu PMSG was administered on 18 October.

Group (v):

GnRH – Three intramuscular injections of synthetic gonadotrophin releasing hormone (GnRH – “Cystorelin”, Abbott), each of 25 µg and spaced at 1,5 hr intervals, were administered on 18 October.

Group (vi):

GnRH / PMSG – GnRH was administered as 3 intramuscular injections, each of 25 µg spaced at 1,5 hr intervals, on 18 October. Two intramuscular injections each of 30 iu PMSG were administered at 0800 hr and 1600 hr each day for 16 days between 19 October and 3 November inclusive.

Observations

Five entire rams fitted with harnesses and marking crayons were put with the ewes on 19 October. Ewes detected in oestrus were recorded and the rams removed on 25 October.

The ovaries of ewes which were not detected in oestrus were examined for evidence of recent ovulation by mid-ventral laparotomy between 25 and 27 October. Corpora lutea which were small or pale in colour were particularly noted.

Blood was taken from the jugular vein of all animals on October 18, 24, 26, 28 and 30, and on November 1, 3 and 7. Plasma was separated by centrifugation and stored at -4°C until required for progesterone assay. Progesterone was assayed by the method of Butcher, Collins & Fugo (1974). The inter-assay coefficient of variation was 16,05%.

Results

Results were taken from 58 ewes. One ewe from the PROG / PMSG (Group (i)) treatment lost its progestagen sponge and one ewe from the PMSG treatment (Group (iv)) died during the experiment.

Results from ewes treated 22 and 35 days post partum, pooled over hormone treatments, are summarised in

Table 1

Effects of post-partum interval on oestrus, ovulation and plasma progesterone

	Mean post-partum interval (days \pm SE)	
	22,3 \pm 0,37	34,6 \pm 0,61
Number of ewes	28	30
Ewes detected in oestrus	3	4
Ewes with corpora lutea*	19	19
Mean ovulation rate*	1,47	1,53
Progesterone production [†] :-		
"None" :		
number of ewes	6	5
peak progesterone (ng/ml \pm SE)	0,64 \pm 0,06	0,62 \pm 0,07
"Normal" :		
number of ewes	12	15
peak progesterone (ng/ml \pm SE)	2,27 \pm 0,31	2,44 \pm 0,26
"Short" :		
number of ewes	8	9
peak progesterone (ng/ml \pm SE)	1,89 \pm 0,17	1,72 \pm 0,15
"Early" :		
number of ewes	2	1
peak progesterone (ng/ml \pm SE)	2,06 \pm 0,21	2,34

* Excluding the PROG / PMS treatment, in which ovulation was not recorded

[†] Defined in the text

Table 1. Post partum interval had no significant effect on oestrus, ovulation or plasma progesterone levels, and there were no significant interactions between post partum interval and hormone treatment. Therefore, results from ewes treated 22 and 35 days post partum have been pooled for subsequent presentation.

The effects of different hormone treatments on oestrus and ovulation are summarised in Table 2. Seven of 9 PROG / PMSG ewes were detected in oestrus and one of the other 2 showed evidence of recent ovulation at laparotomy. None of the 49 ewes in the other treatment groups were detected in oestrus, though 38 ovulated. There were no significant differences between treatments in the number of ewes ovulating or mean ovulation rate.

Four different patterns of progesterone production were recorded during the 20 day period following PMSG or GnRH injection on 18 October. These were defined as: "None" (Plasma progesterone did not increase significantly above the basal level of less than 1.0 ng/ml).

"Normal" (plasma progesterone increased and remained at an elevated level (i.e. above basal) through to day 16 after PMSG or GnRH injection), and

"Short" (Plasma progesterone increased, but did not remain at an elevated level through to day 16 after PMSG or GnRH injection, and

"Early" (plasma progesterone was elevated at the time of the second PMSG injection, but decreased to the basal level by day 12 and remained at this level for at least 6 days).

These patterns of progesterone production, their distribution among the 6 hormone treatment groups, and peak progesterone concentrations (the highest plasma concentration recorded during the 20 day period, usually on days 10 or 12), are presented in Table 2 and Fig. 1.

Eleven ewes, distributed without significant difference among all hormone treatments, showed the "None" pattern of progesterone production. Except for one ewe from Group 1 which exhibited oestrus and was thus not examined for evidence of ovulation these were all ewes which at laparotomy had shown no evidence of recent ovulation.

Some ewes in all treatment groups showed the "Normal" pattern of progesterone production, but the incidence was significantly higher ($P < 0,05$) in PROG / PMSG, PMSG / PROG / PMSG and PMSG / PG / PMSG treatments (72 percent) than in the other 3 treatment groups (21 percent). Peak progesterone concentration among ewes with the "Normal" pattern of production was significantly lower in the PMSG / PROG / PMSG than in other treatment groups. Plasma progesterone remained at a high level on day 20 in 5 ewes which otherwise showed a "Normal" pattern of production (Fig. 1). Three of these in the PROG / PMSG treatment group had mated with entire rams, but one each from the PMSG / PG / PMSG and PMSG groups had not been detected in oestrus.

The "Short" pattern of progesterone production was recorded only in the PMSG, GnRH and GnRH / PMSG treatment groups, but the incidence in these 3 groups was relatively high (77 percent of ewes which ovulated). Within these treatment groups "Short" patterns of progesterone production also had lower mean peak progesterone concentrations than "Normal" patterns (1.8 vs 2,95 ng/ml, $t = 3,71$, $df = 20$, $P < 0,01$).

Only 9 ewes had corpora lutea which were subjectively assessed as small or pale. Three of these had "Normal" patterns of progesterone production, but low peak levels, and the other 6 showed "Short" or "Early" patterns of progesterone production. However, 18 other

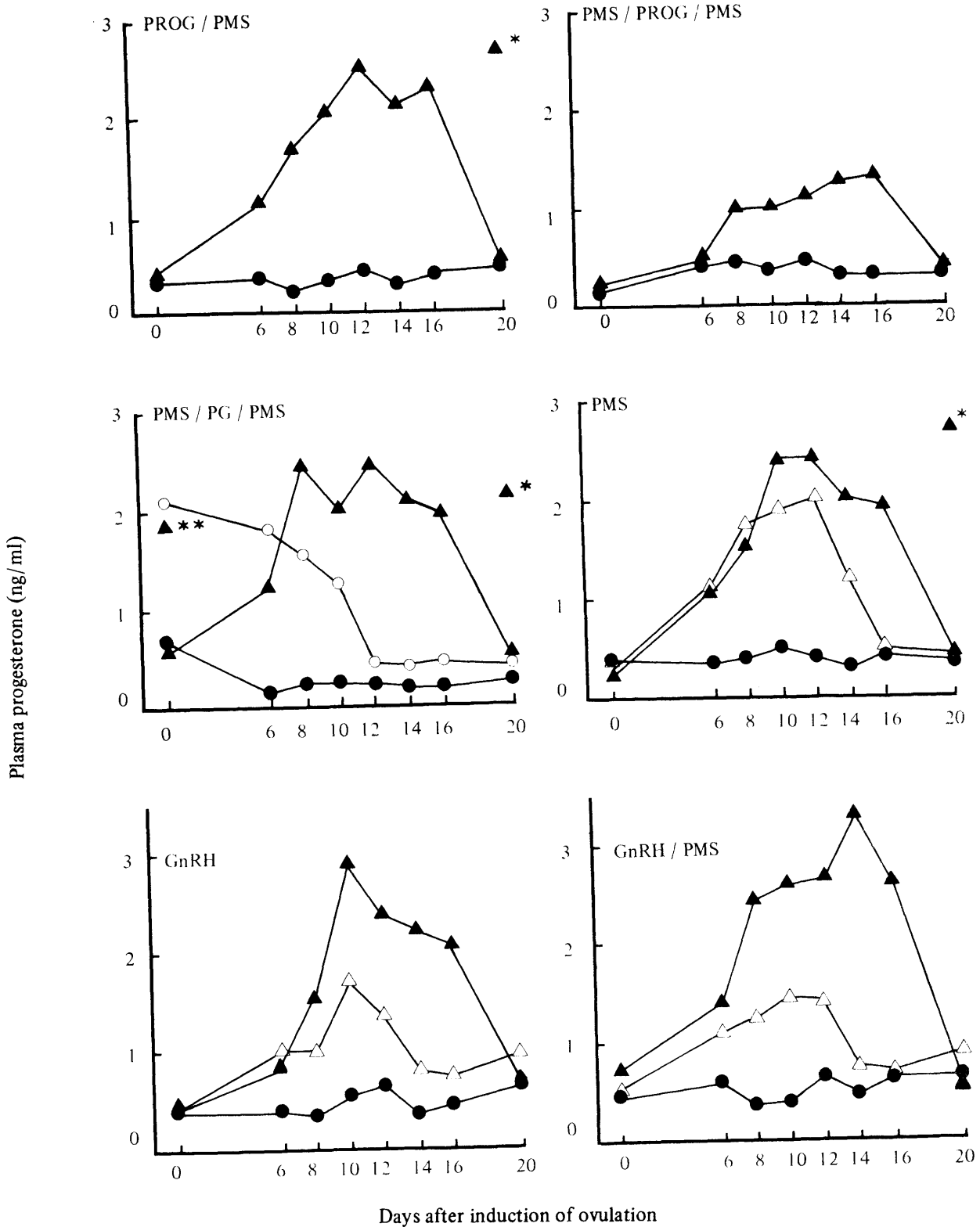


Fig. 1 Plasma progesterone profiles in ewes after ovulation induction during lactation. Where 2 injections of PMS were given the progesterone concentrations refer to samples taken after the second PMS treatment only. The numbers of ewes represented by each curve for "None" (●-●), "Normal" (▲-▲) "Short" (△-△) and "Early" (○-○) patterns of plasma progesterone are presented in Table 2.

** Two ewes which showed elevated progesterone levels at the time of second PMSG injection had "normal" patterns subsequently.

* Three ewes from PROG / PMSG one from PMSG / PG / PMSG, and one from PMSG which showed "normal" patterns of progesterone except that levels remained high on day 20.

Table 2

Effects of hormone treatment on oestrus, ovulation and plasma progesterone

	PROG/PMS	PMS/PROG/ PMS	PMS/PG/ PMS	TREATMENTS PMS	GnRH	GnRH/PMS	P
Number of ewes	9	10	10	9	10	10	
Ewes detected in oestrus	7	0	0	0	0	0	
Ewes with corpora lutea	NR	7	9	7	7	8	NS
Mean ovulation rate	NR	1,14	1,44	1,50	1,29	1,50	NS
Progesterone production :-							
"None"							
number of ewes	1	3	1	1	3	2	NS
peak progesteron (ng/ml \pm SE)	0,56	0,51 \pm 0,02	0,72	0,50	0,74 \pm 0,12	0,75 \pm 0,13	NS
"Normal":							
number of ewes	8	7	6	4	1	1	0,001
peak progesterone (ng/ml \pm SE)	2,62 \pm 0,35	1,40 \pm 0,23	2,68 \pm 0,26	2,53 \pm 0,64	3,32	3,84	0,05
"Short"							
number of ewes	0	0	0	4	6	7	NS
peak progesterone (ng/ml \pm SE)	-	-	-	2,05 \pm 0,16	1,75 \pm 0,12	1,71 \pm 0,24	NS
"Early"							
number of ewes	0	0	3	0	0	0	
peak progesteron (ng/ml \pm SE)	-	-	2,15 \pm 0,15	-	-	-	

NR : not recorded,

NS : not significant

ewes with abnormal patterns of progesterone production or low peak levels had corpora lutea of normal size and colour.

Discussion

The 2 post partum intervals at which ewes were treated in this experiment were both shorter than that at which rebreeding in spring might be expected to be successful (Hunter, 1968), and were selected to investigate changes in the response to hormone therapy within this early period. The fact that there were no significant differences in any of the parameters recorded between ewes treated 22 and 35 days post partum suggests that there may be a relatively abrupt rather than a gradual change to normal reproductive function with increasing time post partum. Although the objective was to investigate changes in response, the post partum stages were selected so that the occurrence of spontaneous ovulations would be minimal. With greater numbers of animals available, it would be desirable to include a greater number of stages so that the changes just prior to the normal onset of oestrus and ovulation can be evaluated.

Although an untreated control group was considered unnecessary under the circumstances of this experiment, obviously spontaneous ovulations could have occurred. However, the progesterone levels on day 0 provide reasonable evidence for the conclusion that ovulations did not take place prior to the initiation of hormonal treatments.

Ewes treated with progestagen and PMSG showed the typical response of lactating ewes treated early post partum – a moderately high incidence of ovulation and oestrus, but low conception. On the evidence of plasma progesterone concentrations remaining elevated instead of returning to basal levels on day 20, a maximum of 3 ewes from this treatment group may have conceived. Eight of 9 ewes showed elevated plasma progesterone concentrations, with a mean peak of 2,6 ng/ml, which were maintained through day 16 after PMSG injection. This is similar to progesterone production recorded by Thorburn, Bassett & Smith (1969) for spontaneous oestrous cycles in non-lactating ewes. Thus there was no evidence from this experiment that impaired conception following early post partum treatment with

progestagen and PMSG was associated with abnormal luteal function.

Two spaced doses of PMSG with progestagen in between (PMSG/PROG/PMSG) were included in the experiment because of preliminary success with this treatment in post partum cattle (Lishman, unpublished). Seven ewes ovulated following the second PMSG injection, but none were detected in oestrus. Further, although corpora lutea had a normal life-span, peak progesterone concentrations were significantly reduced (Fig. 1). If these results can be repeated then a study of the mechanisms involved could perhaps cast some light on the problem of sub-normal luteal function.

Treatment with prostaglandin and PMSG (PMSG/PG/PMSG) was planned on the basis that the initial PMSG injection would induce ovulation and functional corpora lutea, prostaglandin would cause rapid luteolysis, and the second PMSG injection would induce another ovulation. However, this appeared to occur in only 2 of the 10 ewes. Five ewes had basal plasma progesterone concentrations at the time of prostaglandin injection, indicating that the first PMSG injection had failed to induce either ovulation or functional corpora lutea, and there was thus no corpus luteum to be influenced by prostaglandin. The remaining 3 ewes had elevated plasma progesterone concentrations at the time of prostaglandin injection and progesterone levels remained elevated until day 12. This pattern was consistent with normal luteal function following ovulation induced by the first PMSG injection, with no luteolytic effect of prostaglandin. Previous reports on the use of prostaglandin in lactating anoestrous ewes have not been noted, and it is not clear whether the failure of prostaglandin to induce luteolysis was associated with lactation or some other factor. Since 2 of these 3 ewes each had 2 corpora lutea, the dose of prostaglandin (125 μ g) may have been too low (Greyling & van der Westhuysen, 1979).

A high proportion of ewes treated with with PMSG or GnRH ovulated, but in most cases mean peak progesterone concentrations were subnormal, and elevated progesterone levels were not maintained beyond 12 or 14 days after injection. This was similar to responses to GnRH recorded in non-lactating anoestrous ewes by Haresign *et al.* (1975) and Haresign & Lamming (1978). Differences in response to PMSG and GnRH were not significant, but the GnRH treatment tended to induce a higher incidence of "Short" patterns of progesterone production, and both a lower peak concentration and shorter duration of elevated progesterone in these "Short" patterns, than the PMSG treatment.

Twice-daily injections of PMSG for 16 days after GnRH injection had no effect on either the duration or peak concentration of elevated plasma progesterone. The dose or frequency of injection of PMSG may have been insufficient to have a luteotrophic effect. Alternatively, subnormal luteal function may have been predetermined at, or soon after, ovulation and not subject to subsequent influence by luteotrophin. A third possibility lies in the evidence of Denamur, Martinet & Short (1973) that prolactin and LH are both necessary for maintenance of the ovine corpus luteum, and that LH by itself is ineffective. Since prolactin release associated with suckling declines between the second and fifth weeks post partum (McNeilly 1971), there may have been insufficient prolactin release at the post-partum intervals of this experiment to induce a luteotrophic effect with injected PMSG.

The physical appearance of corpora lutea did not provide a reliable indication of progesterone production. All 9 ewes with abnormal corpora lutea (small size or pale colour) showed either reduced peak progesterone concentration or shortened duration of progesterone production, but this also applied to a further 18 ewes in which no visible abnormalities of corpora lutea were recorded.

In spring the resumption of oestrous activity is usually preceded by silent ovulation on one or more occasions prior to the first overt oestrus (Hunter & Lishman, 1967). In the present experiment the high incidence of silent ovulations (38 out of 49 ewes) was thus at least partly due to the treatment regimes applied. However, in the 2 groups where the ewes received 2 injections of PMSG at an interval of 16 days oestrus should have been observed in at least some of the ewes. No acceptable explanation could be found although the poor luteal activity in Group (ii) and the failure to induce luteolysis amongst some ewes Group (iii) may have contributed to the result obtained. In recent years significant advances have been made in the understanding of the mechanisms involved in oestrus and ovulation in the cycling ewe, in contrast, many questions regarding the induction of early re-conception in ewes which lactate during the non-breeding season remain unanswered. The findings reported here perhaps serve only to highlight some of the problems involved.

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