LUTEAL FUNCTION DURING EARLY POST PARTUM IN THE EWE

by

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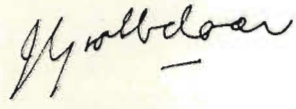
D E C L A R A T I O N

I hereby declare that the results contained in this thesis are from my own original work and has not been previously submitted by me in respect of a degree at any other university



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C O N T E N T S

GENERAL INTRODUCTION

The first step towards the improvement of reproductive performance in established systems or the development of new systems is dependant on (i) an awareness and an understanding of the various reproduction factors and (ii) the interaction of these factors during the different stages throughout the oestrous cycle. Several methods of controlling reproductive activity through the use of exogenous hormones have been investigated and developed (Mauleon, 1976; Haresign, 1978). The majority of these methods could not be successfully applied in practice or gave variable results when applied during early post partum. Because of an intimate association with the reproduction process, a knowledge of the hypothalamic control of anterior pituitary activity, and the luteotrophic action of pituitary hormones must play an important role in developing new methods to manipulate reproductive performance. The results obtained in different studies using a given species cannot be readily extrapolated to other species or to individuals of the same species under different physiological conditions. In sheep, oestrous cycle regulation is complicated further by the seasonality of the sexual activity of the ewe, and to a lesser extent that of the ram (Follet, 1978; Thimonier, 1981). There are large differences between sheep breeds in this respect. The reproductive endocrinology of the early post partum period in sheep breeds maintained under sub-tropical conditions remains to be fully characterized.

An important development in measuring the hormones of reproduction was the development of radioimmunoassays (Niswender, Reichert, Midgley & Nalbandov, 1969; Butcher, 1977). These assays enable the researcher to accurately determine hormonal blood levels of gonadotrophins and gonadal steroids thereby providing a means by which endocrine changes

can be monitored. The discovery that hypothalamic extracts posessed LH and FSH releasing activities led to the identification of the decapeptide structure of gonadotrophin releasing hormone in 1971 (Matsuo, Baba, Nair, Arimura & Schally, 1971). Soon afterwards the molecule was synthesized (Geiger, König, Wissmann, Geisen & Enzmann, 1971). Since then a number of laboratories have prepared a synthetic decapeptide of similar structure to the naturally occurring compound and which has been shown to release both LH and FSH in a number of species. Through the use of GnRH, ovulation or, at least, the surgelike release of the gonadotrophins could be evoked, and this gave new momentum to the study of events during the peri-ovulatory period and also of the ensuing luteal phase. Unfortunately, GnRH-induced ovulation cannot be successfully applied as a new system to regulate reproductive cycles, especially during seasonal- and post partum anoestrous periods. The primary reason is that corpora lutea resulting from GnRH-induced ovulations are either short lived, or the levels of progesterone secretion never attain those associated with normal luteal function (Haresign, 1975). Both these phenomena can be classified as inadequate luteal function. These findings gave new impetus to research on early post partum breeding and focussed attention on events occurring shortly before ovulation and for several days thereafter.

The situation is not completely divorced from the first ovulation after sexual rest. When breeding is restimulated via ram introduction or the use of exogenous hormones a proportion of ewes show inadequate luteal function. This leads to a "wastage" as regards reproduction efficiency. The inadequate luteal function in response to GnRH could be due to inadequate gonadotrophin priming (McGovern & Laing, 1976; Haresign & Lamming, 1978), inadequate steroid priming (Ainsworth, Lachance & Labrie, 1982; Sheffel, Pratt, Ferrel & Inskeep, 1982)

or a deficient luteotrophic effect after ovulation (Barnes, Martinez-Castellano, Kazmer, Wade & Halman, 1982). The use of GnRH as a means to manipulate the breeding cycle of ewes can be enhanced by exogenous hormone treatment which is an absolute prerequisite for the successful practical application of GnRH in the control of reproduction cycles (Haresign, Foster, Haynes, Crighton & Lamming, 1975). The physiological state which prevails during early post partum and lactation in the ewes creates a particularly unfavourable endocrine environment for the manipulation of ovulation, especially when combined with seasonal anoestrus (Haresign, 1978). This is becuase many endocrine parameters are depressed due to an interaction between post partum x lactation x presence of young and season (Mauleon, 1976). Much of the success that has been achieved in the field of controlled breeding has been due to the recognition of these physiological states. Equally important is the ability to recognize the limitations imposed by each physiological state on the artificial manipulation of the breeding cycle and to modify the techniques accordingly. There must then be a system for every set of circumstances or as aptly summed up by Mauleon (1975): "During these years and even now, those decieved have been those who have believed in finding a universal method when it is and must be a reasoned method." These words apply to all new techniques of controlled breeding.

The object of this study was to clarify some of the many problems that preclude the successful use of GnRH as a technique for the control of the breeding cycle in the ewe.

REVIEW OF LITERATURE

The discovery and identification of the decapeptide structure of GnRH by Matsuo et al. (1971) and the synthesis of the molecule (Geiger et al., 1971) opened up a new field of research in endocrinology. This discovery also created new possibilities of controlling the sexual cycles of farm animals.

Today, the ability of synthetic GnRH to induce release of LH and FSH from the ovine pituitary is well established (Arimura, Debeljuk, Matsuo & Schally, 1972; Crighton & Foster, 1972; Foster & Crighton, 1973; Jonas, Salmonsen, Burger, Chamley, Cumming, Findlay & Goding, 1973; Jenkin & Heap, 1974; Ripple, Moyer, Johnson & Mauer, 1974; and many others). The time has arrived for the decapeptide to play an ever increasingly important role in the regulation of reproductive efficiency (Hansel & Convey, 1983).

GnRH AND LUTEAL FUNCTION

The use of GnRH to induce ovulation has progressed through the application of a single injection to a more prolonged administration. The latter probably more closely mimicking the natural situation. Ovulation was induced when GnRH was administered as a single injection to anoestrous ewes (Foster & Crighton, 1973; Haresign, 1975; Haresign et al., 1975; Jenkin, Heap & Symons, 1977; Restall, Kearins & Starr, 1977; Haresign & Lamming, 1978; Wright, Jenkin, Heap & Walters, 1978; Fletcher, Lishman, Thring & Holmes, 1980; McNeilly, Hunter, Land & Fraser, 1981; Ainsworth et al., 1982; Wheaton, Recabarren & Mullett, 1982). The effect of a single injection of GnRH often results in a too low level of luteal function principally as a result of short-lived corpora lutea (Haresign et al., 1975), not only in ewes but also in cattle (Webb, Lamming, Haynes, Hafs & Manns, 1977;

Lishman, Allison, Fogwell, Butcher & Inskeep, 1979; Van der Westhuysen, Coetzer & Greyling, 1980). The use of GnRH as a single injection for the induction of cyclic ovarian activity during early post partum in the ewe (Ainsworth et al., 1982) and in Angora and Boer goat does (Van der Westhuysen, 1979) under practical farming conditions is thus precluded. GnRH injections repeated at short intervals (2 hourly) during post partum anoestrus in an attempt to cause the release of larger quantities of LH over a longer period and simulate the pre-ovulatory LH release more closely were successful and resulted in the formation of corpora lutea in a greater number of ewes than did a single injection (Restall et al., 1977). Fletcher et al. (1980) administered three GnRH injections spaced at 1,5 hour intervals and found that 70% of the ewes ovulated, but this treatment failed to counteract subnormal luteal function. The administration of GnRH every 2 hours for 8 days resulted in ovulation with normal luteal function in all treated ewes (McLeod, Haresign & Lamming, 1982a), but in only 5 out of 20 ewes treated with smaller doses at 2 h intervals for 48 hours (McLeod, Haresign & Lamming, 1982b). A regime whereby GnRH was given as a pulse every 2 hours for 43 - 80 days successfully induced cyclical progesterone activity in anoestrous Romney ewes (McNatty, Ball, Hudson, Gibb & Thurley, 1982a). Ovulations and almost normal luteal function were recorded by Skubiszewski, Przekop, Wolinska, Stupnicka, Wroblewska & Domanski (1982) during mid-anoestrus in ewes injected over 6 consecutive days with small daily doses of GnRH culminating in a dose of 1,5 µg on Day 6.

In post partum beef cows the administration of 500 ng GnRH every 2 h for 4 days resulted in ovulations in response to the releasing hormone, but a large percentage of cows exhibited oestrous cycles of shorter duration than 21 days (Walters, Short, Convey, Staigmiller,

Dunn & Kaltenbach, 1982). At first, the indications were that the pituitary can become refractory to repeated stimulation by GnRH (Crighton, Scott & Foster, 1974; Crighton, Foster, Haresign & Scott, 1975), but McLeod et al. (1982b) suggested that the phenomenon of "down-regulation" may well be the result of using too high a dose-level of GnRH.

Infusion of GnRH resulted in subnormal luteal function (Crighton et al., 1975), and in progesterone levels that were always lower than those recorded during the normal breeding season (Shareh, Ward & Birchall, 1976). If the normal frequency of GnRH secretory episodes is increased from one episode every 3,6 hours to at lease one every 2 hours, cyclic ovarian activity can be restored to seasonally anoestrous sheep (McNatty, Ball, Gibb, Hudson & Thurley, 1982b). This was accomplished by i.v. infusion overy 110 seconds with a total of 500 ng GnRH being given every 2 hours. Infusion of small doses of GnRH for 6 hours per day over 6 days resulted in corpora lutea functioning for 7 days only (Skubiszewski et al., 1982).

Administration of GnRH, preceded by PMSG treatment and thereby exposing the pre-ovulatory follicle to a gonadotrophic stimulus, produced corpora lutea capable of increasing peripheral plasma progesterone concentrations (McGovern & Laing, 1976) although the concentrations are lower than natural mid cycle values (Haresign & Lamming, 1978). In Hereford heifers pretreatment with FSH for 3 days prior to GnRH administration had no effect on the occurrence or lifespan of the induced CL (Lishman et al., 1979). The subnormal luteal function following a single GnRH injection could not be counteracted by small (60 I.U.) twice daily injections of PMSG for 16 days after GnRH administration (Fletcher et al., 1980).

Serial measurements of oestradiol, progesterone and LH around the

time of ovulation in the ewe have demonstrated that the maximum secretion of oestrogen from the pre-ovulatory follicle precedes the LH surge by 12 - 24 h (Scaramuzzi, Caldwell & Moor, 1970). The other steroids secreted by the pre-ovulatory follicle may also act synergistically with oestradiol in inducing ovulation (Baird & Scaramuzzi, 1976). In spite of the evidence quoted above, steroid pretreatment does not consistantly enhance luteal function after GnRH administration. Lewis, Lishman, Butcher, Dailey & Inskeep (1981) could not demonstrate a luteotrophic effect in ewes, pretreated with progestagen impregnated intravaginal pesseries, after a single GnRH injection. McLeod et al. (1982b), recorded a highly significant luteotrophic effect of progesterone priming followed by a multiple injection regime of GnRH. These workers also recorded pregnancy rates of up to 50% in seasonally anoestrous ewes after progesterone treatment followed by GnRH infusions. Ovulation occurred in all the ewes and all but one ewe displayed overt oestrus. Webb et al. (1977), administered 500 ng GnRH to suckled beef cows 20 - 30 days post partum and a second injection 10 days later when the transient rise in plasma progesterone had returned to basal values. The second injection induced normal cyclic progesterone values. Ainsworth et al. (1982), preclude the use of a single injection of GnRH for the successful induction of cyclic ovarian activity without progesterone pretreatment. Oestrogen priming (i.m. injection) and GnRH administration resulted in poor luteal function (Hamilton, Lishman & Lamb, 1979), but in beef cows pretreatment with subcutaneous oestradiol implants eliminated the problem of short oestrous cycles when a multiple GnRH injection treatment was applied (Walters et al., 1982).

The short-term suppression of prolactin does not affect the incidence of ovulation or corpus luteum progesterone production in GnRH treated anoestrous ewes (McNeilly & Land, 1979). The possibility that elevated

levels of prolactin could suppress luteal activity did exist as evidenced by the results of Rhind, Chesworth & Robinson (1978) who reported a reduced output of progesterone by the CL of pregnant ewes at times when serum levels of prolactin were seasonally elevated.

PITUITARY RESPONSE TO GnRH

The pituitary responsiveness is such that a single GnRH injection induces the release of LH in all treated ewes (Haresign et al., 1975), but the total volume released is significantly less than that observed at a natural oestrus (Foster & Crighton, 1975) and amounts to approximately 25% of the total release found at natural oestrus (Haresign & Lamming, 1978). The attempts by Haresign et al. (1975) to augment the induced LH release by increasing the dose of GnRH from 150 µg to 300 µg, failed, although Wheaton et al. (1982) have demonstrated a dose response to GnRH. Thus, in beef cows Webb et al. (1977) recorded a positive linear relationship between dose of GnRH and the area under the LH peak. Treatment with 1 000 ng GnRH resulted in a more sustained rise in plasma LH than 250 and 500 ng GnRH (McLeod et al., 1982a). The pattern of LH secretion also differs significantly with the dose of GnRH used (McLeod et al., 1982b). GnRH injections repeated at short intervals (two hourly) during post partum anoestrus resulted in the release of larger quantities of LH over a longer period (Restall et al., 1977) and similar trends were recorded for post partum beef cows (Walters et al., 1982). In seasonally anoestrous ewes, GnRH injections given at longer intervals (24 h to 48 h) resulted in a rapid decrease of LH secretion after the initial injection. However, if GnRH was administered 96 h later it resulted in a LH release similar to the initial surge (Rippel, Johnson & White, 1974).

The conclusion of Webb, England & Fitzpatrick (1981) that gonadotrophin release from the pituitary gland requires the continual presence of GnRH during the ascending limb of the pre-ovulatory gonadotrophin surge was supported by McLeod et al. (1982a) in an experiment where the administration of GnRH at 2 hour intervals resulted in LH peaks followed by ovulation and overt oestrus. Infusing GnRH in small doses (30 µg total dose over 6 days) led to pre-ovulatory LH peaks most of which were lower than those occurring in naturally ovulating animals (Skubiszewski et al., 1982).

Exogenous treatment with gonadal steroids can alter the pituitary response to GnRH treatment. Progesterone administration for 3 weeks (100 mg/day) or oestradiol (250 µg/day) plus progesterone resulted in a marked decrease in pituitary responsiveness to GnRH injection on the last day of those very high levels of steroid treatment (Wright et al., 1978). Implants containing 375 mg of progesterone also diminished the pituitary responsiveness to GnRH (Wheaton & Mullett, 1982). The work of Quirke, Jennings, Hanrahan & Gosling (1979) indicated that progesterone treatment resulted in sufficient release of LH in response to GnRH to trigger ovulation immediately, in a large proportion of ewes. These findings were complemented by the work of Lewis et al. (1981), using physiological levels of progesterone. Cumming, Buckmaster, Cerini, Cerini, Chamley, Findlay & Goding (1972) made an identical conclusion 9 years previously. However, Wheaton et al. (1982), could not detect any significant increase in LH release in response to GnRH after progesterone pretreatment.

Poultney, Lishman, Louw, Botha & Arangie (1977) reported a positive effect of oestrogen priming prior to GnRH on pituitary responsiveness, both on Day 2 and 15 of the oestrous cycle. Generally, oestrogen administration as a single dose (Haresign & Lamming, 1978; Irvin,

Pflantz, Morrow, Day & Garverick, 1981a) or as divided doses enhanced pituitary responsiveness in terms of LH release after GnRH administration. Wheaton et al. (1982) demonstrated a greater FSH release relative to LH (with no positive effect for LH) using low doses of GnRH and oestradiol pretreatment, while Hoagland (1980) did not record an altered LH response to GnRH infusion 24 h after oestradiol treatment. Under grazing conditions, lambs on oestrogenic clover were found to be more sensitive to GnRH than lambs on non-oestrogenic pastures (Bindon, Adams & Piper, 1982).

As regards gonadotrophin priming prior to GnRH, Lishman et al. (1979) reported that pretreatment with FSH did not alter the pattern of release or maximum concentration of LH.

Both Louw, Lishman, Botha, Arangie, Poultney & Gunter (1976) and McNeilly & Land (1979) found no increase in LH secretion in response to GnRH after suppressing prolactin.

DEFICIENCIES IN PITUITARY LH

The stage of production seems to affect pituitary responsiveness to GnRH so that during pregnancy, the pituitary response decreased progressively with advancing gestation (Jenkin et al., 1977; Wright, Jenkin & Heap, 1981b). In ewes, the LH release on Day 25 post partum was greater than that on Day 12 (Restall et al., 1977). The GnRH induced release of LH and the pituitary content of LH increased with time after parturition (Crowder, Gilles, Tamanine, Moss & Nett, 1982) and returned to values similar to luteal-phase levels of the normal cycle by 21 days post partum (Wright et al., 1981b). In suckled beef cows the LH response to GnRH is not fully restored until 15 - 16 days post partum (Irvin, Zaied, Day & Garverick, 1981b) and weaning further increases the pituitary responsiveness to GnRH (Walters

et al., 1982).

Season seems to affect the pituitary response to GnRH in sheep. Knipe (1981) reported no difference in peak LH plasma concentration and total area under the curve among autumn-lambing ewes at any post partum stage when compared to ewes cycling spontaneously in autumn. Among spring-lambing ewes, however, LH release was significantly lower in early than in late lactation.

THE LUTEOTROPHIC PROCESS

Rothchild (1966) defined the luteotrophic process in the non pregnant mammal as one which promotes the growth of the corpus luteum, and a rate of progesterone secretion, at lease sufficient to prevent ovulation and/or to permit implantation to occur. The formation of the corpus luteum and its subsequent secretory activity are the result of the trophic action of a number of pituitary hormones. The survival of the corpus luteum depends on the outcome of a battle between 2 opposing forces: on the one hand those of the pituitary and the embryo acting in the direction of survival; on the other the uterus and its ally, the follicle acting to cause its dissolution (Denamur, 1974).

Twenty years ago, Short, McDonald & Rowson (1963), failed to demonstrate a convincing luteotrophic action (in vivo) for any of the gonadotrophin hormones LH, FSH and prolactin, even when given in extremely large doses. However, a temporary but small increase in progesterone secretion was observed after injection of LH, FSH and PMSG (Short et al., 1963). In contrast, Domanski, Skrzeczkowski, Stupnicka, Fitko & Dobrowolski (1967), showed that both LH and prolactin, but not FSH stimulate progesterone secretion. Infusion of LH (Cook, Kaltenbach, Niswender, Norton & Nalbandov, 1969) stimulated the rate

of progesterone secretion by increasing both the rate of ovarian blood flow and the concentration of the steroid in the plasma. FSH produced a similar but less pronounced effect, but prolactin had no effect. In ewes with transplanted ovaries McCracken, Uno, Goding, Ichikawa & Baird (1969) demonstrated an increased steroid secretion in response to LH infusions, but recorded no effect of FSH and prolactin on steroid secretion rate. Although the ovarian autotransplant in the ewe is very suitable for the study of the direct local effects of gonadotrophins on ovarian activity, transplantation does prolong the luteal phase (Goding, McCracken & Baird, 1967). Results pertaining to an ovary in which the corpus luteum persisted, may be applicable only to corpora lutea of such nature and should be interpreted as such (Baird & Collet, 1973). Repeated infusions of LH (Collet, Land & Baird, 1973), and HCG in ewes in which the ovary was auto-transplanted to the neck resulted in a temporary increase in secretion of progesterone which returned to basal levels within 60 minutes (Baird & Collet, 1973). This confirmed the finding of Armstrong (1968) that the ovary becomes refractory to the steroidogenic effect of LH. Henricks, Hill, Dickey & Lamond (1973) recorded a stimulation of PMSG on luteal function in beef cows, because of a dose-response relationship between PMSG and the length of time that plasma progesterone remained at high levels after Day 16 of the oestrous cycle. PMSG has both LH and FSH-like properties (Lamond, 1960), thus the gonadotrophin probably exerted a luteotrophic effect on the CL (Henricks et al., 1973). After a comprehensive series of experiments whereby gonadotrophins were administered either through i.m. injection or i.v. infusion, Denamur, Martinet & Short (1973) concluded that prolactin and LH are both necessary for the maintenance of the ovine CL, and that these 2 hormones together, make up the "luteotrophic complex". Prolactin on its own has some luteotrophic

activity, but, LH by itself is completely ineffective, and so is FSH (Denamur et al., 1973).

In an attempt to clarify the confusing and often conflicting results obtained in vivo as regards the trophic action of pituitary hormones, attempts were made to determine (in vitro) the trophic effect of these hormones on luteal tissue. One of the first successful attempts to increase the rate of passage of progesterone from luteal slices in vitro was by Legault-Demare, Mauleon & Suarez-Soto (1960, quoted by Kaltenbach, Cook, Niswender & Nalbandov, 1967) who added PMSG to the incubation medium. Kaltenbach et al. (1967), reported that LH consistantly increased progesterone concentration in vitro, but that prolactin had no effect, even in very high doses. The small stimulatory effect of FSH was attributed to LH contamination of the FSH preparation. So successful were attempts to stimulate progesterone secretion in vitro by means of LH that Hansel (1971) described a bio-assay system for LH based on progesterone secretion from luteal slices.

Today it is accepted that LH is the major luteotrophin in the ewe. Conclusive evidence of this relationship was supplied by Kaltenbach, Craber, Niswender & Nalbandov (1968) who showed that LH is necessary for luteal function following hypophysectomy and Karsch, Cook, Ellincott, Foster, Jackson & Nalbandov (1971) who demonstrated that constant infusions of LH, but not prolactin, extended the lifespan of the corpus luteum.

In a review, Niswender, Suter & Sawyer (1981) proposed a model for the steroidogenic effect of LH. Firstly, LH binds to its plasma membrane receptor and initiates a biological response which activates adenylate cyclase and produces cyclic adenosine monophosphate (cAMP). This is followed by the activation of protein kinase, phosphorylation of steroidogenic enzymes and ribosomes followed by enhanced protein

synthesis. All the actions appear to be involved in the modulation of the steroidogenic response of the luteal cell to LH.

The present study was initiated to investigate the role of PMSG and steroids in enhancing the luteal activity of GnRH-treated ewes.

C H A P T E R I

THE EFFECT OF OESTRADIOL AND PMSG INFUSION DURING EARLY POST PARTUM ON LUTEAL FUNCTION AND PITUITARY RESPONSE IN GnRH TREATED LACTATING EWES DURING SPRING

1. INTRODUCTION

More than 10 years have elapsed since Matsuo et al. (1971) discovered GnRH and Geiger et al. (1971) synthesized the decapeptide molecule. Subsequently it was established that although GnRH induced the release of LH and FSH from the ovine pituitary (Arimura et al., 1972; Crighton & Foster, 1972) a single injection often results in subnormal luteal function (Crighton et al., 1975). This would seem to preclude the use of GnRH without other therapy for the induction of reproductive activity in the anoestrous ewe (Haresign et al., 1975), and further research is needed before GnRH can be considered as a practical aid in controlling livestock reproduction (Quirke et al., 1979). The ovarian response to GnRH in terms of luteal activity was enhanced by gonadotrophin stimulation (PMSG) prior to GnRH administration (McGovern & Laing, 1976), but the progesterone secretion was still less than at natural mid cycle (Haresign & Lamming, 1978). These findings together with those of McNatty et al. (1982b) who infused GnRH supports the theory of Haresign et al. (1975) that the lack of a gonadotrophin stimulus prior to GnRH could be the cause of a lower level of luteal function in ewes receiving a single injection GnRH. The results of Piper & Loucks (1974) and those of Piper & Wells (1974) demonstrated heavier corpora lutea and in the latter case also higher ovarian plasma progesterone concentrations in ewes infused and injected with LH during the mid luteal phase of the oestrous cycle. This raised the question of whether PMSG infusion after GnRH

administration would also result in elevated progesterone levels. As regards pituitary response to GnRH in ewes primed with PMSG, Haresign & Lamming (1978) reported a positive effect, but Lishman et al. (1979) could not alter the pattern of release or maximum concentration of LH in beef cows primed with FSH. Follicle development is not only dependant on pituitary hormones, but also on the ovarian steroid hormones (Richards, 1980). It is possible then that the lack of suitable steroid priming prior to GnRH may also be causative in subnormal luteal function after GnRH administration (Ainsworth et al., 1982).

Many endocrine parameters are depressed due to an interaction between post partum, lactation, suckling and season (Mauleon, 1976). The object of this study was to:

(i) Determine the luteotrophic effect of PMSG infusion before and after, and that of E2 plus PMSG before a single GnRH injection in early post partum lactating ewes during Spring.

(ii) To characterize the LH surge in response to GnRH and to determine tonic levels of LH.

2. PROCEDURE

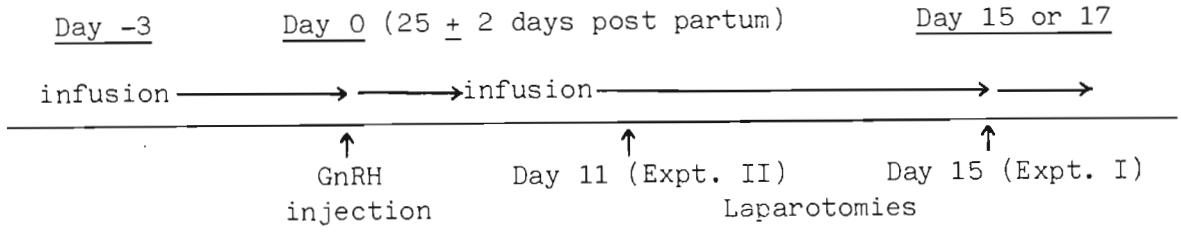
The first of two experiments was conducted during late Spring (November, 1979, Experiment I) and in order to establish any differences in response due to season a second trial was conducted during early spring (August - September, 1980, Experiment II).

Multiparous lactating S.A. Mutton merino ewes, 25 $\pm$ 2 days post partum, were used as experimental animals. The ewes together with their lambs, were housed in individual pens on a raised slattered floor in an enclosed building. The ewes were fed according to NRC standards (1975) on a ration containing 2,71 Mcal ME/kg DM and 11,5% DCP on

a DM basis. The lambs had ad lib access to a creep ration containing 2,5 Mcal ME/kg DM and 9,5% DCP on a DM basis. Both the ewes and lambs had free access to drinking water.

2.1 Experimental layout and treatments

During both experiments all the ewes were infused intravenously with normal saline (0,9% NaCl), as either a control infusion or as a carrier for exogenous hormones. The general experimental procedure can be illustrated as follows:



The treatments applied and infusion schedule are shown in Table I.

TABLE I : Treatments applied and infusion schedule followed during both Expt. I (late spring) and Expt. II (early spring). Each ewe was injected i.m. with GnRH on Day 0.

Group	n	Infusion schedule and treatment
SOS	15	Control. Saline infusion Day -3 to Day 15 or 17*
POS	12	PMSG infusion Day -3 to Day 0, and saline Day 0 to Day 15 or 17
EPOS+	7	PMSG infusion Day -3 to Day 0, and Oestradiol infusion Day -1 to Day 0 and saline Day 0 to Day 15 or 17
SOP	13	Saline infusion Day -3 to Day 0 and PMSG infusion Day 0 to Day 15 or 17
POP	12	PMSG infusion Day -3 to Day 15 or 17

* Day 15 Expt. I and Day 17 Expt. II

EPOS+ only during Expt. II

All the ewes received 1 ml of GnRH (Hoechts - Receptal 1 ml =

0,0042 mg buserelin acetate) on Day 0, which corresponded to 25 ± 2 days post partum. The GnRH injection on Day 0 signalled the change in infusion treatment for the POS, SOP and EPOS groups. The infusion was at a rate of 500 ml saline per 24 h. PMSG was administered at a rate of 200 I.U./24 h. This dose was in a range expected to induce growth of follicles and oestrogen synthesis, but not ovulation. In the EPOS group estradiol was infused at a rate of 50 µg over 24 h, 15 µg during the first 12 h and 35 µg during the second 12 h.

2.2 Infusion technique

The ventral neck area of the sheep was clipped, shaved and disinfected and the skin on the site of infusion infiltrated with local analgesic. Cannulation of the jugular was found to be more efficient when the animal was restrained in the standing position rather than when recumbent. A 2,8 mm (12 gauge) hypodermic needle, 110 mm long, was inserted caudally into the jugular vein and a polyethylene catheter (I.D. 1,4 mm x O.D. 1,9 mm, Intramedic, cat No. 7440) introduced into the lumen of the vein through this needle. Approximately 150 mm of the cannula was passed into the vein, the needle was removed and a 1,422 mm (17 gauge) needle (shortened to 40 mm) was inserted into the exposed end of the cannula. The cannula was then flushed with 1 ml heparinized saline (500 units/ml, 0,9% saline) and a small rubber cap was placed over the hub of the needle. The point of entry of the cannula through the skin was sealed with cotton-wool soaked in flexible collodion (S.A. Druggists) and the shaved area was sprayed with a film of topical antiseptic (Surgispray, Novo Industries). A 50 mm x 50 mm square of adhesive plaster (Elastoplast) was moistened with anaesthetic ether and pressed down firmly over the wound. A 50 mm wide strip of masking tape was then wound around the neck of the sheep to shield completely the point of entry into the skin and

to prevent soiling. After removal of the rubber cap from the hub of the needle, the catheter was connected to a saline bag (1 litre capacity, Viaflex container, Baxter) via a Plexitron "intravenous infusion" set (60 drops/ml, Baxter) with a small Hoffman clamp as flow regulator. The saline bag was suspended from a hook tied to a nylon cord (5 mm diameter) and the one end was attached to a linen strap placed around the body of the sheep and situated just behind the shoulders. To counterbalance the mass of the full saline bag and also to keep the nylon cord taut a weight (1,4 kg for 1 000 ml saline) was attached to the free end of the cord (Fig. 1). This maintained a constant "head" between the withers of the ewe and the liquid reservoir, both in the standing and recumbent positions. To prevent the ewes from turning around in their individual pens they were each fitted with a halter and tied to the feed trough.

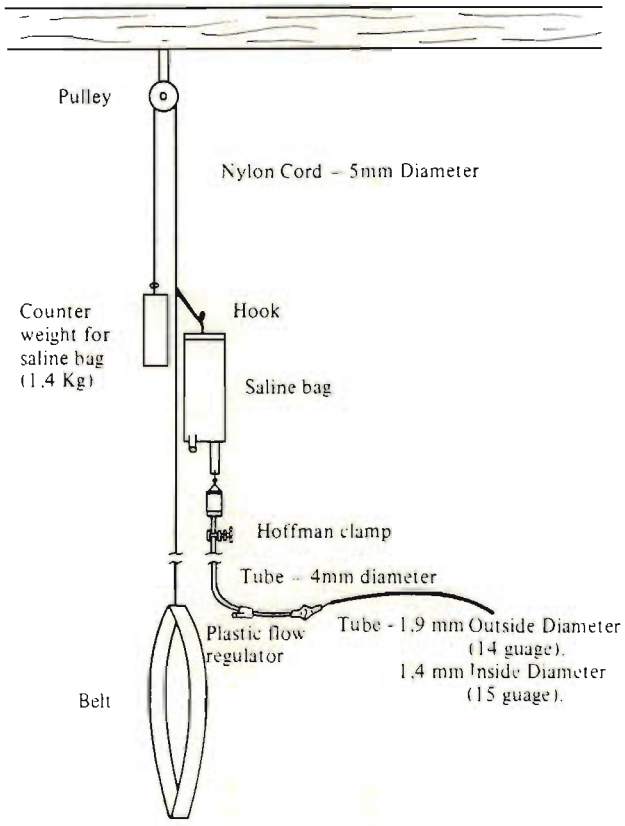


FIG. 1 : Schematic representation of the infusion apparatus for the administration of exogenous hormones in saline (0,9% NaCl).

Daily blood samples were drawn into heparinized syringes by disconnecting the infusion apparatus at the junction between the cannula and the connector drip (Fig. 1) after interrupting the flow via the plastic flow regulator.

The main problem encountered was that of suckling lambs chewing at the tubing and thereby severing the delivery tube. This could be prevented by encasing the tube within thick-walled 18 mm Tygon tubing. Slitting the outer tube along its length allowed the cannula to be exteriorized for sampling. The patency of the catheter was maintained in all cases, except where the saline flow was interrupted for more than 30 minutes. In such cases patency could be restored in some instances by forcing heparinized saline under pressure through the catheter using a 10 ml disposable syringe. If this failed the cannulation process was repeated on the opposite side of the neck.

The accumulation of fibrin at the tip of the cannula eventually (after 7 days or more) prevented the withdrawal of blood samples although infusion was not interrupted. In such cases needle puncture of the jugular on the opposite side was employed.

No infection at the site of entry into the body was observed, but fibrosis of the adjacent tissue occurred in a few animals.

2.3 Blood sampling

Blood samples were collected from the jugular vein via the infusion catheter. A "waste" sample of approximately 2 ml was drawn first to clear the catheter of infusion fluids. Heparinized syringes were used to collect the blood, after which it was transferred to centrifuge tubes containing one drop of heparinized saline (500 units/ml of 0,9% NaCl). The tubes were capped with parafilm and tilted gently to facilitate the mixing of the blood and heparin. Within approximately

15 minutes the blood was centrifuged, the plasma aspirated with pasteur pipettes and stored at -15°C in tightly capped plastic vials until assayed.

Blood samples for progesterone (10 cc) were taken daily, samples (6 cc) to determine the LH peak after GnRH injection every 30 minutes for 8 hours, and samples (6 cc) used to monitor tonic LH were taken every 15 minutes for two hours on Days -1 and 4 during Experiment II.

2.4 Pre-ovulatory LH and luteal progesterone in spontaneously cycling, non-lactating ewes (CYC-ewes)

Commencing on the same day as Experiment II non-lactating ewes were teased hourly with vazectomized rams. After the first signs of oestrus, blood samples were drawn every 30 minutes for an 18 hour period in order to characterize the pre-ovulatory LH peak in these ewes. A total of 10 ewes was sampled, but in only 4 were the LH levels elevated after the beginning of oestrus. Plasma LH levels were already elevated when oestrus commenced in the 6 ewes that were eliminated. The pattern of progesterone secretion was established from daily blood samples taken for the 17 days after oestrus.

2.5 Ovarian examination

All the ewes were laparotomized on Day 15 (Experiment I) or Day 11 (Experiment II) according to the technique of Lamond & Urquhart (1961) and the number of corpora lutea present, noted and described.

2.6 Preparation of saline and hormonal solutions for infusion

The bags (Viaflex, Baxter) were filled with 500 ml normal saline (0,9% NaCl), all the air expelled, and the bags boiled for 10 minutes. The PMSG (Tuco, 6 000 I.U.) was dissolved in 0,5% phenol and the solution made up to 30 ml.

This solution was kept at 4°C. A fresh solution was made every 48 hours. Just prior to infusion, 1 ml (200 I.U.) PMSG solution was

added to the saline bag. The bags were changed at 09h00 daily. The oestradiol (Ostratien [1, 3, 5 (10)] - diol - [3, 176] Merck), solution was prepared by dissolving 5 mg oestradiol 17b in 100 ml ethanol. This stock solution was stored at 4°C. Prior to use, 10 ml of stock was diluted to 100 ml with normal saline. One milliliter of this dilution thus contained 5 µg oestradiol 17b. For the infusion of 15 µg over the first 12 hour period, 6 ml (30 µg) was injected into the saline bag at 09h00. At 21h00 4 ml (20 µg) was added and thus 35 µg was infused over the second 12 hour period which ended at 09h00 on Day 0.

2.7 Radioimmunoassays

The plasma was assayed for progesterone according to the method of Butcher (1977). For LH the method of Niswender et al. (1969), validated for this laboratory by Lishman (1972), was used. For the determination of the tonic LH levels greater sensitivity was attained by diluting the anti LH serum to 1 : 160 000 instead of 1 : 100 000, and the incubation of the anti LH serum with the standards and the unknown samples was increased from 24 h to 48 h.

Pooled plasma samples were included in every assay for the determination of intra and inter assay variation. The same pool plasma was used for both Experiment I and II.

2.8 Statistical analyses

2.8.1 An analysis of variance was conducted as for a simple randomized design using the following parameters:

2.8.1.1 Progesterone concentration

2.8.1.1.1 The area under the progesterone curve from:

(i) Day 1 - 7: As a measure of luteal function during the early luteal phase, as corpora lutea from post partum GnRH treated cows did not continue to develop beyond this stage (Kesler, Weston, Pimental, Troxel, Vincent &

Hixon, 1981).

(ii) Day 1 - 11: As an indication of quantitative secretion for the period from ovulation to peak production.

(iii) Day 1 - 15 (Experiment I) or 1 - 17 (Experiment II): As a measure of total progesterone secretion.

2.8.1.1.2 Maximum concentration minus basal value as a parameter of qualitative luteal function.

2.8.1.1.3 The number of days on which levels of 2 ng/ml plasma or higher were recorded as an indication of quantitative secretion at appreciable levels.

All these concentrations were analysed per se and also when expressed per CL. The results for the 2 experiments were examined separately and the 2 experiments were also combined.

2.8.1.2 LH concentration

2.8.1.2.1 The total area under the LH secretional curve for all the treatments and also after combining the values of the groups infused with either saline or PMSG at the time of GnRH injection.

2.8.1.2.2 The maximum LH values.

2.8.2 The number of ewes with active corpora lutea and number of corpora lutea observed during laparotomy were compared between treatments by means of a Chi-square test.

2.8.3 The inter assay and intra assay coefficient of variation based on plasma pool concentrations was calculated for the progesterone and LH assays and also for the recovery percentage of labelled progesterone (Terblanche & Labuschagne, 1980). A between-assay analysis of variance was applied to test for possible between year variation in progesterone concentration of pool plasma.

2.8.4 The results were also analysed as for a completely randomized

design with repeated measures over time. The repeated measures were accommodated by conducting a mixed model analysis of variance with animals within treatments being considered a random effect. The mean sums of squares of the latter was used as error term for testing differences between treatments, whilst the residual mean square was used for testing "sub-plot" (time & time x treatment) effects.

2.8.5 Preplanned orthogonal comparisons amongst response curves

Tests for regression heterogeneity were conducted by a method proposed by Deaver (personal communication). Analysis showed the response of progesterone and log LH over time could be adequately described by third degree polynomials. Each treatment and relevant combination of treatments "animals within treatments" were considered random effects, whilst time was viewed as a continuous independant variable. An example of such an analysis and contrast between curves was:

Assume 3 treatments,

(i) for each treatment apply the following analyses:

Source	df
Animals within T_1	n_1
Time Linear mean	1
Quadratic mean	1
Cubic mean	1
Remainder (T_1)	

(ii) After drawing the various response curves, preplanned comparisons among the curves could be:

T_1 vs T_2, T_3

T_2 vs T_3

The following analyses are applied first:

	df
Treatments	2 (Treatments T_1, T_2 & T_3)
Animals within treatments	N_T ($N_T = n_1 + n_2 + n_3$)
Time Linear	1
Quadratic	1
Cubic	1
$Remainder_{(T)}$	

and,

	df
Treatments	1 (Treatments T_2 & T_3)
Animals within treatments	N_{23} ($N_{23} = n_2 + n_3$)
Time Linear	1
Quadratic	1
Cubic	1
$Remainder_{(23)}$	

First contrast: T_1 vs T_2, T_3.

Remainder	df	SS	MS	F
T_2, T_3	V_{23}	remainder (T_{23})		
T_1	V_1	remainder (T_1)		
Total	$V_{23} + V_1$	remainder ($T_{23} + T_1$)		
T_1, T_2, T_3	V_{123}	remainder (T)		
Difference				*

* Calculate F value (MS difference/MS total). If this F value is significant, the analysis indicates that analysing the two response curves (T1 and T2, T3) separately, resulted in an appreciably better fit than using the overall pooled curve (T1, T2, T3). Heterogeneity of regression then occurred or the response curves were not parallel.

Second contrast: T_2 vs T_3

Remainder	df	SS	MS	F
T_2	V_2	remainder (T_2)		
T_3	V_3	remainder (T_3)		
Total	$V_2 + V_3$	remainder ($T_2 + T_3$)		
T_2, T_3	V_{23}	remainder (T_2, T_3)		
Difference				*

* Calculate F value

2.8.6 Under the assumption of equal treatment group variances, the observations at any one point were analysed as a one-way analysis of variance followed by selected specific comparisons (Bonferroni's method, Millar [1966]). The different analyses of variance are then obviously not independant so that the overall tendency will have been toward erroneously claiming a greater number of "significant" results than would be indicated by the nominal Type 1 error (Gill, 1978).

3. RESULTS

3.1 Ovarian examination

At laparotomy on Day 15 (Experiment I) and Day 11 (Experiment II), 81,4% of the ewes had macroscopically active corpora lutea, that were neither pale nor small. In 18,6% of the ewes where no functional CL could be observed, the mean maximum progesterone level, within 12 days after GnRH treatment was 1,3 ng/ml plasma as compared to 2,9 ng/ml for those ewes with normal corpora lutea. The number of ewes with short-lived corpora lutea (lower progesterone levels after Day 7 than during the first 3 days) were 5 out of a total of 59. Of these, 3 were from the SOS group and one each from the EPOS and

SOP groups respectively. The percentage of ewes in each group, having active corpora lutea was as follows:

Group	% Ewes with active CL's
SOS (Control)	55,7*
SOP (PMSG after GnRH	76,9
EPOS (E_2 + PMSG before GnRH)	71,4
POS (PMSG before GnRH)	100,0*
POP (PMSG before and after GnRH)	91,7

* Difference significant ($0,25 < P < 0,5$)

A comparison between the number of ewes, in which active corpora lutea were present, in the groups which received only saline prior to GnRH (SOP and SOS), or only PMSG (POS and POP) were as follows:

Group	%	n
SOS + SOP	71,4	20 out of 28
POS + POP	95,8	23 out of 24

The difference is significant ($0,25 < P < 0,50$) and indicates that PMSG pretreatment did in fact stimulate a higher percentage of ewes to ovulate in response to GnRH. (Assuming an even distribution of CL's not observed during ovarian examination).

The average number of corpora lutea in those ewes where a CL was present at laparotomy were:

SOS	1,4
SOP	1,5
EPOS	1,8
POS	1,5
POP	1,8

The differences between the groups were small and non-significant

and the PMSG dose administered clearly was not high enough to stimulate multiple ovulations.

3.2 Intra and inter assay variation

3.2.1 Progesterone

The intra assay coefficients of variation for 28 duplicate pairs was 7,67%. The inter assay CV's for Experiment I and II were as follows:

	Plasma pool progesterone (ng/ml): Mean ± SeM	CV
Expt. I	3,05 ± 0,11 (n = 20)	15,60%
Expt. II	3,10 ± 0,08 (n = 36)	15,81%

	Recovery % of labelled progesterone: Mean ± SeM	CV
Expt. I	90,62 ± 0,96	4,74%
Expt. II	95,45 ± 0,76	4,54%

An analysis of variance to test for variation between years was conducted on the pooled plasma values, as shown below.

Analysis of variance: between years pooled plasma progesterone concentration

Source	df	SS	MS	F
Years	1	0,0333	0,0333	0,1433
Error	54	12,5457	0,2323	N.S.
Total	55	12,5790		

$\bar{x}$ = 3,07 ± 0,06 ng/ml CV = 15,65%

There was no year effect on the assayed values for the progesterone concentration of the pooled plasma.

3.2.2 Luteinizing hormone

All samples for Experiment I were included in one assay to avoid inter assay variation and no intra assay CV could be calculated as

only one pooled plasma sample was erroneously included in the assay. During Experiment II the mean concentration and the intra assay CV were:

Assay	No. of "pool" samples	Mean Conc. ± SeM (ng/ml)	CV %
Tonic LH	38	1,73 ± 0,03	7,92
LH peaks after GnRH	18	1,71 ± 0,05	14,71
Repeats and natural LH peaks	10	1,83 ± 0,10	13,14

The inter assay CV was 13,08% and the mean of the pooled plasma was 1,74 ± 0,03 ng/ml plasma.

3.3 Plasma progesterone

3.3.1 Progesterone secretion curves

The changes in mean daily progesterone concentration for the various treatment groups (Fig. 2 and Fig. 3) demonstrated the improved luteal function in the 2 groups which received PMSG after GnRH administration (SOP and POP groups) and to a lesser extent for ewes infused with PMSG before GnRH (POS).

There is a striking resemblence between Figs. 2 and 3 for the treatments which were applied in two consecutive years. The pre- and post peak slopes are very similar for the same treatments, exactly the same order of magnitude for the treatments is maintained, the peak concentration is reached on exactly the same day for the SOP and POP groups and peak values are very similar. The curve describing secretion of progesterone in the ewes that ovulated naturally (CYC - Fig. 3) closely resembles the curve for the POS group both in magnitude and shape, but the maximum values are markedly lower than for the treatment groups which received PMSG after GnRH. There was some suggestion that pretreatment with oestradiol - 17b during the first 6 days resulted in improved luteal activity, but this trend

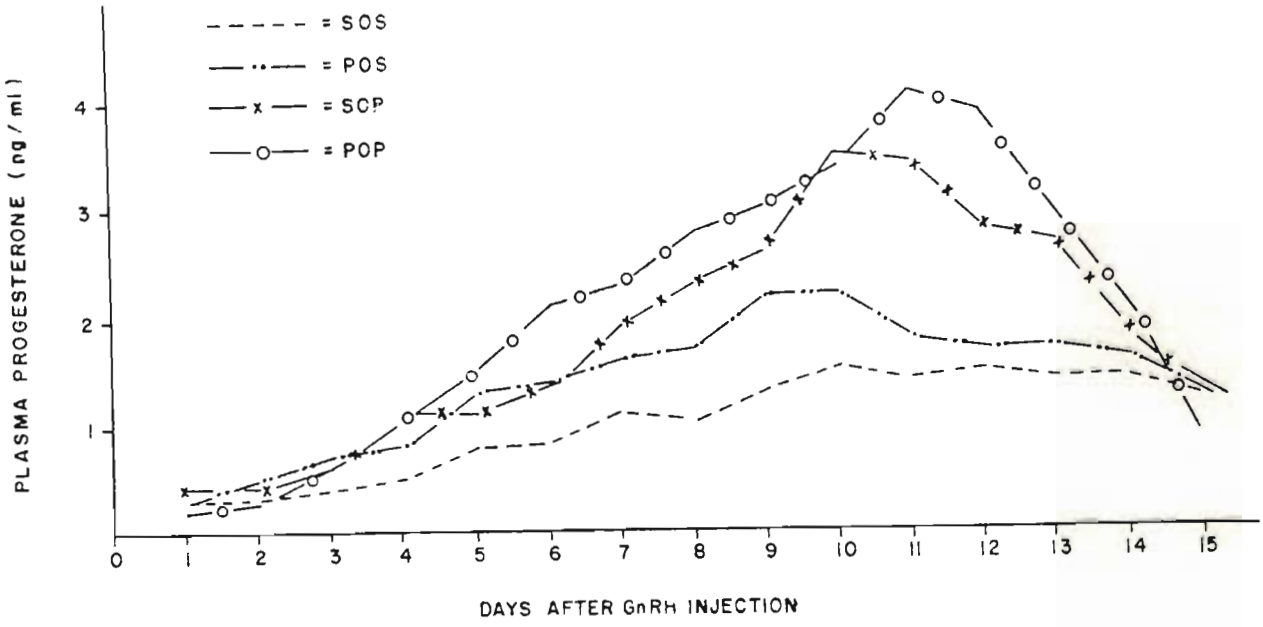


FIG. 2 : Plasma progesterone concentration (ng/ml) during early post partum for the control ewes (SOS), ewes infused with PMSG prior to (POS), after (SOP) and prior to and after (POP) GnRH injection (Experiment I).

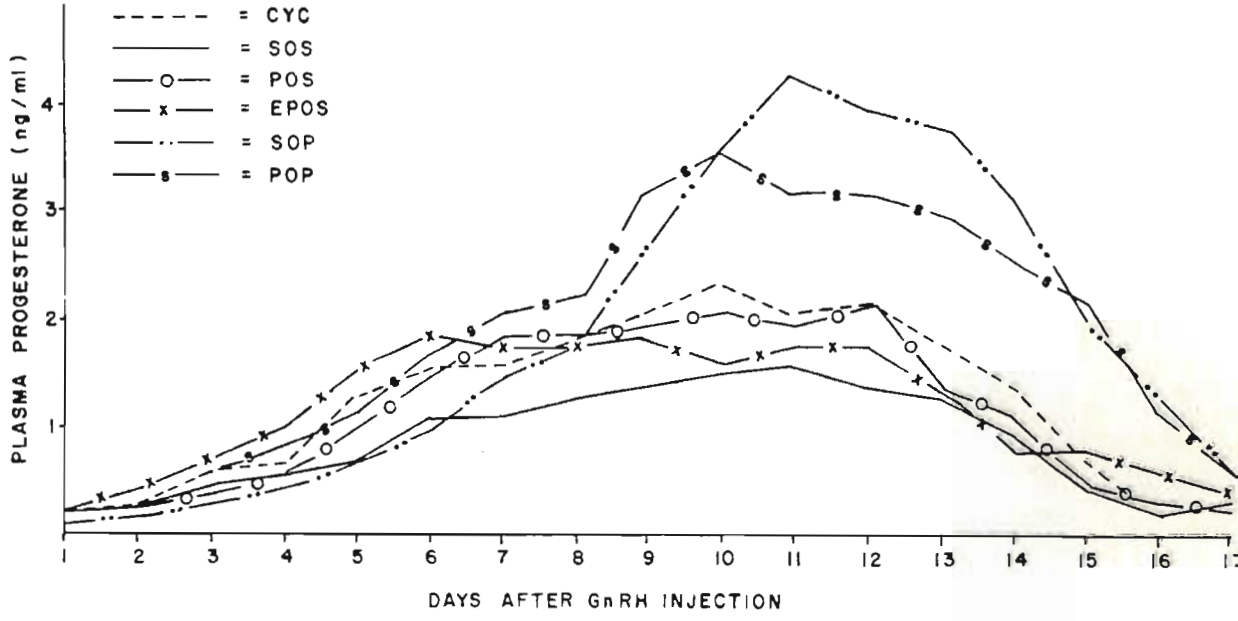


FIG. 3 : Plasma progesterone concentration (ng/ml) for spontaneously cycling non lactating ewes (CYC), and ewes during early post partum, viz. control ewes (SOS) infused with PMSG prior to (POS), after (SOP), prior to and after (POP) and PMSG plus E2 infused prior to (EPOS) GnRH injection (Experiment II).

was not maintained after Day 6.

3.3.2 Total progesterone secretion

The areas under the progesterone secretional curves were used as a measure of total luteal activity. The values for the areas are expressed in arbitrary units. The results are presented as a total of the CL's observed at laparotomy, and are also expressed as a fraction of the number of observed CL. Dividing the calculated area by the number of corpora lutea in some cases did result in a change in order between the treatments and in the significance of treatment differences.

3.3.2.1 Progesterone secretion from Day 1 to Day 7

PMSG infusion prior to and after GnRH injection (POP group) resulted in a mean area under the progesterone secretional curves for Day 1 to Day 7 of up to twice the value for the SOS (Control) group (Table 2). When expressed per CL the mean area for the POP treatment is still nearly double that of the SOS group. There were only minor differences between the POS and SOP treatments.

TABLE 2 : Area under the progesterone curve from Day 1 - 7 (Expt. I) for the control ewes (SOS) and ewes infused with PMSG prior to (POS), after (SOP) and prior to and after (POP) GnRH administration.

Group	n	Treatment mean ± SeM		As % of SOS mean	
		As is	Per CL	As is	Per CL
POP	5	7,10 ± 0,49	4,82 ± 0,72	204,0	176,5
POS	7	5,82 ± 1,27	4,68 ± 1,32	167,2	171,4
SOP	5	5,57 ± 0,46	4,45 ± 0,76	160,0	163,0
SOS	5	3,48 ± 0,32	2,73 ± 0,39	100,0	100,0
		$\bar{x}$ = 5,52 ± 0,48	$\bar{x}$ = 4,21 ± 0,49		

None of the differences in Table 2 were significant.

In Experiment II (Table 3) the oestradiol priming prior to GnRH injection resulted in an area significantly greater ($P < 0,05$) than the control (SOS) group, but the difference became non significant when the values were adjusted for the number of corpora lutea observed. The mean values for the other treatments were slightly lower during Experiment II than during Experiment I.

TABLE 3 : Area under the progesterone curve, Day 1 - 7 (Expt. II) for the control ewes (SOS) and ewes infused with PMSG prior to (POS), after (SOP), prior to and after (POP) and PMSG plus E2 infused prior to (EPOS) GnRH administration.

Group	n	Treatment mean ± SeM		As % of SOS mean	
		As is*	Per CL	As is	Per CL
EPOS	7	6,60 ± 1,42	4,72 ± 1,31	182,0	158,4
POP	6	5,23 ± 1,42	2,99 ± 0,65	144,5	100,3
POS	5	4,90 ± 1,19	3,09 ± 0,39	135,4	103,7
SOP	7	4,00 ± 0,85	3,29 ± 0,94	110,5	110,4
SOS	8	3,62 ± 0,40	2,98 ± 0,22	100,0	100,0

$\bar{x} = 4,82 \pm 0,48$ $\bar{x} = 3,40 \pm 0,37$

* EPOS SOS ($p < 0,05$)

A combination of the Day 1 to Day 7 areas for both experiments (Table 4) confirmed the trends established from the results in Tables 2 and 3.

3.3.2.2 Progesterone secretion from Day 1 to Day 11

The most significant feature of the progesterone secretion from Day 1 to Day 11 is that the ranking order of the treatments changed in comparison to Days 1 - 7. PMSG infusion after GnRH appeared to stimulate the CL to a larger extent than PMSG infusion prior to the releasing hormone injection

(Tables 5 and 6). This is the complete opposite of the position from Day 1 to Day 7 where the mean of the POS group was higher than for the SOP group (Tables 2 and 3).

TABLE 4 : Area under the progesterone curve, Days 1 - 7 (Expt. I and II) for the control ewes (SOS), and ewes infused with PMSG prior to (POS), after (SOP), prior to and after (POP) and PMSG plus E2 prior to (EPOS) GnRH administration

Group	n	Treatment mean ± SeM As is*	Per CL	As % of SOS mean As is	Per CL
EPOS	7	6,60 ± 1,42	4,72 ± 1,31	184,9	163,9
POP	11	6,08 ± 0,83	3,82 ± 0,54	170,3	132,6
POS	12	5,44 ± 0,93	4,02 ± 0,79	152,4	140,0
SOP	12	4,65 ± 0,56	3,78 ± 0,63	130,3	131,3
SOS	13	3,57 ± 0,27	2,88 ± 0,19	100,0	100,0

$\bar{x}$ = 5,10 ± 0,34 $\bar{x}$ = 3,75 ± 0,30

* EPOS and POP > SOS ($p < 0,05$)

TABLE 5 : Area under the progesterone curve, Days 1 - 11 (Expt. I) for the control ewes (SOS) and ewes infused with PMSG prior to (POS), after (SOP) and prior to and after (POP) GnRH.

Group	n	Treatment mean ± SeM As is*	Per CL	As % of SOS mean As is	Per CL
POP	5	19,53 ± 2,50	12,83 ± 1,49	230,0	194,9
SOP	5	16,90 ± 2,01	13,60 ± 2,94	195,5	206,6
POS	7	13,53 ± 2,62	10,95 ± 2,60	159,4	166,4
SOS	5	8,49 ± 0,97	6,58 ± 0,87	100,0	100,0

$\bar{x}$ = 14,44 ± 1,15 $\bar{x}$ = 10,99 ± 1,14

* POP > SOS ($p < 0,01$)

SOP > SOS ($p < 0,05$)

In both experiments, PMSG treatment before and after GnRH (POP group) resulted in the largest mean area, but because of the slightly higher ovulation rate of this group, adjusting for the number of CL's changed the rank order (Tables 4 and 5).

TABLE 6 : Area under the progesterone curve, Days 1 - 11 (Expt. II) for the control (SOS) and ewes infused with PMSG prior to (POS), after (SOP), prior to and after (POP) and PMSG plus E2 prior to (EPOS) GnRH administration.

Group	n	Treatment mean ± SeM		As % of SOS mean	
		As is*	Per CL	As is	Per CL
POP	5	17,66 ± 4,48	9,43 ± 1,93	193,0	123,6
SOP	7	15,05 ± 2,42	12,38 ± 2,77	164,5	162,3
EPOS	6	13,16 ± 3,18	10,39 ± 3,45	143,9	136,2
POS	5	12,76 ± 3,01	8,09 ± 0,54	140,5	106,0
SOS	7	9,15 ± 0,91	7,63 ± 1,02	100,0	100,0

$\bar{x}$ = 13,35 ± 1,25 $\bar{x}$ = 9,67 ± 1,04

* POP > SOS ($p < 0,01$)

A combination of the areas, for Day 1 to 11, of Experiments I and II (Table 7) shows that the apparent luteotrophic effect of oestradiol (EPOS group) during the first 7 days (Table 4) was no longer evident. Instead, PMSG seemed to exert a trophic action during the critical period for luteal survival viz. from Day 7 to 11.

The trophic effect of the gonadotrophin was demonstrated by the finding that even after division of the total quantity of progesterone by the number of CL, the PMSG treatment after GnRH resulted in a significantly greater progesterone secretion than for the control group (Table 7).

3.3.2.3 Progesterone secretion from Day 1 to 15 (Experiment I) or Day 17 (Experiment II)

These results present very sound evidence that PMSG does

exert a luteotrophic effect, very much more so during the presence of the corpus luteum, than as a treatment prior to the induction of ovulation and subsequent formation of a CL.

The total progesterone secretion (area under the curve) for Experiment I and II and the combined values for the two Experiments (Tables 8, 9 and 10) indicate, as envisaged, the same trends as during the first 11 days.

The pre- and post GnRH PMSG treatment (POP) and post GnRH (SOP) treatment was proved superior to the other treatments during both experiments.

TABLE 7 : Areas under the progesterone curve, Days 1 - 11 (Expt. I and II) for the control ewes (SOS), and ewes infused with PMSG prior to (POS), after (SOP), prior to and after (POP) and PMSG plus E2 prior to (EPOS) GnRH administration

Group	n	Treatment mean ± SeM		As % of SOS mean	
		As is*	Per CL**	As is	Per CL
POP	10	18,59 ± 2,44	11,12 ± 1,28	209,6	154,4
SOP	12	15,70 ± 1,61	12,89 ± 1,95	177,0	179,0
POS	12	13.21 ± 1,89	9,76 ± 1,54	148,9	135,6
EPOS	6	13,16 ± 3,18	10,39 ± 3,45	148,4	144,3
SOS	12	8,87 ± 0,65	7,20 ± 0,69	100,0	100,0

$\bar{x}$ = 13,81 ± 0,84 $\bar{x}$ = 10,23 ± 0,75

* POP > SOS ($p < 0,01$) ** SOP > SOS ($p < 0,05$)

> POS ($p < 0,05$)

SOP > SOS ($p < 0,01$)

The mean area for the ewes that ovulated naturally (CYC) is greater than that of the POS and SOS groups (Table 9), but smaller than for the post GnRH PMSG treatment groups (POP and SOP).

TABLE 8 : Areas under the progesterone curve, Days 1 - 15 (Expt. I) for the control ewes (SOS) and ewes infused with PMSG prior to (POS), after (SOP) and prior to and after (POP) GnRH administration.

Group	n	Treatment mean ± SeM As is*	Per CL**	As % of SOS mean As is	Per CL
POP	6	26,91 ± 5,13	20,43 ± 2,53	225,9	178,6
SOP	6	23,64 ± 4,15	21,07 ± 4,17	198,5	184,2
POS	7	20,32 ± 3,09	16,48 ± 3,03	170,6	144,1
SOS	7	11,91 ± 1,98	11,44 ± 2,01	100,0	100,0

$\bar{x}$ = 20,34 ± 1,81 $\bar{x}$ = 17,28 ± 1,61

* POP > SOS ($p < 0,01$) ** SOP > SOS ($p < 0,05$)

SOP > SOS ($p < 0,05$)

TABLE 9 : Areas under the progesterone curve, Days 1 - 17 (Expt. II) for ewes that ovulated naturally (CYC), control ewes (SOS) and ewes infused with PMSG prior to (POS), after (POS), prior to and after (POP) and PMSG plus E2 prior to (EPOS) GnRH administration.

Group	n	Treatment mean ± SeM As is*	Per CL**	As % of SOS mean As is	Per CL
POP	6	31,84 ± 6,65	16,83 ± 3,04	228,9	142,5
SOP	7	31,71 ± 5,46	21,95 ± 5,18	228,0	185,9
EPOS	7	22,49 ± 4,46	16,65 ± 4,61	161,7	140,9
CYC	5	20,97 ± 3,21	◊	150,8	-
POS	5	19,42 ± 4,56	12,26 ± 0,38	139,6	103,8
SOS	8	13,91 ± 1,42	11,81 ± 1,50	100,0	100,0

$\bar{x}$ = 23,26 ± 1,85 $\bar{x}$ = 15,97 ± 1,62

◊ CYC group were not laparotomized

* POP and SOP > SOS ($p < 0,05$)

** SOP > SOS ($p < 0,05$)

From the results in Table 9 it appears that pretreatment with PMSG

has very little advantage as regards stimulating luteal activity over an entire cycle. The POS treatment resulted in a slightly smaller mean area as the CYC group, but when expressed per CL it is very similar to the SOS group (controls).

3.3.3 Maximum progesterone values

The maximum progesterone concentration in the plasma is an indication of luteal activity and therefore quality of luteal function during the peak production. The values presented here are the mean of the peaks within a treatment group minus the mean value on Day 0 for that group. The peak concentrations are presented as totals per animal and also expressed per CL observed during laparotomy (Tables 11, 12 and 13). As could be expected, the results follow very closely the trend as shown by the areas under the progesterone curve (Table 10).

Table 10 : Areas under the progesterone curve, Days 1 - 15 (Expt. I and II) for control ewes (SOS), and ewes infused with PMSG prior to (POS), after (SOP), prior to and after (POP) and PMSG plus E2 prior to (EPOS) GnRH.

Group	n	Treatment mean ± SeM Per CL	As % of SOS mean Per CL
SOP	12	20,53 ± 3,22	186,8
POP	11	17,31 ± 2,07	157,5
EPOS	7	15,05 ± 4,09	136,9
POS	12	14,38 ± 1,98	130,8
SOS	13	10,99 ± 1,16	100,0

$\bar{x}$ = 15,59 ± 1,09

SOP > SOS ($p < 0,01$)

The corpora lutea of the ewes pretreated with PMSG (POS , Table 12)

had the ability to secrete the same maximum concentration of progesterone per ml plasma as the ewes that ovulated naturally (CYC), but the former had higher (N.S.) peak values than the control ewes (SOS). PMSG treatment prior to GnRH thus stimulated the corpora lutea to at least equal or better the capacity of the ewes that ovulated naturally.

TABLE 11 : The maximum plasma progesterone concentration (ng/ml) minus the basal value (Expt. I) for the control ewes (SOS), and ewes infused with PMSG prior to (POS), after (SOP) and prior to and after (POP) GnRH administration.

Group	n	Treatment mean ± SeM		As % of SOS mean	
		As is*	Per CL	As is	Per CL
POP	6	3,71 ± 0,63	2,98 ± 0,53	272,8	201,4
SOP	6	3,39 ± 0,66	2,97 ± 0,79	249,3	200,7
POS	7	2,36 ± 0,34	2,01 ± 0,40	173,5	135,8
SOS	7	1,36 ± 0,32	1,48 ± 0,36	100,0	100,0

$\bar{x}$ = 2,64 ± 0,24 $\bar{x}$ = 2,32 ± 0,26

* POP and SOP > SOS ($p < 0,01$)

After expressing the values in Table 11 per CL the differences became non significant.

The results (Table 10) indicate that a larger volume of progesterone was secreted due to PMSG treatment specifically after GnRH injection (SOP; POP). Both post GnRH and, pre- and post GnRH PMSG infusion gave rise to significantly ($p < 0,01$) higher progesterone peaks (Table 13). After allowing for the number of corpora lutea the peak value obtained with PMSG only after GnRH (SOP group) is still 2,3 times higher than the group not receiving PMSG (SOS group).

TABLE 12 : The maximum plasma progesterone concentration (ng/ml) minus the basal value (Expt. II) for ewes that ovulated naturally (CYC), control ewes (SOS) and ewes infused with PMSG prior to (POS), after (SOP) prior to and after (POP) and PMSG plus E2 prior to (EPOS) GnRH administration.

Group	n	Treatment mean ± SeM		As % of SOS mean	
		As is*	Per CL**	As is	Per CL
SOP	7	4,74 ± 0,98	3,23 ± 0,88	292,6	224,3
POP	6	4,23 ± 0,80	2,20 ± 0,38	261,1	152,8
EPOS	7	2,49 ± 0,39	1,83 ± 0,42	153,7	127,1
POS	5	2,34 ± 0,43	1,56 ± 0,13	144,4	108,3
CYC	5	2,32 ± 0,42	-	143,2	-
SOS	8	1,62 ± 0,24	1,44 ± 0,28	100,0	100,0

$\bar{x}$ = 2,96 ± 0,25 $\bar{x}$ = 2,06 ± 0,23

* SOP and POP > SOS ($p < 0,01$)
> EPOS, POS and CYC ($p < 0,05$)

** SOP > SOS ($p < 0,01$)

TABLE 13 : The maximum plasma progesterone concentration (ng/ml) minus the basal value (Expt. I and II) for control ewes (SOS) and ewes infused with PMSG prior to (POS), after (SOP), prior to and after (POP) and PMSG plus E2 prior to (EPOS) GnRH administration.

Group	n	Treatment mean ± SeM	As % of SOS mean
		As per CL	As per CL
SOP	12	4,54 ± 0,61	237,7
POP	11	4,37 ± 0,46	228,8
POS	12	2,68 ± 0,28	140,3
EPOS	7	2,67 ± 0,42	140,0
SOS	13	1,91 ± 0,16	100,0

$\bar{x}$ = 3,24 ± 0,19

SOP and POP > EPOS, POS and SOS ($p < 0,01$)

3.3.4 Continued secretion at appreciable levels

The ability of the corpora lutea to continuously secrete progesterone so as to maintain a concentration of at least 2 ng/ml plasma over several days was compared for the treatment groups (Table 14). The superiority of the SOP and POP treatment is clearly demonstrated and the difference between these two treatments strengthens the theory that PMSG infusion after GnRH exerts a luteotrophic effect to a far greater extent than PMSG prior to GnRH.

TABLE 14 : The mean number of days plasma progesterone levels were equal to or exceeded 2 ng/ml plasma (Expressed per CL, Expt. I and II combined) for the control ewes (SOS) and ewes infused with PMSG prior to (POS), after (SOP) prior to and after (POP) and PMSG plus E2 (EPOS) prior to GnRH administration.

Group	n	Treatment mean ± SeM	As % of SOS mean
SOP	12	3,94 ± 0,81	635,5
POP	11	3,73 ± 0,66	601,6
EPOS	7	3,24 ± 1,53	552,5
POS	12	2,51 ± 0,88	404,8
SOS	13	0,62 ± 0,24	100,0

$\bar{x}$ = 2,71 ± 0,35

SOP and POP > SOS ($p < 0,01$)

EPOS > SOS ($p < 0,05$).

3.3.5 Repeated measures analyses

The repeated measures analysis of the results obtained in Expt. I indicated a significant effect ($p = 0,0003$) of PMSG treatment after GnRH (Table 15). The effect of PMSG also resulted in a significant difference over time in plasma progesterone concentration.

TABLE 15 : Mixed model analysis of variance for plasma progesterone concentration with days as repeated measures (Expt. I).

Source[2]	df	SS	MS	F	Prob	Tab F[3]
Per 1	1	10,02	10,02	2,58	0,13	-
Per 2	1	46,32	46,32	11,90**	0,003	-
Per 1 x Per 2	1	0,12	0,12	0,03	0,86	-
Ewes : Treatments	18	70,04	3,89	2,58		
Days	14	220,61	15,76	35,07**	-	4,41
Per 1 x days	14	4,06	0,29	0,65 N.S.	-	4,41
Per 2 x days	14	38,94	2,78	6,19*	-	4,41
Per 1 x Per 2 x days	14	1,63	0,12	0,26 N.S.	-	4,41
Error (b)	248	111,42	0,50			

[2] Per 1 : PMSG prior GnRH

Per 2 : PMSG after GnRH

[3] Tab F : Tabulated $F_{1,18}$ for conservative F tests, Winer (1962)

* $p < 0,05$

** $p < 0,01$

Using the conservative test of significance described by Winer (1962) (number of days/number of days = 1; error (b)/number of days = 18), the effect of time on concentration of progesterone was found to be highly significant. The significant interaction ($p<0,05$; conservative test) between PMSG after GnRH, and days, is important and indicates a difference in the response over time to the various treatments applied during that period (Table 15).

In Experiment II the treatments applied did not have a significant effect on plasma progesterone concentration (Table 16). However, as indicated by the conservative F-test, "Days" had a significant effect on the response curves of the various treatments ($p < 0,01$). The absence of a significant treatment by day effect (conservative test) would

tend to suggest homogeneous regression curves for the different treatments. As the interaction was quite near to significance and bearing in mind that a very conservative F-test was used, it was decided to carry out individual tests on the slopes resulting from the various treatments applied in this experiment.

TABLE 16 : Mixed model analysis of variance for plasma progesterone concentration with days as repeated measures (Expt. II).

Source	df	SS	MS	F	Prob.	Tab F[2]
Treatment	5	79,99	15,99	2,07 N.S.	0,10	-
Ewe x Trt.	25	193,18	7,73	14,01	0,0001	-
Days	16	320,29	20,02	36,29**	-	4,24
Trt. x days	80	110,83	1,38	2,51 N.S.	-	2,60
Error	400	220,66	0,55	-	-	-

[2] Tab F : Tabulated F for conservative F tests, Winer (1962)

** $p < 0,01$

In order to examine the response curves of treatments over time, separate analyses were carried out in which the sums of squares for time were subdivided up to the 5th degree. The orthogonal components as well as tests of significance of each treatment/treatment combination were determined (Table 17). Again, the conservative test (Winer, 1962) was used.

Up to the cubic term the goodness of fit for all treatments accounted for such a large percentage of the variance amongst days that it was decided to concentrate on the cubic term. For example, the 5th grade could be significant, but the 3rd grade most often resulted in an acceptable fit.

TABLE 17 : The orthogonal components of time and tests of significance of each treatment/treatment combination for plasma progesterone concentration.

Group	Degree of polynomial: Sums of squares (days)							% Var[3] 1 - 3 Degree
	Total	Linear	Quad	Cub	Quard	Quin	Higher Order	
Expt. I								
SOS	16,31	13,99**	1,14	0,87	0,01	0,00	0,29	98,10
POS	36,29	19,94**	12,29**	2,23*	0,27	0,08	1,48	94,96
SOP	86,73	44,91**	19,85**	18,60**	0,31	0,78	2,28	96,10
POP	128,98	55,49**	42,21**	26,88**	1,06	0,00	3,34	96,58
Expt. II								
CYC	47,62	0,98	42,20**	1,81*	0,94	0,47	1,22	94,47
SOS	23,35	0,41	19,43**	1,18*	1,18*	0,65	0,48	95,15
POS	45,22	0,67	38,99**	1,08	2,92	0,14	1,42	90,01
EPOS	24,92	0,00	22,53**	0,43	0,43	0,03	1,50	92,15
SOP	206,18	64,46**	73,88**	56,67**	0,27	8,30*	2,59	94,59
POP	102,73	22,73	64,39**	12,62**	0,90*	0,47	1,63	97,08

[3] % Variance accounted for by the first to third order polinomial degrees

* $p < 0,05$ (conservative F-test)

** $p < 0,01$ (conservative F-test)

The method suggested by Deaver (personal communication) was applied to gain a preliminary insight into the differences between the various curves (Fig. 4 and 5). The preplanned contrast for Experiment I (effect of treatment after GnRH) gave the following results:

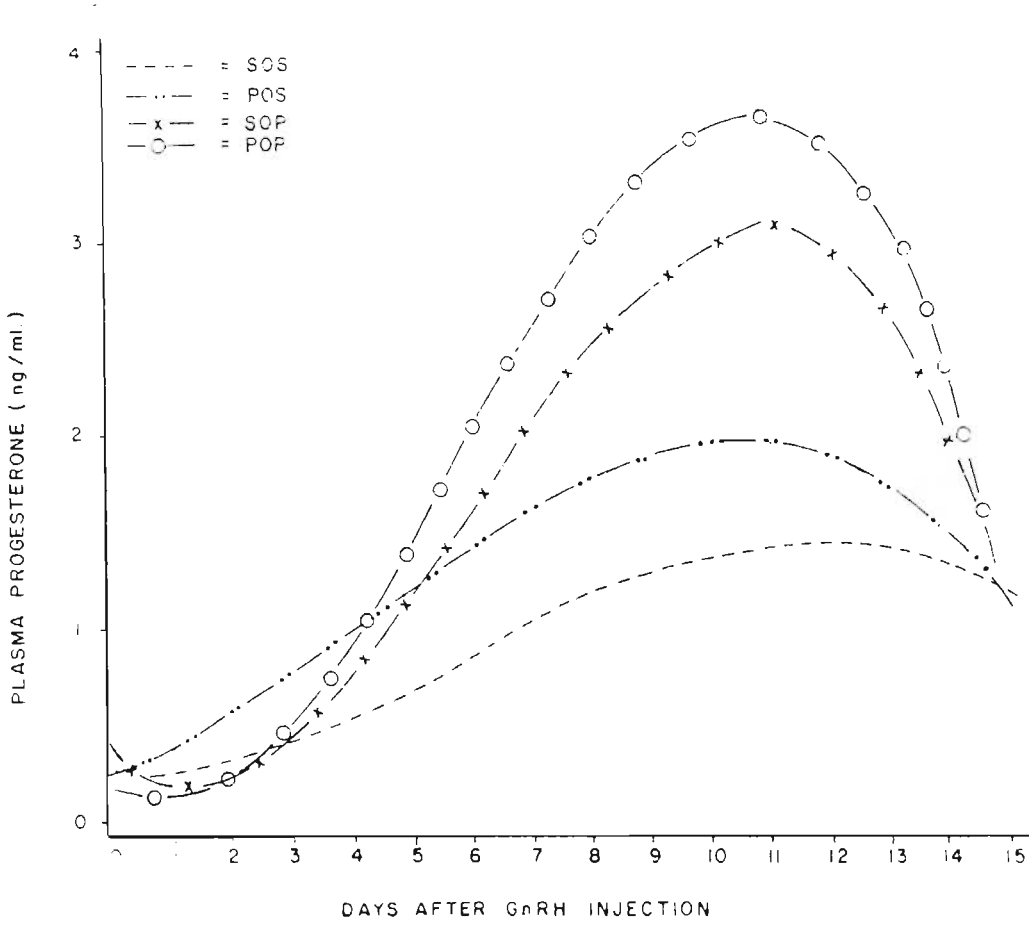


FIG. 4 : Estimated regression of the progesterone concentration (ng/ml) on days during early post partum for the control ewes (SOS), ewes infused with PMSG prior to (POS), after (SOP) and prior to and after (POP) GnRH injection (Experiment I).

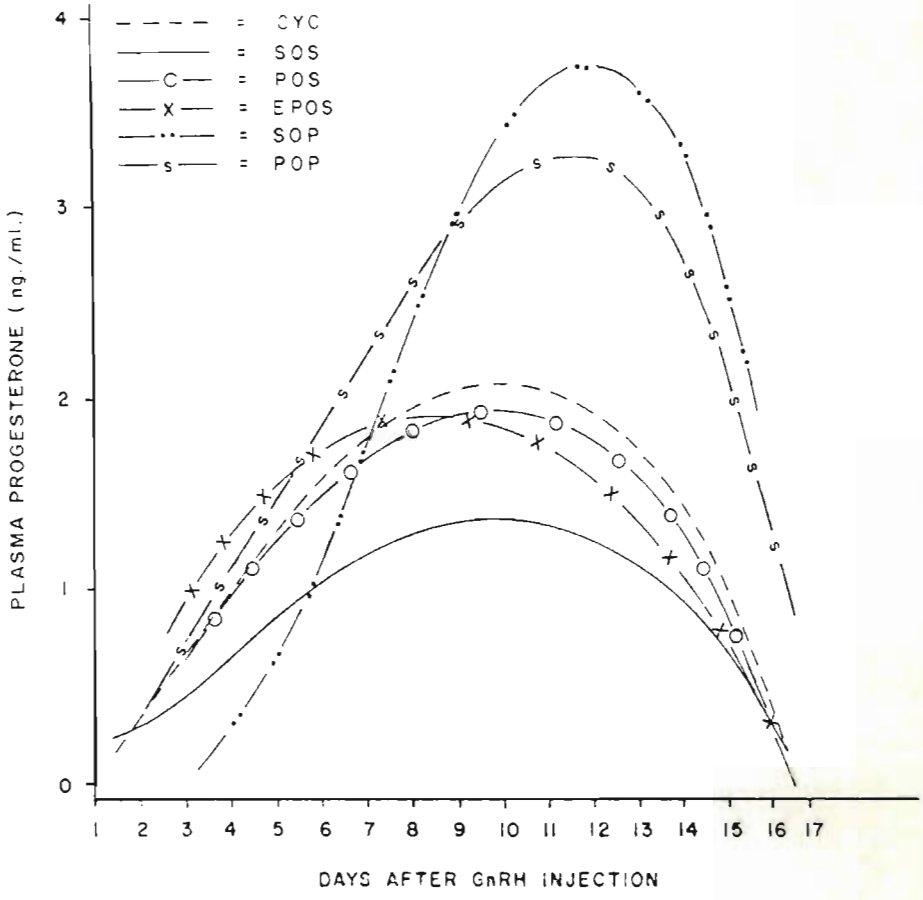


FIG. 5 : Estimated regression of the progesterone concentration (ng/ml) on days during early post partum for spontaneously cycling non lactating ewes (CYC), and ewes during early post partum, viz. control ewes (SOS), ewes infused with PMSG prior to (POS), after (SOP), prior to and after (POP) and PMSG plus E2 infused prior to (EPOS) GnRH injection (Experiment II).

(i) Preplanned orthogonal comparisons among the various response curves for the treatment groups, Experiment I.

(a) SOS vs POP, SOP and POP

Remainder	df	SS	MS	F
POS, SOP & POP (pooled)	252	146,64		
SOS	72	10,07		
Total	324	156,71	0,48	
SOS, POS, SOP & POP (pooled)	327	172,90		
Difference	3	16,19	5,40	11,25**

∴ Response curve of control ewes (SOS) is not parallel to the pooled response curves of POS, SOP & POP treatments

(b) POS vs SOP & POP

Remainder	df	SS	MS	F
SOP & POP (pooled)	147	93,07		
POS	102	29,39		
Total	249	122,46	0,49	
POS, SOP & POP (pooled)	252	146,64		
Difference	3	24,18	8,06	16,45**

∴ Response curve of ewes infused with PMSG prior to GnRH (POS) is not parallel to the pooled response curves of SOP & POP treatments

(c) SOP vs POP

Remainder	df	SS	MS	F
SOP	72	42,83		
POP	72	47,50		
Total	144	90,33	0,63	
SOP & POP (pooled)	147	93,07		
Difference	3	2,74	0,91	1,44 N.S.

∴ Response curve of ewes infused with PMSG after GnRH (SOP) is parallel to the response curve of the ewes infused with PMSG prior to and after GnRH injection (POP group).

(ii) Preplanned orthogonal comparisons among the various response curves for the treatment groups during Experiment II.

(a) SOP vs CYC, SOS, POS, EPOS & POP

Remainder	df	SS	MS	F
CYC, SOS, POS, EPOS & POP (pooled)	397	178,78		
SOP	93	99,58		
Total	490	278,36	0,57	
CYC, SOS, POS, EPOS, SOP & POP (pooled)	493	345,05		
Difference	3	66,69	22,23	39,00**

∴ Response curve of ewes infused with PMSG after GnRH (SOP) is not parallel to the pooled response curves of CYC, SOS, POS, EPOS & POP

(b) POP vs CYC, SOS, POS & EPOS

Remainder	df	SS	MS	F
CYC, SOS, POS & EPOS (pooled)	317	83,52		
POP	77	70,01		
Total	394	153,53	0,39	
CYC, SOS, POS, EPOS & POP (pooled)	397	178,78		
Difference	3	25,25	8,42	21,59**

∴ Response curve of ewes infused with PMSG prior to and after GnRH (POP) is not parallel to the pooled response curves of CYC, SOS, POS & EPOS

(c) SOS vs CYC, POS & EPOS

Remainder	df	SS	MS	F
CYC, POS & EPOS (pooled)	221	67,76		
SOS	93	12,16		
Total	314	79,91	0,25	
CYC, SOS, POS & EPOS (pooled)	317	83,52		
Difference	3	3,61	1,20	4,80**

∴ Response curve of control ewes (SOS) is not parallel to the pooled response curves CYC, POS & EPOS

(d) EPOS vs CYC & POS

Remainder	df	SS	MS	F
CYC & POS (pooled)	157	40,55		
EPOS	61	24,00		
Total	218	64,55	0,30	
CYC, POS & EPOS (pooled)	221	67,75		
Difference	3	3,20	1,07	3,59*

∴ Response curves of ewes infused with PMSG + E2 prior to GnRH (EPOS) is not parallel to the pooled response curves of CYC & POS

(e) CYC vs POS

Remainder	df	SS	MS	F
POS	77	26,06		
CYC	77	14,40		
Total	154	40,46	0,26	
CYC & POS (pooled)	157	40,55		
Difference	3	0,09	0,03	0,12 N.S.

∴ Response curves of spontaneously cycling ewes (CYC) is parallel to the response curve of ewes pretreated with PMSG (POS)

The non-parallelism between the response curves of the control group and those of the PMSG treatment groups verifies the trophic effect of PMSG on luteal function. The superior luteotrophic effect of the post GnRH PMSG infusion is also re-established by the preplanned orthogonal comparisons.

In order to establish during which intervals the points on the response curves differed significantly, simultaneous inferences on the means were made by the method of Bonferroni (Millar, 1966) and the points that proved to be significantly different plotted graphically (Fig. 6, 7 and 8 for Experiment I and in Figs. 9, 10 and 11 for Experiment II).

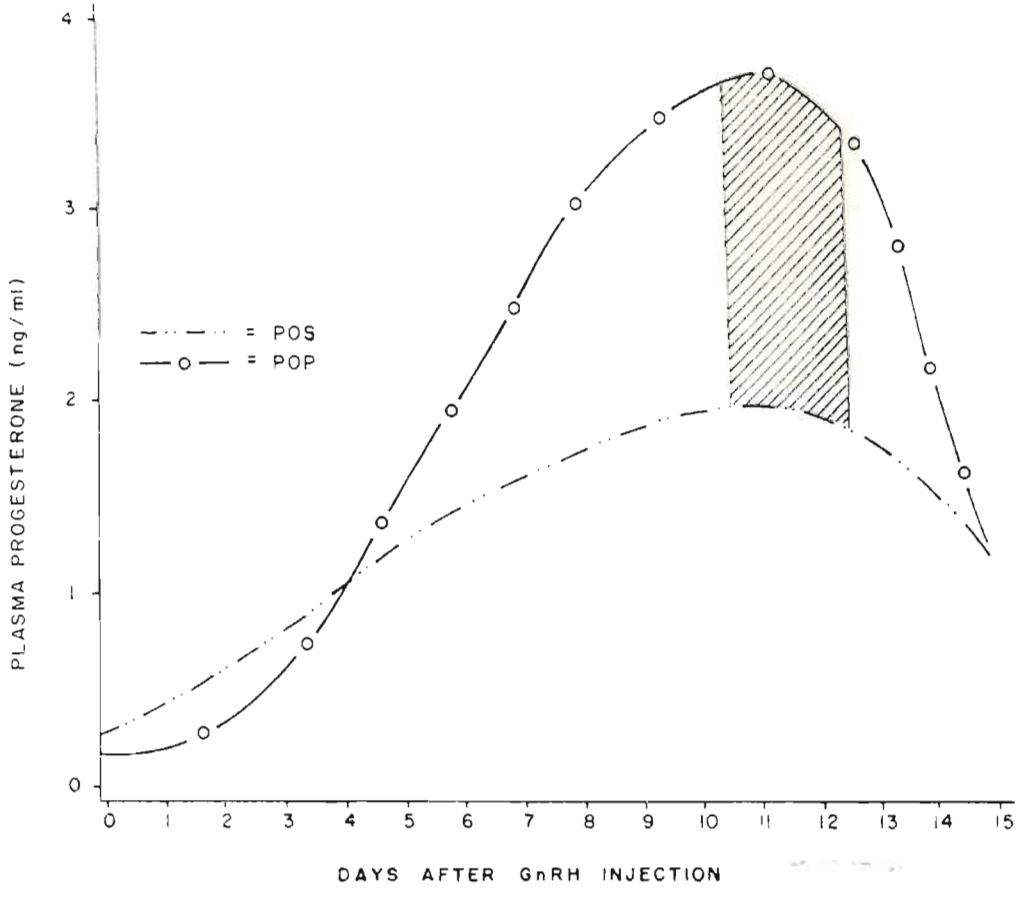


FIG. 6 : Estimated regression of the progesterone concentration on days of the groups receiving PMSG before GnRH (POS) and for those ewes treated both before and after GnRH (POP). The shaded area indicating differences ($p < 0,05$) in concentration of the daily mean values (Experiment I).

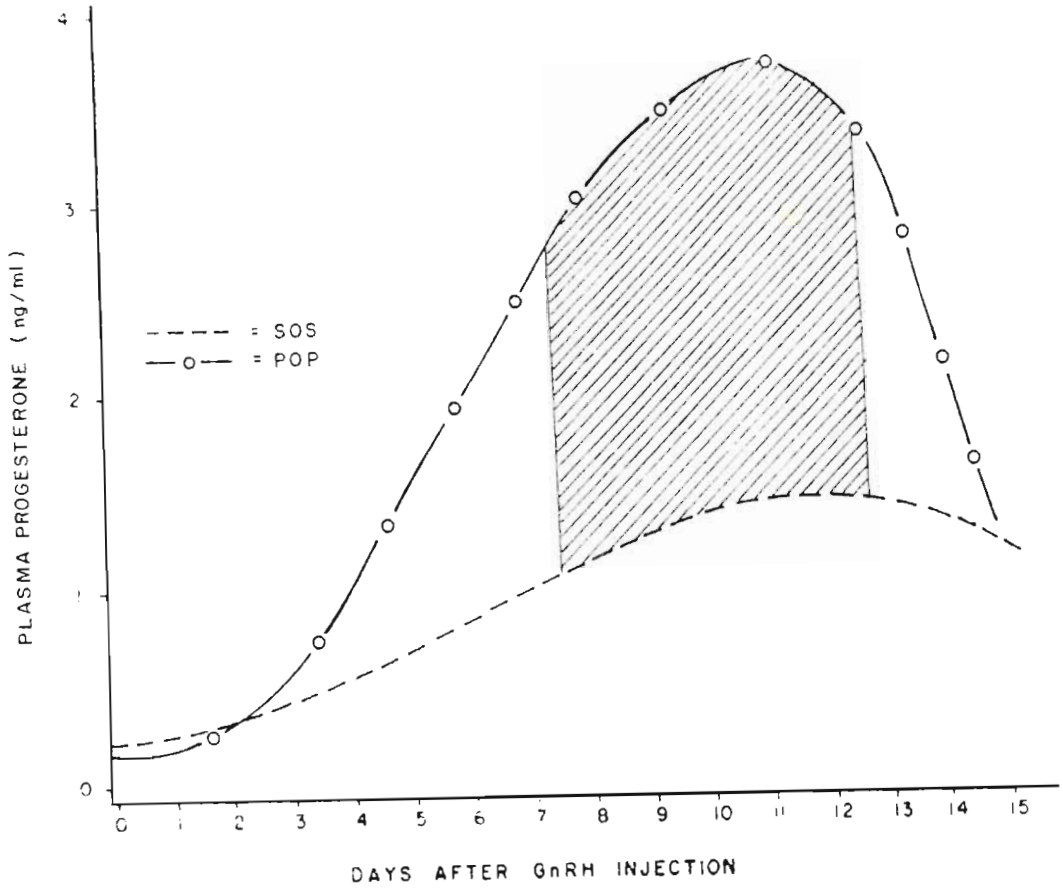


FIG. 7 : Estimated regression of the progesterone concentration on days of the control group (SOS) and for those ewes treated with PMSG both before and after GnRH (POP). The shaded area indicating significant differences ($p < 0,05$) in concentration of the daily mean values (Experiment I).

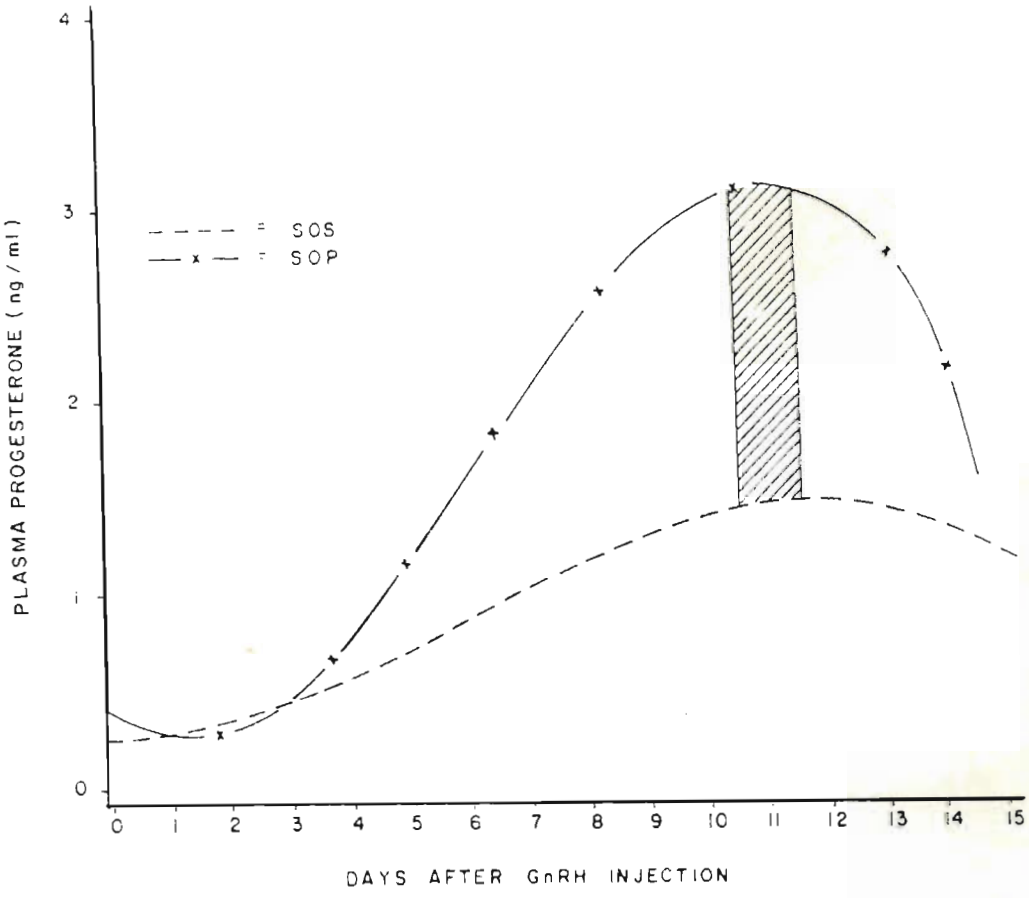


FIG. 8 : Estimated regression of the progesterone concentration on days of the control group (SOS) and for those ewes treated with PMSG after GnRH (SOP). The shaded area indicating significant differences ($p < 0,05$) in concentration of the daily mean values (Experiment I).

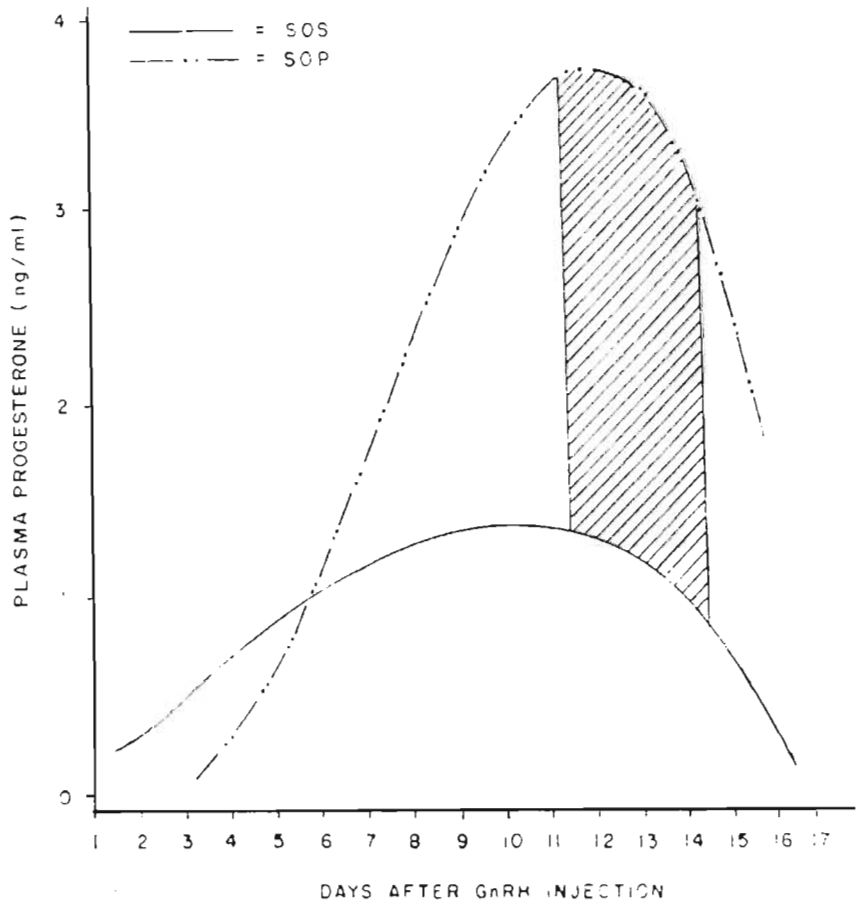


FIG. 9 : Estimated regression of the progesterone concentration on days of the control group (SOS) and for those ewes treated with PMSG after GnRH (SOP). The shaded area indicating significant differences ($p < 0,05$) in concentration of the daily mean values (Experiment II).

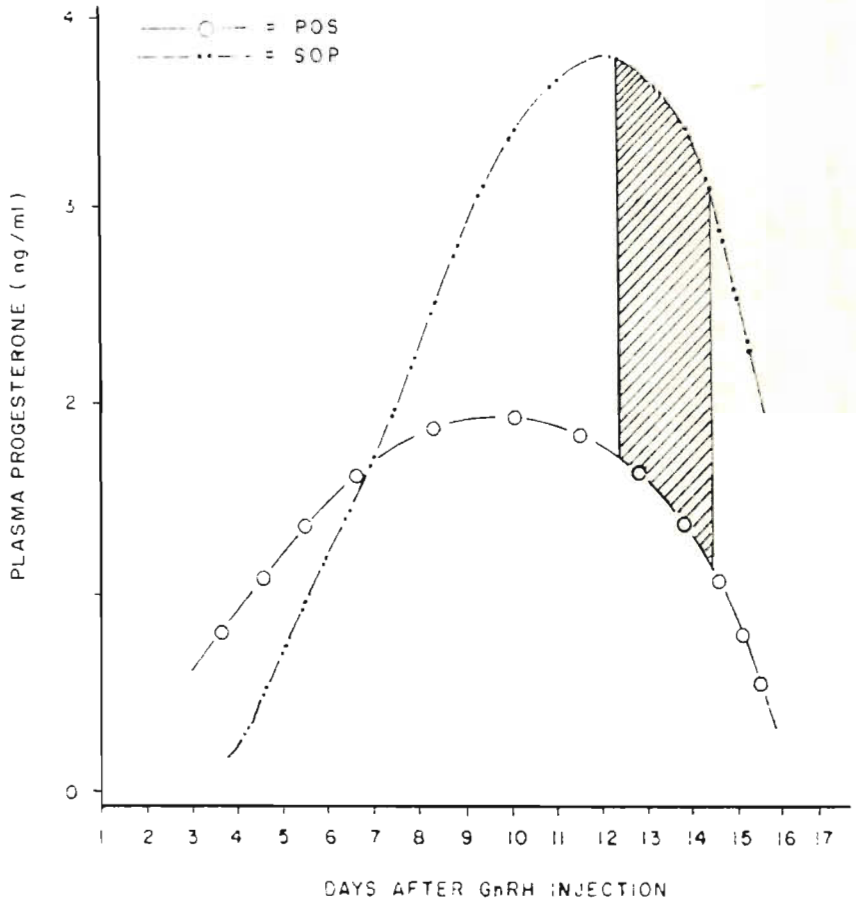


FIG. 10 : Estimated regression of the progesterone concentration on days of the ewes treated with PMSG before GnRH (POS) and those ewes treated after GnRH (SOP). The shaded area indicating significant differences ($p < 0,05$) in concentration of the daily mean values (Experiment II).

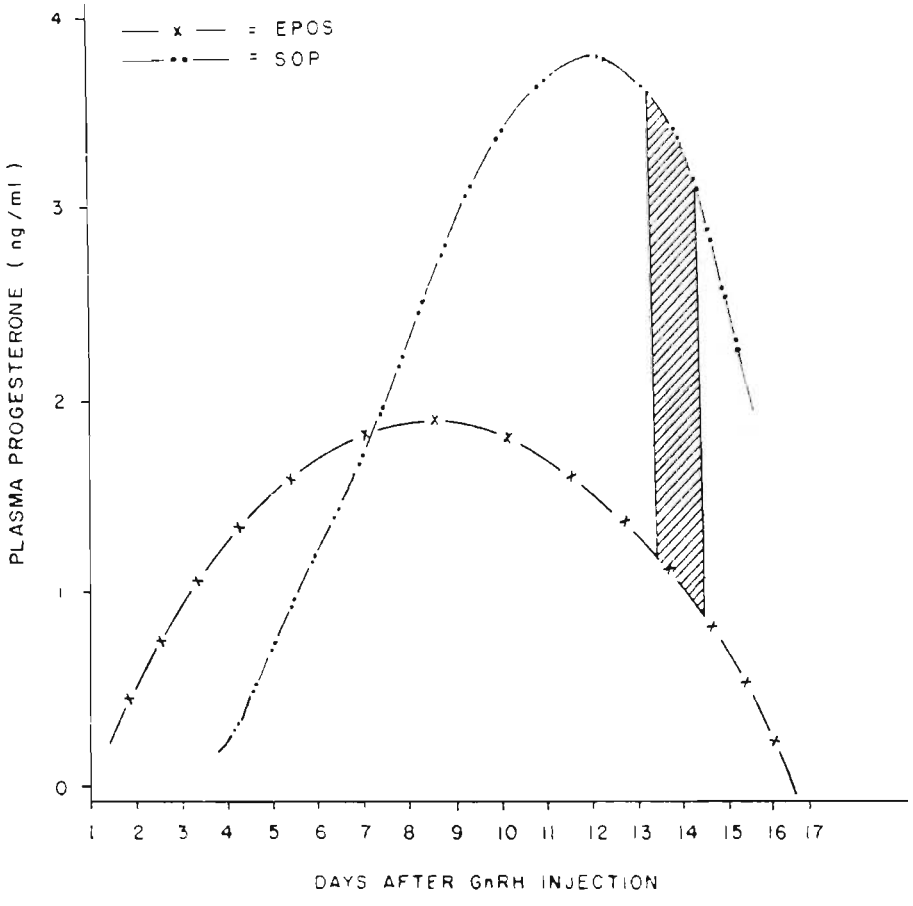


FIG. 11 : Estimated regression of the progesterone concentration on days of the ewes treated with both E2 and PMSG before GnRH (EPOS) and those ewes treated with PMSG after GnRH (SOP). The shaded area indicating significant differences ($p < 0,05$) in concentration of daily mean values (Experiment II).

3.4 Plasma luteinizing hormone

3.4.1 LH secretion curves

From the LH secretion curves after GnRH treatment (Fig. 12 for Experiment I and Fig. 13 for Experiment II) it is apparent that peak concentration for all the treatment groups were reached at approximately the same time (150 minutes after releasing hormone injection). The control group (SOS) reached the highest levels in both experiments with the ewes treated both before and during GnRH administration (POP) being the lowest. PMSG infusion at the time of GnRH injection apparently suppressed LH release. This effect is clearly demonstrated (Fig. 14) where the LH concentration of the ewes infused with saline (SOS and POS) at GnRH injection, or with PMSG (SOP and POP) were combined. The very high correlation of r = 0,98 between experiments for the saline groups (SOS + POS) and a correlation of r = 89 between the PMSG groups (SOP + POP) supports this conclusion. Furthermore, the ewes that received no PMSG at all (SOS), always showed higher values than the SOP group. It is also evident that E2 infusion during the 24 h preceding GnRH, in addition to PMSG infusion (EPOS) suppressed LH release to levels very much lower than for the control group (SOS, Fig. 13). As suspected, the pre-ovulatory LH curve of ewes ovulating naturally (CYC) differs greatly from the GnRH-induced LH curves (Fig. 15). The pre- and post peak slopes are different, the peak values were recorded much later after the values first became elevated and the values remained elevated for an average of 10 hours after the first increase for the ewes that ovulated naturally.

3.4.2 Total LH secretion

The areas under the LH secretional curves were used as a measure of total LH release in response to releasing hormone (Table 18, Experiment I and Table 19, Experiment II). The values for the areas

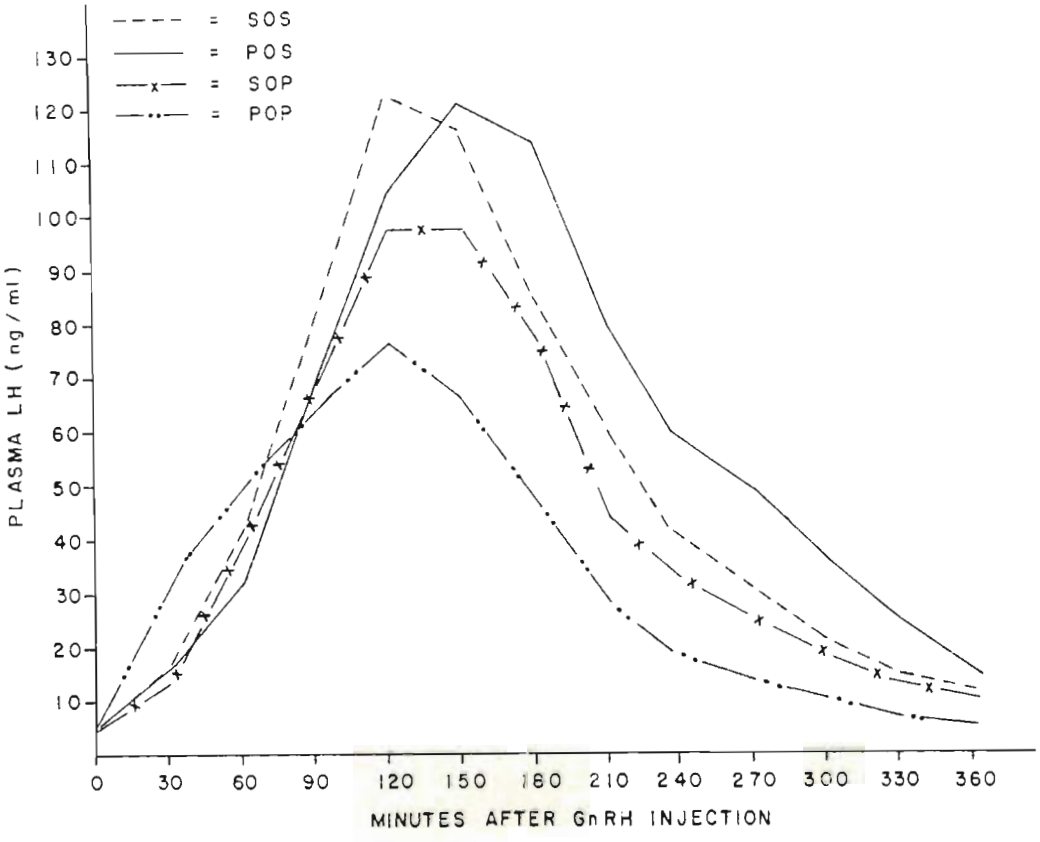


FIG. 12 : Plasma LH concentration (ng/ml) of the control ewes (SOS), ewes infused with PMSG before (POS), during and after (SOP) and before, during and after (POP) GnRH i.m. injection (Experiment I).

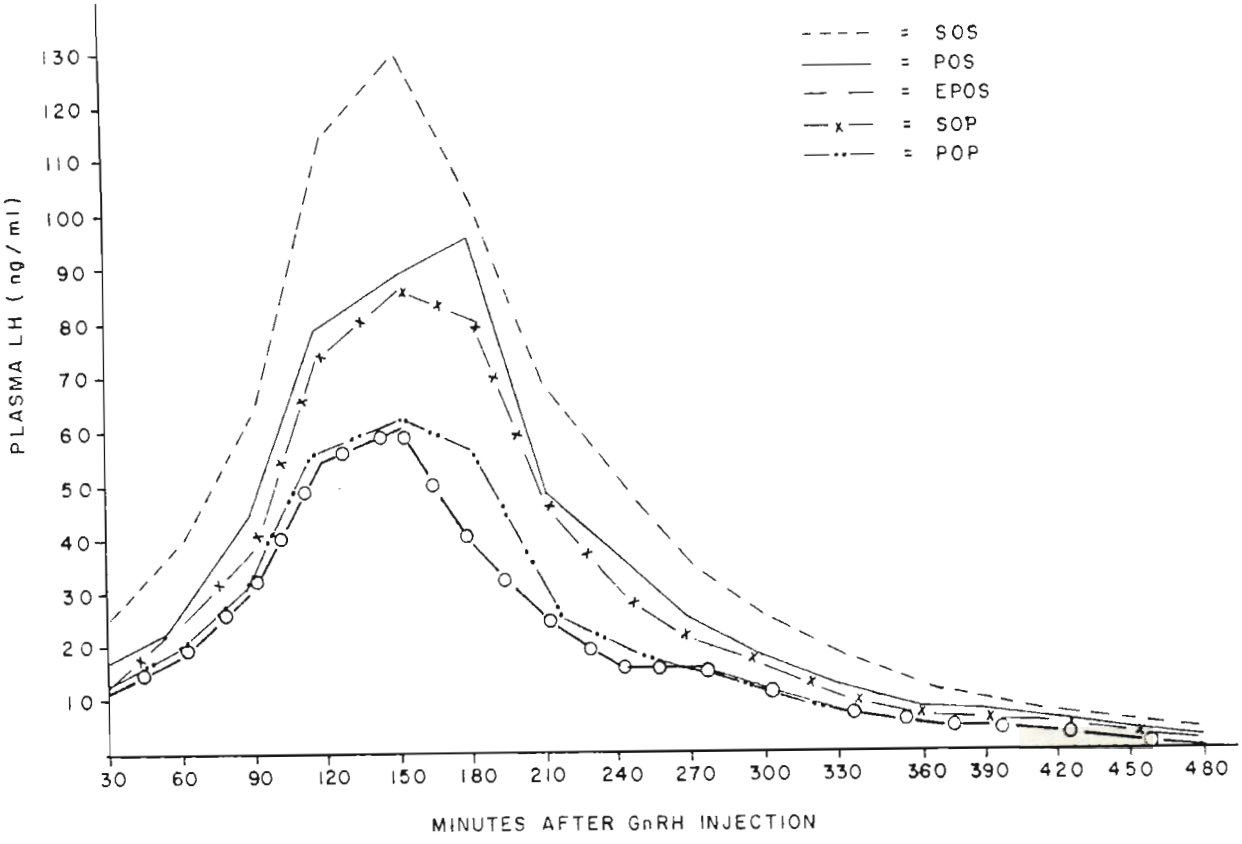


FIG. 13 : Plasma LH concentration (ng/ml) of the control ewes (SOS), ewes infused with PMSG before (POS), during and after (SOP), before, during and after (POP) and infused with PMSG + E2 before (EPOS) GnRH i.m. injection (Experiment II).

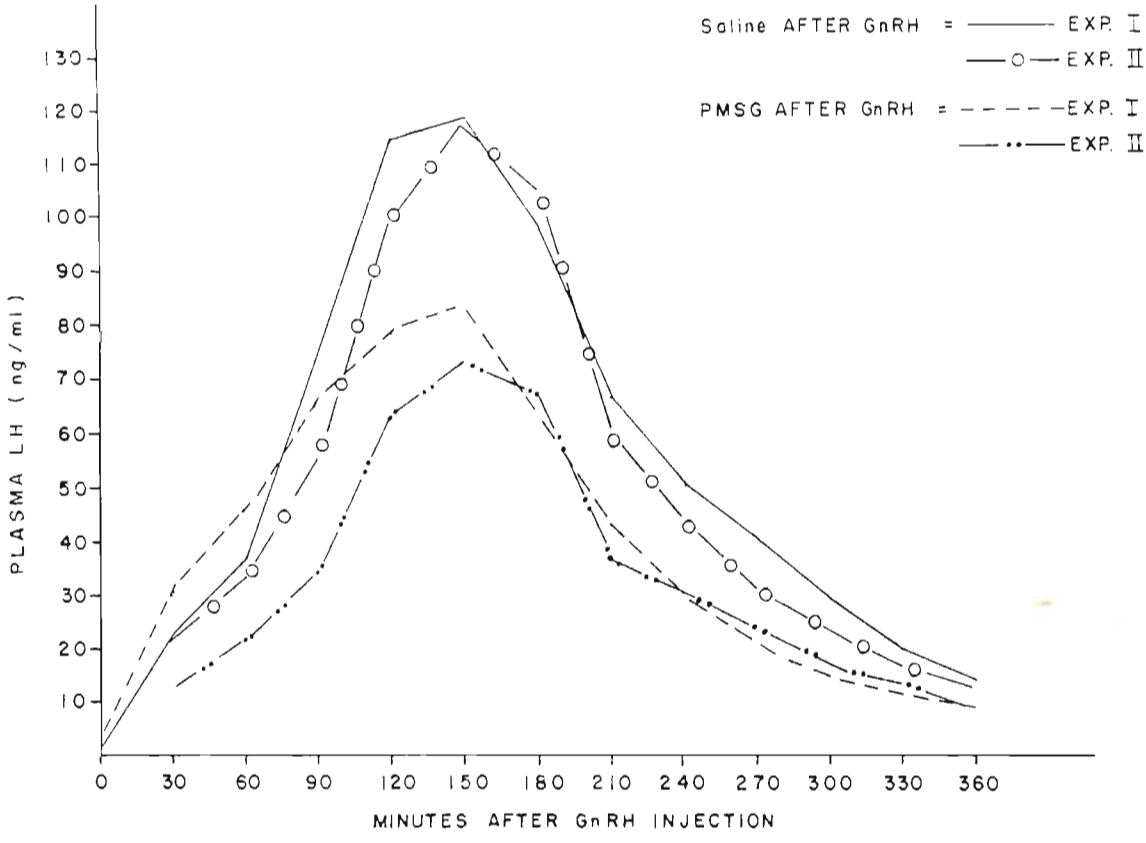


FIG. 14 : Mean plasma LH concentration (ng/ml) of ewes infused with PMSG (SOP + POP) or saline (SOS + POS) during and after GnRH injection.

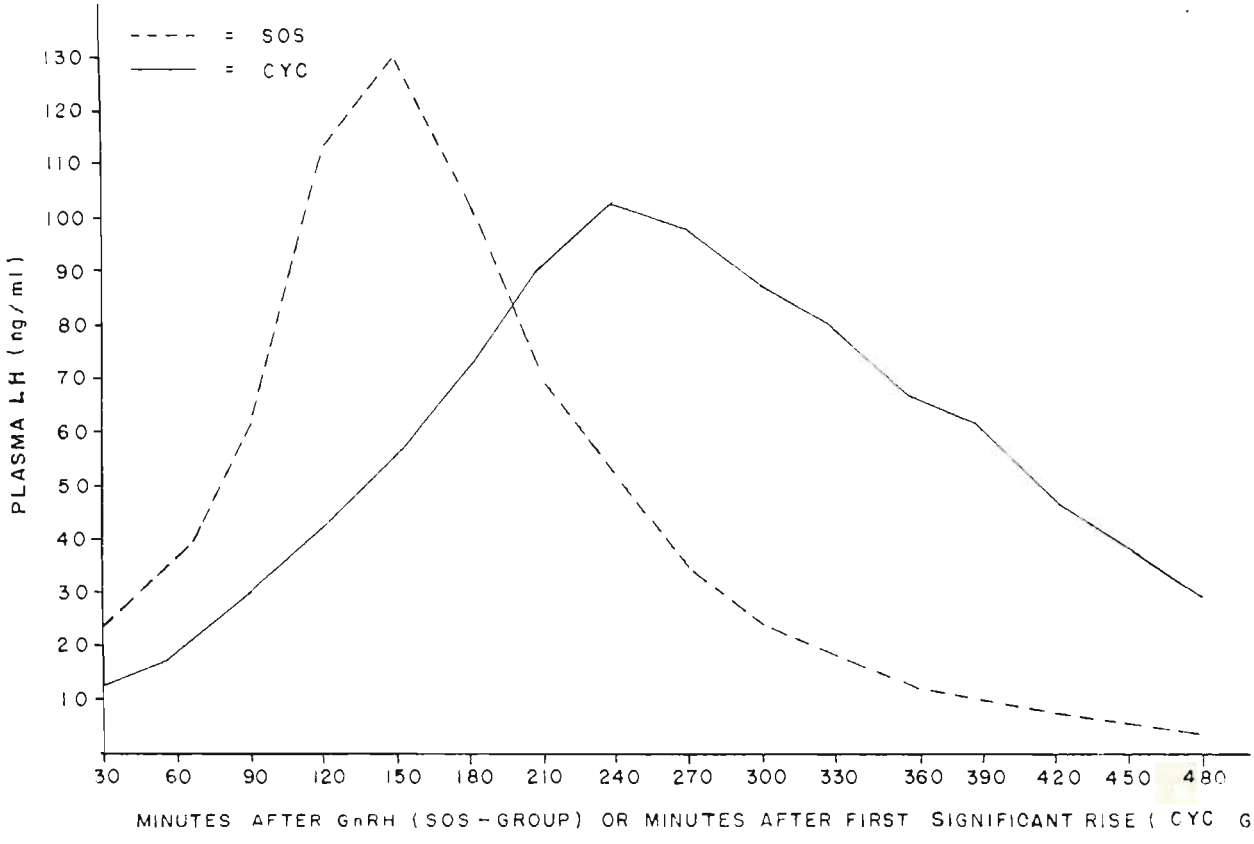


FIG. 15 : Pre-ovulatory LH curve for ewes ovulating naturally (CYC) and GnRH induced ovulations for the control ewes (SOS).

are expressed in arbitrary units. The pituitary response recorded for the treatment groups during Experiment I is not significantly different, there is however a marked tendency for the groups infused with saline during and after GnRH (SOS and POS) to be higher.

TABLE 18 : Area under the LH curve for the control ewes (SOS), ewes infused with PMSG before (POS), during and after (SOP) and before, during and after (POP) an i.m. GnRH injection (Expt. I).

Group	n	Treatment mean ± SeM	As % of SOS mean
POS	7	722,24 ± 168,67	110,6
SOS	7	652,79 ± 107,89	100,0
SOP	6	569,87 ± 95,38	87,2
POP	6	434,65 ± 82,94	66,6

$\bar{x}$ = 602,01 ± 72,00

The value for ewes that ovulated naturally (CYC group) were included in Experiment II (Table 19) and as could be expected from the prolonged duration of the LH peak for these ewes (Fig. 15) the area under the curve was significantly greater than for the other treatment groups.

Oestradiol infusion during the 24 h prior to GnRH treatment suppressed the area under the LH peak in relation to the saline (SOS) group (Table 19). The POP group again had the smallest area under the LH curve (Table 19) as during Experiment I (Table 18). PMSG infusion during and immediately after GnRH injection significantly suppresses LH release in response to releasing hormone (Table 21) and although the values during Experiment I (Table 20) are not significantly different the same trend was observed as in Experiment II.

TABLE 19 : Area under the LH curve for the ewes ovulating naturally (CYC), infused with PMSG before (POS), during and after (SOP), before, during and after (POP) and infused with PMSG + E2 before (EPOS) an i.m. GnRH injection (Expt. II).

Group	n	Treatment mean ± SeM	As % of SOS mean
CYC	4	813,32 ± 127,20	114,1
SOS	8	712,60 ± 95,29	100,0
POS	8	507,09 ± 84,81	71,2
SOP	7	498,19 ± 78,86	68,6
EPOS	8	378,07 ± 98,39	53,1
POP	8	337,08 ± 69,97	47,3

$\bar{x}$ = 516,73 ± 36,93

CYC > EPOS and POP ($p < 0,01$)

SOS > EPOS and POP ($p < 0,01$)

CYC > POS and SOP ($p < 0,05$)

TABLE 20 : Area under the LH curve of ewes infused with saline (SOS + POS) or PMSG (SOP + POP) during and after GnRH administration (Expt. I)

Groups	n	Treatment mean ± SeM	As % of G Mean
SOS + POS	14	687,52 ± 96,86	115,5
SOP + POP	12	502,27 ± 100,59	84,43

TABLE 21 : Area under the LH curve of ewes infused with saline (SOS + POS) or PMSG (SOP + POP) during and after GnRH administration (Expt. II).

Groups	n	Treatment mean ± SeM	As % of G mean
SOS + POS	16	609,85 ± 66,81	118,7
SOP + POP	15	417,64 ± 54,78	81,3

SOS + POS > SOP + POP ($p < 0,05$)

3.4.3 Repeated measures analysis

For the repeated measures analysis the plasma LH levels were transformed to the logarithmic scale as it was found that this was a better description of the regression of LH over time than the absolute values. Treatments had no significant effect on plasma LH concentration during Experiment I (Table 22).

TABLE 22 : Mixed model analysis of variance for plasma LH concentration (log scale) with time as repeated measures (Expt. I).

Source	df	SS	MS	F	Prob.	Tab F[2]
Treatment	3	23,33	7,78	1,48 N.S.	0,25	-
Ewes	22	115,43	5,25	28,86	0,0001	-
Time	12	312,92	26,07	143,46**	-	7,94
Trt x Time	36	14,26	0,40	2,18 N.S.	-	3,05
Error (b)	264	47,99	0,18			

[2] Tab f: Tabulated for for conservative F-tests, Winer (1962)

** $p < 0,01$

However, as indicated by the conservative F-test, "time" had a significant effect on the response curves of the various treatments. The absence of a significant treatment by time effect suggests homogeneous regression curves for the different treatments.

In the repeated measures analysis of Experiment II (Table 23 - the CYC group, ewes ovulating naturally, were excluded because of their vastly different LH curve, Fig. 15), treatments had a significant effect ($p = 0,0008$) on plasma LH concentration. Using the conservative F-test (number of times/number of times; Error (b)/number of times), time significantly influenced the response curves. The slopes, however, were homogeneous, as suggested by the N.S. Treatment x Time F-value

(Table 23).

TABLE 23 : Mixed model analysis of variance for plasma LH concentration (log scale) with time as repeated measures (Expt. II)

Source	df	SS	MS	F	Prob.	Tab F[2]
Treatment	4	58,25	14,56	4,13**	0,008	-
Ewes	30	105,78	3,53	35,83	0,0001	-
Time	15	471,22	31,41	319,25**	-	7,56
Trt x Time	60	7,94	0,13	1,34 N.S.	-	2,69
Error (b)	450	44,28	0,10			

[2] Tab F: Tabulated F for conservative F-tests, Winer (1962)

** $p < 0,01$

TABLE 24 : The orthogonal components of time and tests of significance of each treatment/treatment combination for plasma LH concentration (log scale) during Expt. II.

	Degree of polynomial							
Group	Sums of squares (time)							% Var.[2] 1 - 3 Degree
	Total	Linear	Quad	Cub	Quard	Quin	Higher Order	
SOS	135,43	88,52**	27,15**	16,97**	0,41*	0,44*	1,94	97,94
POS	92,64	57,38**	17,41**	15.31**	0,16	0,68	1,68	97,26
EPOS	68,12	44,85**	6,01**	14,33**	1,08*	0,16	1,68	95,70
SOP	93,77	52,59**	25,25**	13,77**	0,26	0,41	1,48	97,70
POP	99,73	66,15**	11,03**	19,06**	0,98*	0,36	2,14	96,50

* $p < 0,05$ and ** $p < 0,01$ (Conservative F-test)

[2] % Variance accounted for by the first to the third polinomial degrees

In order to examine the response curves of treatments during Experiment

II, over time, as was done for the progesterone concentration, separate analyses were carried out in which the sums of squares for time were subdivided as far as the 5th degree. The orthogonal components of time as well as tests of significance of each treatment/treatment combination are presented in Table 24. The conservative F-test (Winer, 1962) was used.

The fit (until the cubic term) of the sums of squares accounted for approximately 97% of the variance due to time for all the treatment groups.

The method of Deaver (personal communication) was applied to detect differences among the estimated regression curves of LH concentration on time (Fig. 16 - Experiment I and Fig. 17 - Experiment II). The preplanned contrasts for Experiment II (Experiment I, no significant differences) gave the following results:

(a) EPOS vs SOS, POS, SOP & POP

Remainder	df	SS	MS	F
SOS, POS, SOP & POP (pooled)	432	50,33		
EPOS	87	12,04		
Total	519	62,37	0,12	
SOS, POS, EPOS, SOP & POP (pooled)	522	64,81		
Difference	3	2,44	0,81	6,75**

∴ Response curve of ewes infused with PMSG + E2 before GnRH (EPOS) is not parallel to the pooled response curves of SOS, POS, SOP & POP

(b) POP vs SOS, POS & SOP

Remainder	df	SS	MS	F
SOS, POS & SOP (pooled)	312	36,24		
POP	117	11,43		
Total	429	47,67	0,11	
SOS, POS & SOP (pooled)	432	50,33		
Difference	3	2,66	0,89	8,09**

∴ Response curve of ewes infused with PMSG before, during and after GnRH (POP) is not parallel to the pooled response curves of SOS, POS & SOP

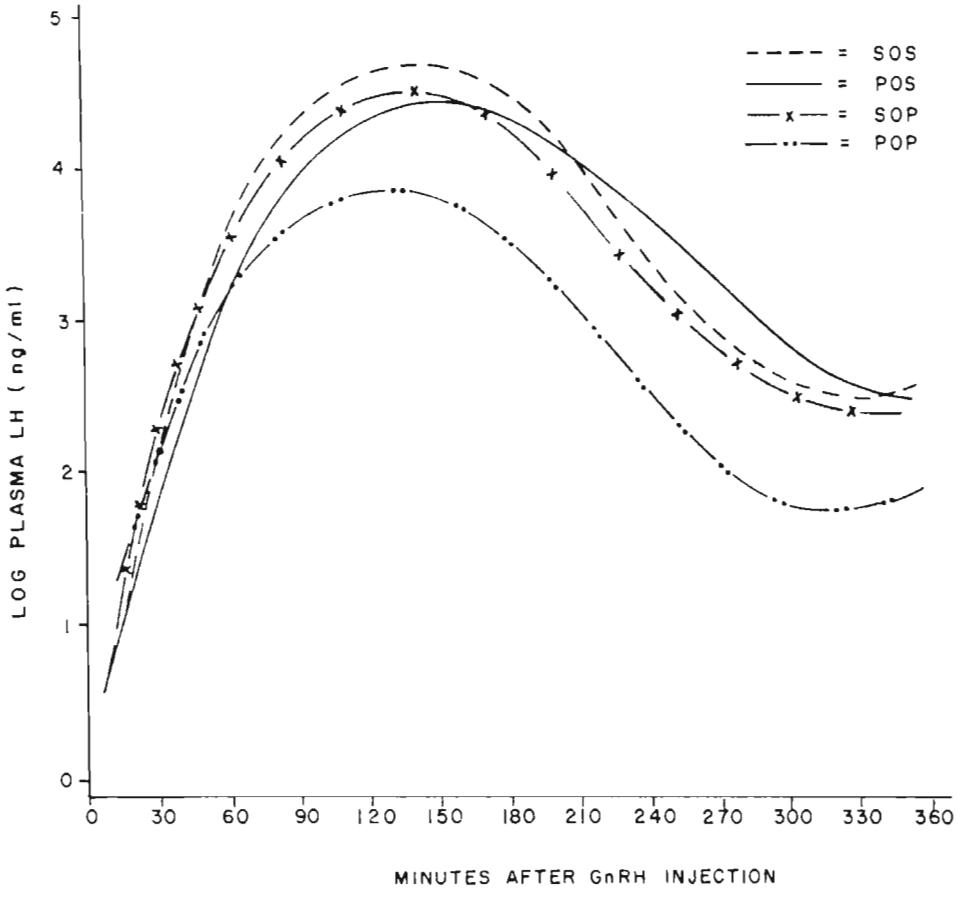


FIG. 16 : Estimated regression of pre-ovulatory LH curves on time (logarithmic concentration) of the control ewes (SOS), ewes infused with PMSG, before (POS), during and after (SOP), and before, during and after (POP) GnRH injection (Experiment I).

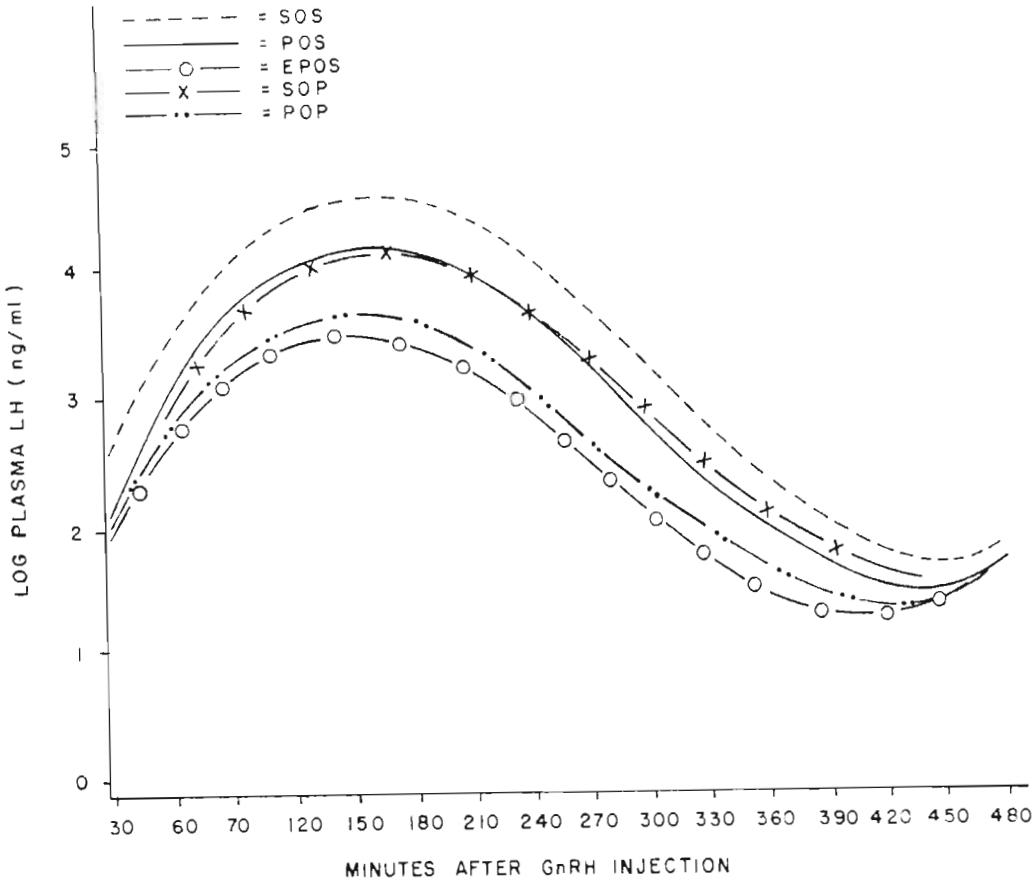


FIG. 17 : Estimated regression of pre-ovulatory LH curves on time (logarithmic concentration) of the control ewes (SOS), ewes infused with PMSG, before (POS), during and after (SOP), before, during and after (POP) and infused with PMSG + E2 before (EPOS) GnRH injection (Experiment II).

(c) SOS vs POS & SOP

Remainder	df	SS	MS	F
SOP & POS (pooled)	192	27,02		
SOS	117	8,32		
Total	309	35,34	0,11	
SOS, POS & SOP (pooled)	312	36,24		
Difference	3	0,90	0,30	2,73 N.S.

∴ Response curve of control ewes (SOS) is parallel to the pooled response curves of POS & SOP

(d) SOP vs POS

Remainder	df	SS	MS	F
POS	102	10,06		
SOP	87	16,32		
Total	189	26,38	0,14	
SOP & POS (pooled)	192	27,02		
Difference	3	0,65	0,22	1,57 N.S.

∴ The response curve of ewes infused with PMSG during and after GnRH (SOP) is parallel to the response curve of ewes infused with PMSG before GnRH (POS).

Points on the response curves for Experiment II where the means (log LH concentration) were significantly different, after simultaneous inferences had been made according to the method of Bonferroni (Millar, 1966) and plotted graphically (Fig. 18 and 19 - the means did not differ significantly during Experiment I).

3.4.4 Tonic plasma luteinizing hormone

In general PMSG raised the tonic LH level before and after ovulation (Table 25). The E2 infusion had no significant effect on tonic LH levels.

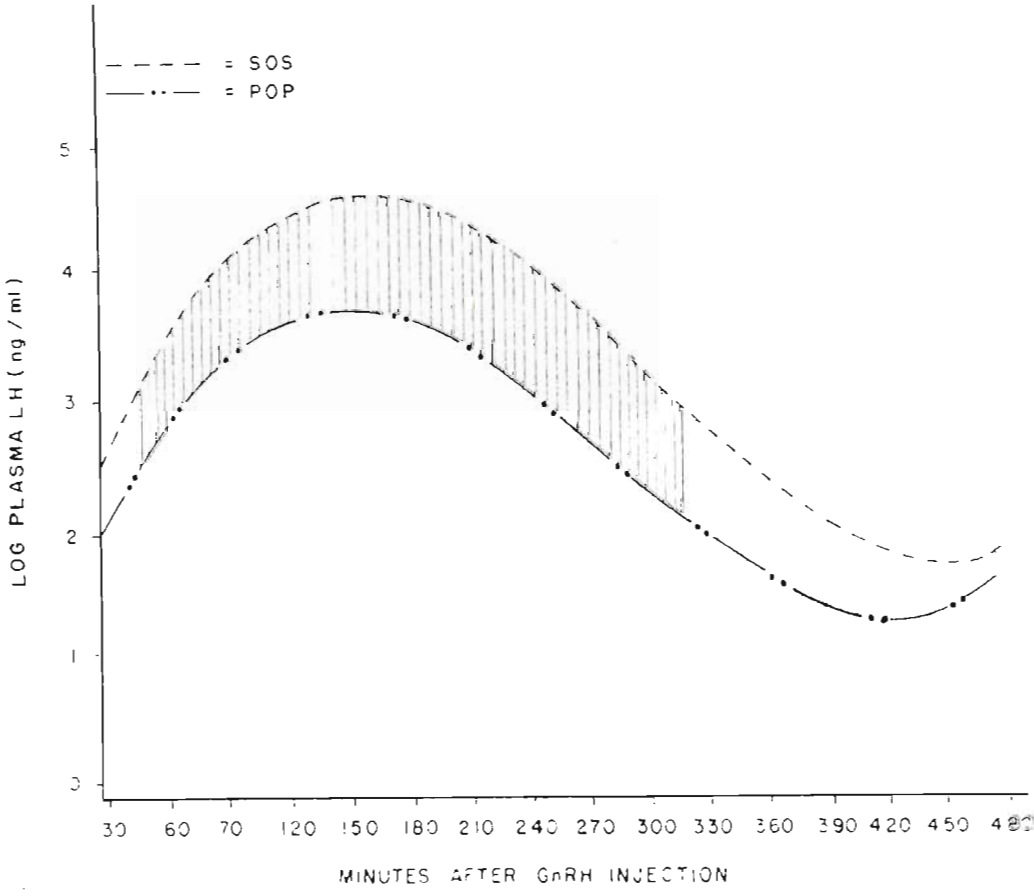


FIG. 18 : Estimated regression of the pre-ovulatory LH curves on time of the control ewes (SOS) and ewes infused with PMSG before and after GnRH (POP) (logarithmic concentration) with the shaded areas indicating significant differences ($p < 0,05$) between the means of sampling periods (Experiment II).

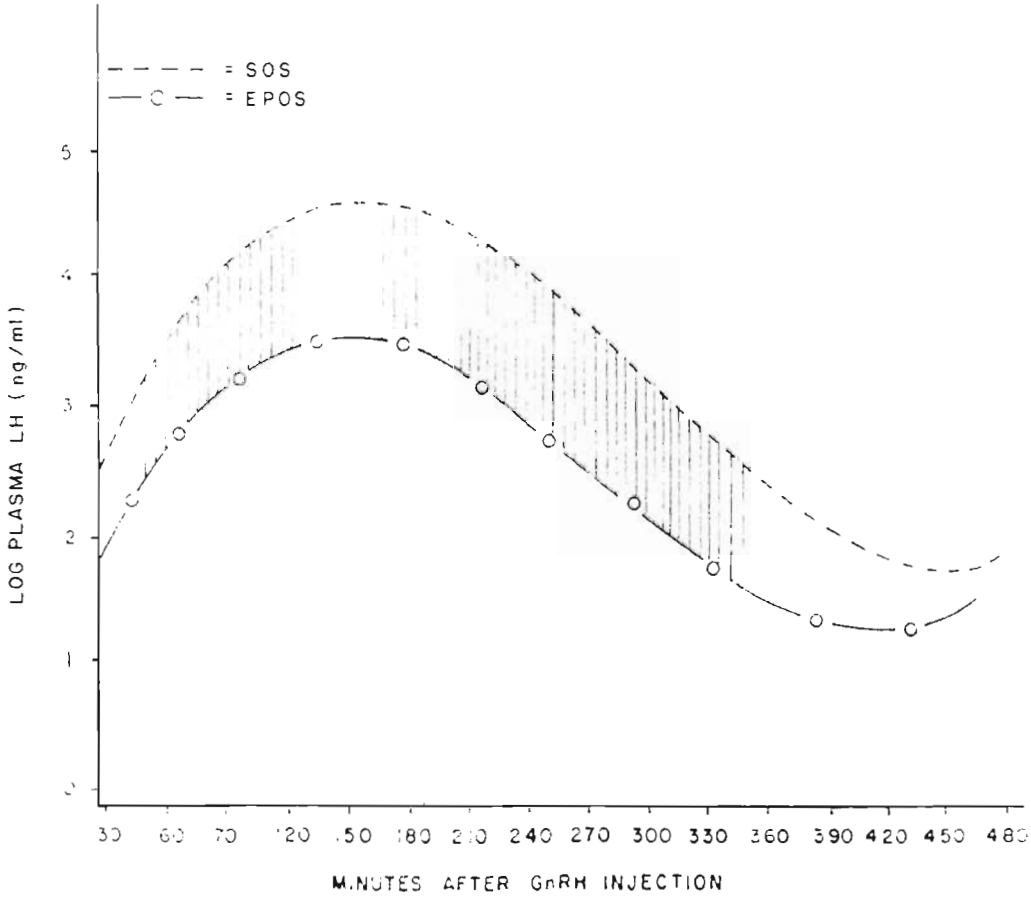


FIG. 19 : Estimated regression of the pre-ovulatory LH curves on time of the control ewes (SOS) and ewes infused with PMSG + E2 before GnRH (EPOS) (logarithmic concentration) with the shaded areas indicating significant differences ($p < 0,05$) between the means of sampling periods (Experiment II).

TABLE 25 : The mean tonic LH concentration on Days -1 and 4 (Day 0 = GnRH) of ewes infused with either saline, PMSG or PMSG + E2 at time of sampling.

Group	Day	n	Infusion*	Mean ± SeM
EPOS	-1	7	EP	2,45 ± 0,37
POP	-1	8	P	2,13 ± 0,23
POS	-1	8	P	1,91 ± 0,16
SOS	-1	8	S	1,87 ± 0,20
SOP	-1	8	S	1,74 ± 0,11
POP	4	8	P	2,01 ± 0,13
SOP	4	8	P	1,98 ± 0,12
EPOS	4	8	S	1,68 ± 0,22
POS	4	8	S	1,66 ± 0,29
SOS	4	8	S	1,59 ± 0,20
$\bar{x}$ P		32	P	2,01 ± 0,16
$\bar{x}$ S		40	S	1,71 ± 0,20

* EP = Oestradiol + PMSG

P = PMSG

S = Saline

Mean of P infusion > mean of S infusion ($p < 0,05$)

4. DISCUSSION

4.1 Ovarian activity

Ovarian examination at laparotomy showed that the quantity of PMSG infused prior to GnRH did not result in a higher ovulation rate. However, more ewes were stimulated to ovulate (71,4% vs. 95,8%) due to priming of the pre-ovulatory follicle by the gonadotrophin. The priming effect could be related to the very low LH and FSH status of the ewe during early post partum (Restall & Starr, 1977), which can be aggrevated by the suckling effect (Thimonier, 1973 as quoted by Restall et al., 1977). A higher release of prolactin (Walton, McNeilly, McNeilly & Cunningham, 1977), may be also implicated in

ovulation failure, although Louw et al. (1976) and McNeilly & Land (1979) could not alter LH levels by the short term suppression of prolactin. The high percentage of ewes that ovulated after the single GnRH injection corresponds with the observations of Haresign et al. (1975), and Crighton (1977), who stated that ovulation could be evoked consistently in anoestrous ewes by using a single injection of GnRH. Kesler et al. (1981), reported 8 out of 12 cows ovulated while McLeod et al. (1982b) noted that only one of 20 ewes did not ovulate and Ainsworth et al. (1982), achieved ovulation in 13 out of 16 ewes.

4.2 Luteal function

The values pertaining to plasma progesterone secreted by corpora lutea resulting from GnRH-induced ovulations in this study tended to be higher than most published results. Whereas Haresign et al. (1975), observed that a single injection of GnRH resulted in a very low level of luteal function (mainly manifested as short-lived corpora lutea) the incidence of short-lived corpora lutea in the present study was very low (5 out of 59 ewes). A corpus luteum was termed "short-lived" if the plasma progesterone levels were found to be lower after Day 7, and remained lower for at least 3 days, than the level during Days 3 - 5. Unfortunately the observations during the laparotomies were of little value in supplying additional evidence as regards the early regression of corpora lutea. The ovarian examinations were conducted too long after GnRH administration and only information as regards active corpora lutea could be gained. Because of the relatively high mean maximum plasma progesterone (1,3 ng/ml) in the 10 ewes where no CL could be observed (5 of which were short-lived) it can be assumed that some corpora lutea were deep within the ovary and were not visible during laparotomy, or else had regressed prematurely. The progesterone values in the ewes

where no CL's were observed correspond remarkably well with those reported on by Wright, Geytenbeek, Clarke & Findlay (1983a) who recorded values of 1,3 ng/ml for ewes treated with GnRH and showed subnormal luteal function. The occurrence of inadequate luteal function in terms of low plasma progesterone was not as drastic when compared to cyclic ewes (see Figs. 2 and 3) nor as poor as the response obtained by Haresign & Lamming (1978) and McNeilly & Land (1979) or the complete absence of luteal activity as reported by Haresign et al. (1975). Fletcher et al. (1980), using the same breed (S.A. Mutton merino) as used in this study reported subnormal peak progesterone concentrations in 70% of the treated ewes. The discrepancy could be due to the different doses and types of synthetic releasing hormones used. Fletcher et al. (1980), gave 3 x 25 µg injections (Cystorelin-Abbot) spaced at 1,5 hour intervals whereas a single injection (Receptal - Hoechst's) of 1 ml (4,2 µg Buserelin) was administered in the present study. Webb et al. (1977) also drew attention to differences in potency between different synthetic gonadotrophin releasing hormones. The possibility of breed differences is very real since Haresign et al. (1975), and Haresign & Lamming (1978), used Clun Forest ewes, while McNeilly & Land (1979) utilized Scottish Blackface ewes. Both breeds could be classified as having a short breeding season, while the S.A. Mutton merino may not be completely sexually inactive during Spring (Botha & Morgenthal, 1980).

In spite of the lower incidence of short-lived CL's and higher plasma progesterone values than recorded elsewhere the control ewes (SOS) in the present study did show subnormal luteal function. The plasma progesterone secretion curves (Fig. 3) suggest lower values than for cycling ewes (CYC) which is also demonstrated by non parallelism of the response curve (SOS) with the pooled response curves of the

cyclic ewes (CYC), ewes infused with PMSG prior to GnRH (POS) and also the ewes infused with both PMSG and E2 prior to GnRH (EPOS). The response curves of the latter three treatment groups were very similar (Fig. 5).

As regards priming before GnRH, both McGovern & Laing (1976) and Haresign & Lamming (1978) showed that PMSG treatment prior to GnRH resulted in corpora lutea capable of increasing plasma progesterone concentration, as was the case in this study (Figs. 2 and 3). The effect was not merely a result of a higher ovulation rate, as no significant differences between treatments for ovulation rate existed. Furthermore, after expressing the progesterone concentration as a function of the number of CL's, the ewes infused with PMSG before GnRH (POS) still had a 30% greater area under the plasma progesterone secretional curve than the control ewes infused with saline only (SOS, Table 10). This trend was maintained throughout the luteal phase (Table 4 and 7). The luteotrophin in sheep is a combination of LH and prolactin (Denamur et al., 1973) and follicular development prior to ovulation is a direct function of gonadotrophin stimulation (Dufour, Cahill & Mauleon, 1979). FSH stimulates the growth and development of the follicles (Greep, 1961) and the action of LH is well established (Short, 1964). Receptors for LH first appear in the thecal cells of small follicles and as the number of receptors increase there is a marked increase in the LH binding capacity of the follicle (Carson, Findlay, Burger & Trounsen, 1979) and after ovulation the number of receptors for LH and the peripheral concentration of progesterone are highly correlated (Diekman, O'Callaghan, Nett & Niswender, 1978). The action of prolactin in the development of the follicle is not clear (Baird & McNeilly, 1981), but all the ewes in the present study most probably were subjected to a prolactin

stimulus evoked by suckling (Fell, Beck, Brown, Catt, Cumming & Goding, 1972; Lamming et al., 1972). Prolactin is known to be present in high levels during early post partum in lactating ewes (Louw et al., 1976). The lower level of luteal function in ewes receiving only GnRH (Figs. 2 and 3) possibly may be related to the lack of a stimulus prior to ovulation (Haresign et al., 1975), the stimulus being gonadotrophin priming of the pre-ovulatory follicle (Haresign & Lamming, 1978). Ovarian acyclicity in post partum ewes is probably due to failure of follicular development as a result of inadequate release of LH, reflecting inadequate release of GnRH (Wright, Geytenbeek, Clarke & Findlay, 1981a). The fact that the infusion of GnRH over short periods initiated cyclic ovarian activity in anoestrous sheep (McNatty et al., 1982b) is in itself support for the theory that gonadotrophin stimulation prior to GnRH is beneficial to luteal function. PMSG administration at a very low level over a period of 72 hours in the present study was applied in order to exert a gonadotrophic effect on the latent follicles so as to stimulate their development and thereby give rise to corpora lutea capable of secreting higher levels of progesterone. The infusion of PMSG resulted in higher tonic LH levels (Table 25) which could thus counteract the inhibitory effects of ovarian hormones on the recovery of the hypothalamo-pituitary axis in the post partum ewes (Wright, Stelmasiak & Anderson, 1983b). Plasma LH levels in post partum ewes are significantly less than those associated with pre-ovulatory follicular development in cyclic ewes (Wright et al., 1983a). The response in terms of plasma LH in post partum ewes to hourly GnRH injections for 48 h demonstrated that these lower levels were not due to insensitivity of the pituitary (Wright et al., 1983a), but rather to a lack of LH.

The administration of GnRH to the post partum ewes in this study evoked an LH peak in all ewes. This agrees with the observations of Haresign et al. (1975), while Hamilton et al. (1979) recorded an LH release in a very high percentage of ewes. Values significantly lower (Foster & Crighton, 1975) or representing only 25% of total release found at natural oestrus (Haresign & Lamming, 1978) have been recorded after GnRH injection. In this study (Table 19) the total release (area under the curve) for the ewes ovulating naturally (CYC) was also substantially greater over an 8 hour period than for the GnRH treated ewes.

As regards events during the peri-ovulatory period, the available evidence seems to suggest that a lower luteal function cannot be ascribed to an inadequate pre-ovulatory LH surge (Crighton et al., 1975; Wright et al., 1983a). It would appear that lower than normal luteal function is due rather to inadequate gonadotrophin priming prior to GnRH than sub-optimal release of LH itself (Haresign & Lamming, 1978). Occurrence of ovulation thus also depends more on the status of the follicle in the ovary and not the magnitude of the pre-ovulatory LH peak (McNeilly & Land, 1979). The inadequate follicular development, according to Wright et al. (1983a), in GnRH treated ewes could be a reflection of inappropriate GnRH treatment manifested as a direct antagonistic action of GnRH on the ovary (Sharpe, 1980), or the action of some other factor associated with post partum anoestrus such as elevated plasma prolactin levels (Wright et al., 1981b). From the results of the present study (Fig. 4 and 5) it would appear that gonadotrophin priming prior to GnRH certainly plays a role in subsequent luteal function. In those ewes infused with PMSG in combination with oestradiol prior to GnRH (EPOS; Table 10), the E2 evoked no additional effect. The total area under the secretional curve was

nearly identical to that of the ewes receiving PMSG but no E2 (POS; Table 10) and the preplanned orthogonal comparisons between the response curves of the two treatments indicated no significant differences. Unfortunately, the levels of oestrogen were not monitored. A single injection of 50 µg oestradiol benzoate 7 hours prior to GnRH resulted in only basal levels (less than 0,5 ng/ml) of progesterone (Haresign & Lamming, 1978), and in beef cows Lishman et al. (1979) could not alter the incidence of, or life span of GnRH-induced corpora lutea by pretreating with oestradiol plus FSH. In the study reported here, the ewes infused with PMSG + E2 (EPOS) had a significantly greater area under the secretional curve for the first 7 days after GnRH than the control ewes (SOS; Table 4), but from Day 10 onwards the plasma progesterone declined to very much the same level of the control ewes (SOS; Fig. 3). This resulted in a significant difference between the daily mean values on Day 14 with the ewes receiving PMSG after GnRH (SOP; Fig. 11). Exactly the same trend was described by Lishman et al. (1979), in beef cows where FSH and oestradiol treatment prior to GnRH tended to increase the progesterone secretion during the first week, only to drop to levels recorded for controls on Day 10. As receptors for LH first appear in the thecal cells of follicles (Carson et al., 1979) and the receptor numbers and concentration of progesterone are highly correlated (Diekman et al., 1978) these elevated levels of plasma progesterone were not totally unexpected. The same pattern was observed in a comparison between PMSG infusion prior to and after GnRH (POS vs. SOP, Fig. 10). The PMSG before GnRH could have promoted the number of luteal cells, resulting in "normal" luteal activity during the first part of the luteal phase, but for this trophic effect to continue, additional PMSG is required (Figs. 6, 10 and 11).

The beneficial effect of PMSG, which has both LH and FSH like properties, (Lamond, 1960) after GnRH on luteal function was clearly demonstrated. Significant differences were identified in the repeated measures analyses (Table 15), the orthogonal response curves suggested possible differences (Fig. 4 and 5), the total progesterone secretion was higher than for the control (SOP vs. SOS; Table 10) and the means on the response curves differed significantly during several days (Figs. 6 to 11). During an earlier study the subnormal luteal function resulting from GnRH administration could not be counteracted by twice daily injections of PMSG (Fletcher et al., 1980). Only 60 I.U. was injected per 24 h (200 I.U. in this study) and as mentioned earlier a different mode of administration and synthetic releasing hormone was used by Fletcher et al. (1980). Other workers also reported a luteotrophic effect of gonadotrophin administration during the luteal phase. As long ago as 1963, using sheep, Short et al. (1963) demonstrated a small and temporary luteotrophic effect after administrating large amounts of LH, FSH and PMSG. Similar results were recorded by Domanski et al. (1967), Kaltenbach et al. (1968), Cook et al. (1969), Karsh et al. (1971), Henricks et al. (1973), Piper & Loucks (1974), Piper & Wells (1974), Guthrie & Knudsen (1981) and Barnes et al. (1982). As the number of granulosa cells do not increase after Day 2 of the oestrous cycle (McClellan, Diekman, Abel & Niswender, 1975) PMSG must have exerted a direct trophic effect on the luteal cells, as normal circulating levels of LH are required for the maintenance of the normal numbers of receptors in the luteal tissue (Diekman, 1978, quoted by Niswender et al., 1981). The possibility arises that PMSG infusion in the present study substituted for the "normal circulating levels of LH." An increase in serum concentration of LH through an i.v. injection results in a dramatic increase in total

number of receptors for LH (Suter, Fletcher, Sluss, Reichert & Niswender, 1980) and thus it also can be speculated that the infusion of PMSG resulted in an increase in "LH like" activity in the plasma. No significant differences in plasma progesterone were recorded between ewes infused after GnRH (SOP) and those infused both before and after (POP) in fact the mean total progesterone secreted (Table 10) was very similar for the 2 treatment groups. The results seem to indicate that gonadotrophin priming both before and after GnRH is not necessary (Figs. 4 and 5), although there is a suggestion of a slight beneficial effect during the early luteal phase (Fig. 5).

The above evidence demonstrates that PMSG infusion did enhance luteal function in terms of progesterone secretion and that the GnRH injection in the control group of ewes did result in subnormal luteal function. An important question that remains to be answered is whether this enhanced luteal function can support pregnancy, and also how does nutrition during late pregnancy influence luteal function subsequently induced with GnRH? In view of the long half life of PMSG (c. 21 h) it would also be of interest to know if infusion of this gonadotrophin can be substituted by injections.

4.3 Pituitary response

Exogenous hormone treatment depressed pituitary response during the present study (Table 19, Fig. 14). Although treatments had no effect on Log LH plasma concentration in Experiment I (Table 22) significantly lower ($p < 0,01$) values in log LH plasma concentration were recorded in Experiment II (Table 23). Haresign & Lamming (1978) reported a significant increase of LH in PMSG primed ewes in response to GnRH, but the doses, routes of administration and timing of the treatments differed from those reported here. In beef cows primed with small

doses of FSH, Lishman et al. (1979) could not alter the pattern of release or maximum concentration of LH resulting from GnRH administration. The FSH treatment was applied through twice daily injections over 3 days, a protocol very similar to the gonadotrophin administration during this study where PMSG was infused over 3 days. PMSG injections however, can reduce the pituitary response to GnRH in beef heifers (Ford & Stormshak, 1975).

It would appear that LH release in response to GnRH is affected to a greater extent if PMSG is infused at the time of releasing hormone administration (Fig. 14, Table 20 and 21). The amount of LH released by the pituitary depends on (Restall et al., 1977):

(i) Change of pituitary sensitivity to GnRH.

(ii) Change in pituitary LH content.

(iii) Increased (or decreased) synthesis of LH. It could be that the pituitary was desensitised by the gonadotrophic action of PMSG, as the response was noted too soon for the treatments to have affected the pituitary content or rate of synthesis over such a short time span.

Whereas in Experiment I, PMSG pretreatment did not influence the pre-ovulatory LH release, in Experiment II when combined with E2 the LH release was significantly suppressed in comparison to the control ewes (Fig. 19). A phenomenon ascribed to the suppression of the hypothalamic pituitary axis by high levels of steroids as during pregnancy in humans (Friedman, Gaeke, Fang & Kim, 1976). Synthetic oestrogen apparently can also depress the pituitary secretion of LH (Thomson, Arfani & Taymor, quoted by Friedman et al., 1976), but generally oestrogen is found to stimulate the pituitary response to GnRH when administered by injection. Poultney et al. (1977) found this to be so when using lactating ewes during autumn and Haresign

& Lamming (1978) recorded a similar effect in anestrous ewes. Hamilton et al. (1979) injected a total of 30 µg oestradiol benzoate over a period of 12 hours and significantly improved the LH release after two spaced GnRH injections, but there was evidence of a double LH peak, suggesting a possible oestrogen-induced and a GnRH-induced peak or 2 GnRH-induced peaks. In contrast to the present finding Wheaton et al. (1982) reported that the release of E2 from subcutaneous implants for 8 days prior to GnRH did not affect the pre-ovulatory LH peak. The findings have been explained on the basis that the repeated stimulation of the pituitary with GnRH causes the gland to become refractory (Crighton et al., 1974; Crighton et al., 1975) and the "down" regulation of the pituitary is the result of administering high doses of GnRH (Knobel, 1980).

4.4 Tonic plasma LH

Episodic releases of tonic LH could not be accurately detected during the current study. The sampling period of 2 hours, with samples every 15 minutes was of too short a duration. During the breeding season the pulses occur "about every 2 hours" (Baird, Swanston & Scaramuzzi, 1976), but only occasionally during anoestrus. They are frequent during the pre-ovulatory period of the oestrous cycle (Scaramuzzi & Baird, 1977). Yuthasastrakosol, Palmer & Howland (1977) recorded an episodic LH peak every 5,6 hours during mid anoestrus, every 6,9 hours during late anoestrus and peaks every 1,5 hours during Days 3 and 14 of the oestrous cycle. During Days 9 and 10 of the oestrous cycle a peak was recorded every 24 hours. Furthermore, in the breeding season during the luteal phase, episodic pulses of LH occur at intervals of approximately 3 hours 20 minutes and approximately every hour during the follicular phase, but with decreased amplitude (Baird, 1978). A rule of thumb as regards the sampling

time so as to be able to interpret results regarding episodic releases with confidence (E.K. Inskeep & D.R. Deaver, personal communication) is to sample for at least 3 times the length of the expected time between pulses. The frequency of sampling should not be less than 5 or 6 data points per cycle. Obviously, the procedure followed in this study was inappropriate.

Administration of LH to ewes stimulated the secretion of oestradiol (McCracken et al., 1969) and HCG, an LH-like stimulus also evokes an increase in circulating oestradiol (Karsch, Legan, Ryan & Foster, 1978), leading to elevated LH levels as illustrated with the infusion of PMSG (Table 25). Simultaneous infusion of E2 with PMSG did not affect tonic LH level (Table 25, EPOS vs. POP).

The possibility that PMSG cross-reacted with the LH antibody in the assay is ruled out by comparing the levels (Table 25) between the POS (PMSG before GnRH) treatment group and the SOS (control) treatment group for both Days -1 and 4. The same order of magnitude is maintained.

CHAPTER II

THE EFFECT OF LOW PROTEIN DURING LATE PREGNANCY AND PMSG INJECTION ON THE ACTIVITY OF GnRH-INDUCED CORPORA LUTEA IN LACTATING EWES

1. INTRODUCTION

The tendency for ewes with greater body mass at parturition to ovulate sooner after lambing than lighter animals (Hunter & Lishman, 1967; Vosloo, Hunter & Carstens, 1969) suggests that this is either an inherent tendency of larger animals or that nutrition prior to parturition may play a role (Lishman, Stielau & Botha, 1974a). A high protein supplement reduced the number of ewes that returned to service (Croker, Lightfoot & Marshall, 1976), whereas a low level of nutrition during early post partum can delay oestrus and suppress ovulation, but not reduce progesterone levels (Shevah, Black & Land, 1975). Also, underfeeding of beef cows during pre- and post partum can delay onset of oestrous cycles following partus (Dunn, Ingalls, Zimmerman & Wiltbank, 1969; Whitman, Remmenga & Wiltbank, 1975).

Treatment with GnRH, 15 days post partum, has been found to reduce the period to first oestrus from 49 to 40 days (Hamilton & Lishman, 1979), but conception was not affected. PMSG treatment before or after GnRH infusions has a marked luteotrophic effect (Chapter I, Figs. 2 and 3) resulting in progesterone secretion at least equal to values recorded in cyclic ewes. Although these levels compare favourably with normal levels it is not known if such GnRH-induced corpora lutea can support pregnancy. Furthermore, it would be of interest to fat lamb producers to establish whether a developing embryo could "rescue" GnRH induced corpora lutea.

The administration of PMSG by s.c. injection is far less cumbersome than infusion and in view of the relative long half life of this

gonadotrophin in sheep (McIntosh, Moor & Allen, 1975) twice daily injections would seem sufficient to maintain elevated biologically active levels in the ewe. The present study was initiated to determine the effect on luteal function of:

(i) a low level protein intake during late pregnancy

(ii) twice daily PMSG administration and

(iii) A.I. after GnRH injection in early post partum ewes.

2. PROCEDURE

During the summer of 1981/82, S.A. Mutton merino ewes were treated with GnRH to induce ovulation. The effect on GnRH-induced luteal function of a diet low in protein during late pregnancy and of PMSG when administered via subcutaneous injections was investigated. The ewes grazed kikuyu pasture (Pennesetum clandestinum) with a DCP content of 9,7% on a DM basis (Grobbelaar & Botha, 1983), or were fed a ration low in protein, during the last 4 weeks of pregnancy (the normal and low protein groups respectively). The low protein ration consisted of:

72% Voermol

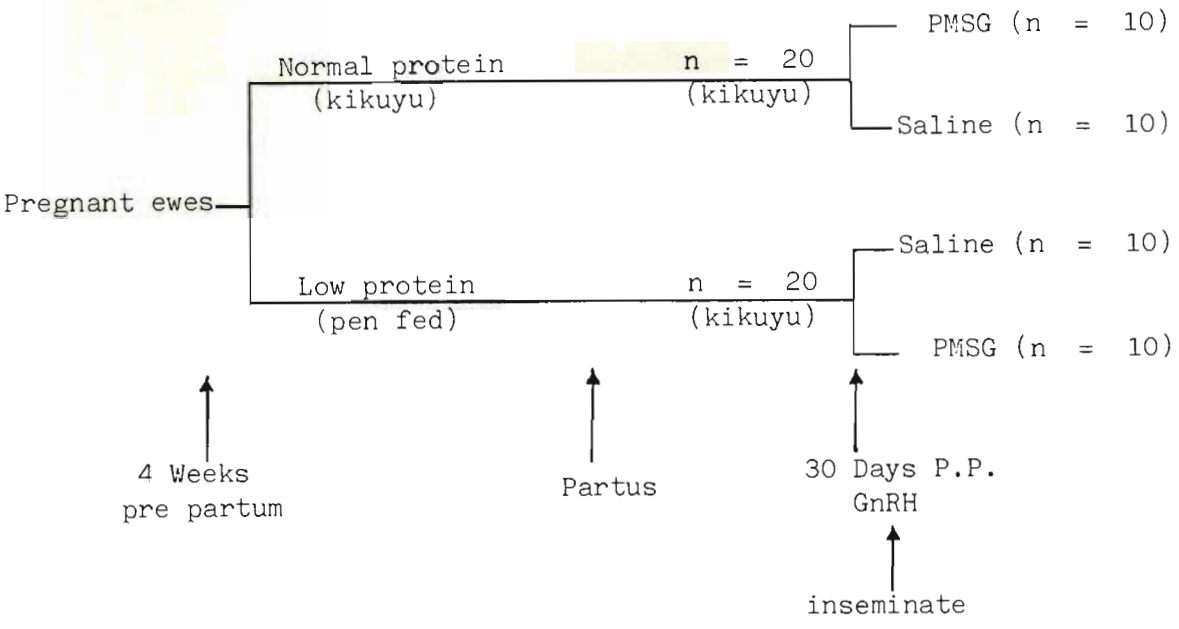
14% Calorie 3 000

14% Maize meal

The daily amount fed was 1 400 g and the estimated crude protein intake was only 53 g per day which is less than 30% of the requirements (N.R.C., 1975). At lambing 20 ewes from both the normal and low protein treatment groups that lambed within 4 days of each other were selected for further study. During lactation all the ewes were grazed on kikuyu for the duration of the experiment.

2.1 Experimental layout and treatments

The experimental layout can be illustrated as follows:-



Ovulation was induced in all the ewes on Day 30 ± 2 post partum (11h00 on 13.1.82) by means of an i.m. injection of 1 ml GnRH (Hoechts - Receptal 1 ml = 0,0042 mg buserelin acetate). At 07h00 and 16h00 (21 h and 32 h after GnRH) on Day 31 post partum all the ewes were inseminated with fresh semen. For the subsequent 16 days, half the ewes (n = 10) that had received the low protein diet prior to lambing and half the ewes (n = 10) fed normal protein levels were given 200 I.U. PMSG per 24 h. The PMSG was administered as 100 I.U. gonadotrophin in sterile water at 06h00 and 18h00 by means of a s.c. injection in the inner thigh of the ewes. The other half of the ewes was injected with normal saline. Alternate thighs were used for each injection. Every 48 h from the 2nd to the 16th day after GnRH injection, blood samples were taken by venipuncture into heparinized syringes. The samples were centrifuged, the plasma aspirated and frozen until assayed for progesterone concentration. From Day 15 to 22 after GnRH injection oestrous detection was carried out with the aid of vasectomized rams.

The body mass of the ewes was determined at lambing and at 42 ± 2 days post partum.

3. RESULTS

3.1 Mass of the ewes

The mean body mass (kg) of the ewes which received the low or normal diets were:

	Lambing	42 ± 2 Days Post partum
Low protein	58,1 ± 1,3 kg	59,0 ± 1,5 kg
Normal protein	67,5 ± 1,6 kg	63,5 ± 1,5 kg

At lambing the difference in body mass between the treatment groups was 16,2%, but this difference diminished to only 7% at 42 days post partum.

The mean birth mass of lambs born to ewes fed recommended levels of protein was 4,8 ± 0,12 kg (n = 29), while the lambs of ewes fed 30% of NRC recommendations (protein) averaged 4,2 ± 0,17 kg (n = 29). Similar distribution of sexes and multiples occurred in the two treatment groups.

3.2 Incidence of oestrous and pregnancy rates

Nineteen of the 40 ewes showed oestrus between Day 16 and Day 20 after GnRH injection. No trends could be observed as regards level of protein intake during late pregnancy or PMSG administration in relation to observed oestrus. It is possible that where no oestrus was detected the ewes experienced a "silent" ovulation. This conclusion is supported by the finding that the progesterone concentration in the plasma of the ewes on Day 21, where oestrus was detected, was the same as where no oestrus was observed viz. 0,79 ± 0,09 ng/ml plasma vs. 0,75 ± 0,22 ng/ml. These values indicate that the oestrous and non-oestrous ewes were in the same luteal stage on Day 21 after GnRH.

None of the ewes lambed as a result of the artificial insemination after GnRH administration.

3.3 Plasma progesterone

The level of protein intake during late pregnancy did not influence the mean plasma progesterone levels (Fig. 1) nor the total quantity of progesterone secreted (Table 1).

The markedly higher plasma progesterone concentrations in the control group indicated that PMSG stimulated luteal function (Fig. 2, Table 2).

TABLE 1 : The mean area under the progesterone curves of lactating ewes fed normal or low protein diets during late pregnancy and injected with GnRH 30 days post partum (Days 2 - 8 and 2 - 16).

Group	n	Mean ± SeM	As % of G mean
Day 2 - 8	40	3,50 ± 0,23	
Normal protein	20	3,57 ± 0,41	101,9
Low protein	20	3,43 ± 0,34	98,1
Day 2 - 16	40	16,60 ± 1,05	
Normal protein	20	16,50 ± 2,05	99,4
Low protein	20	16,69 ± 1,85	100,5

TABLE 2 : The mean area under the progesterone curves of lactating ewes injected with PMSG or saline twice/24 h for 16 days after GnRH administration 30 days post partum (Days 2 - 8 and 2 - 16).

Group	n	Mean ± SeM	As % of G mean
Day 2 - 8	40	3,50 ± 0,23	
PMSG	20	4,38 ± 0,98	125,2
Saline	20	2,62 ± 0,22	74,8
Day 2 - 16	40	16,60 ± 1,05	
PMSG	20	22,27 ± 1,74	134,2
Saline	20	10,93 ± 1,12	65,8

PMSG > Saline (Day 2 - 8; $p < 0,05$ and Day 2 - 16; $p < 0,01$)

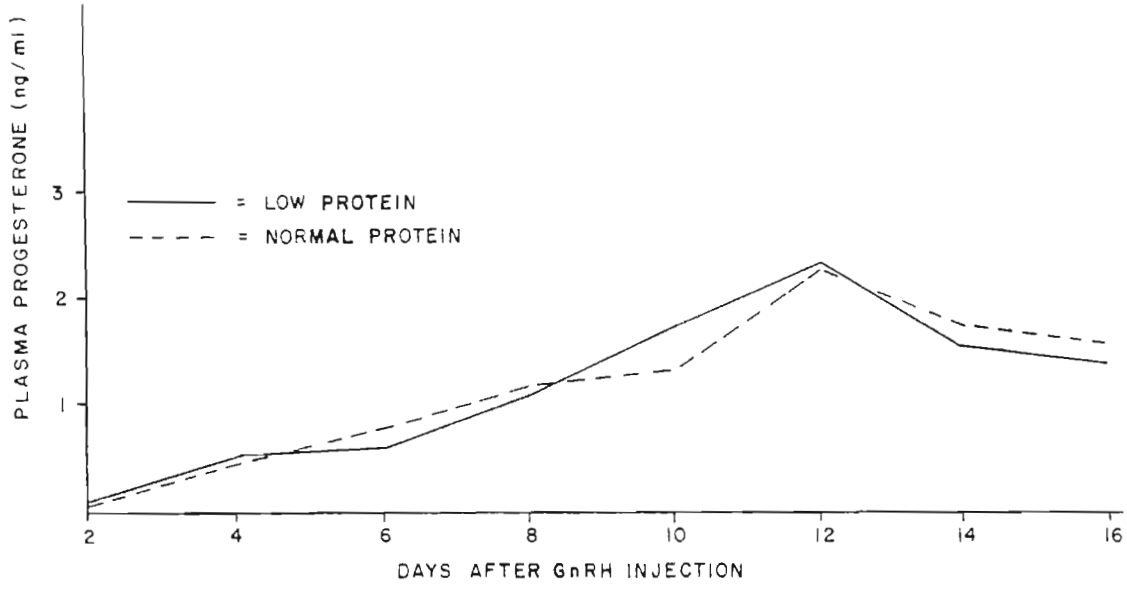


FIG. 1 : Mean plasma progesterone concentration (ng/ml) of lactating ewes fed a normal or low protein diet during late pregnancy and injected with GnRH 30 days post partum.

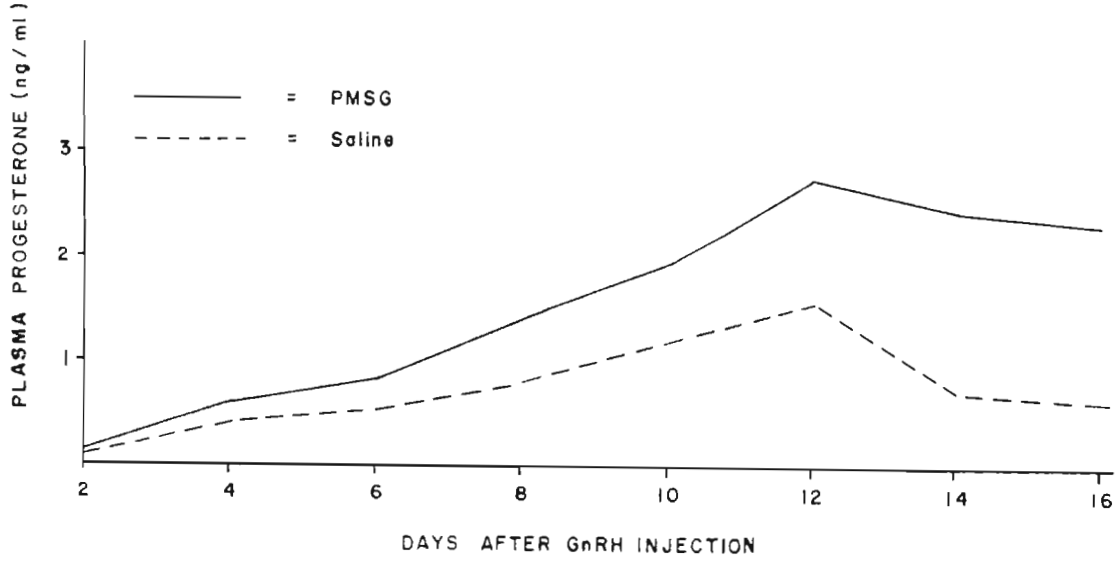


FIG. 2 : Mean plasma progesterone concentration (ng/ml) of lactating ewes injected with PMSG or saline twice/24 h for 16 days after administration of GnRH 30 days post partum.

3.4 Repeated measures analyses

Because of the near identical results the data (Fig. 1) of the normal and low protein groups was not subjected to the repeated measures analysis.

TABLE 3 : Mixed model analysis of variance for plasma progesterone concentration with days as repeated measures for lactating ewes injected twice/24 h with PMSG or saline for 16 days after GnRH administration 30 days post partum.

Source	df	SS	MS	F	Prob.	Tab F[2]
Treatment	1	53,36	53,36	152,6	0,0001	-
Ewe x Trt.	38	55,65	1,46	4,2	0,0001	-
Days	7	123,84	17,69	50,60**	-	7,35
Trt. x Days	7	30,31	4,33	12,39**	-	7,35
Error (b)	264	92,31	0,35			

[2] Tab F: Tabulated F for conservative F-test, Winer (1962)

** $p < 0,01$

PMSG treatment consisting of 2 x 100 I.U. s.c. injections per 24 h evoked a highly significant luteotrophic effect ($p = 0,0001$; (Table 3). Using the conservative test of significance (Winer, 1962), number of days/number of days; error (b)/number of days, the effect of time on plasma progesterone concentration was highly significant. This was expected in view of the curve-like secretional pattern of progesterone during the luteal phase. However, of greater importance was the significant interaction ($p < 0,01$; conservative test) between PMSG and days. This indicated a difference in the response of the 2 treatments during the luteal phase and non-homogeneous regression curves for the treatments.

The orthogonal components of the response curves of treatment over

days were calculated and the tests of significance for the two treatments (PMSG and Control) were conducted (Table 4). Analyses dividing the sums of squares up to the 5th degree were applied.

TABLE 4 : The orthogonal components of time and tests of significance of each treatment for plasma progesterone concentration.

Group	Degree of polynomial							
	Sums of squares (days)							% Var.[2] 1-3 Degree
	Tot.	Linear	Quad.	Cub.	Quard.	Quin	Higher Order	
PMSG	12818,84	11330,76**	577,18**	529,72**	30,76*	229,04*	121,37	99,70
Control	2911,99	866,61**	1219,70**	408,26*	12,38*	307,28**	97,76	85,67

[2] % Variance accounted for by the first to the third order polinomial degrees

* $p < 0,05$

** $p < 0,01$ (conservative F-test [Winer, 1962])

A very large percentage of the variance was accounted for by the sums of squares up to the cubic term.

It is evident from the estimated regression curves (Fig. 3) that PMSG resulted in a highly significant ($p < 0,01$) difference in the response over time as indicated by the significant ($p < 0,01$) treatment x days interaction (Table 3).

The differences between the means of each sampling point as established by the method of simultaneous inferences of Bonferroni (Millar, 1966) were significant from Day 6 - 16 (Fig. 3).

4. DISCUSSION

Although the protein deficiency during this study was very real so

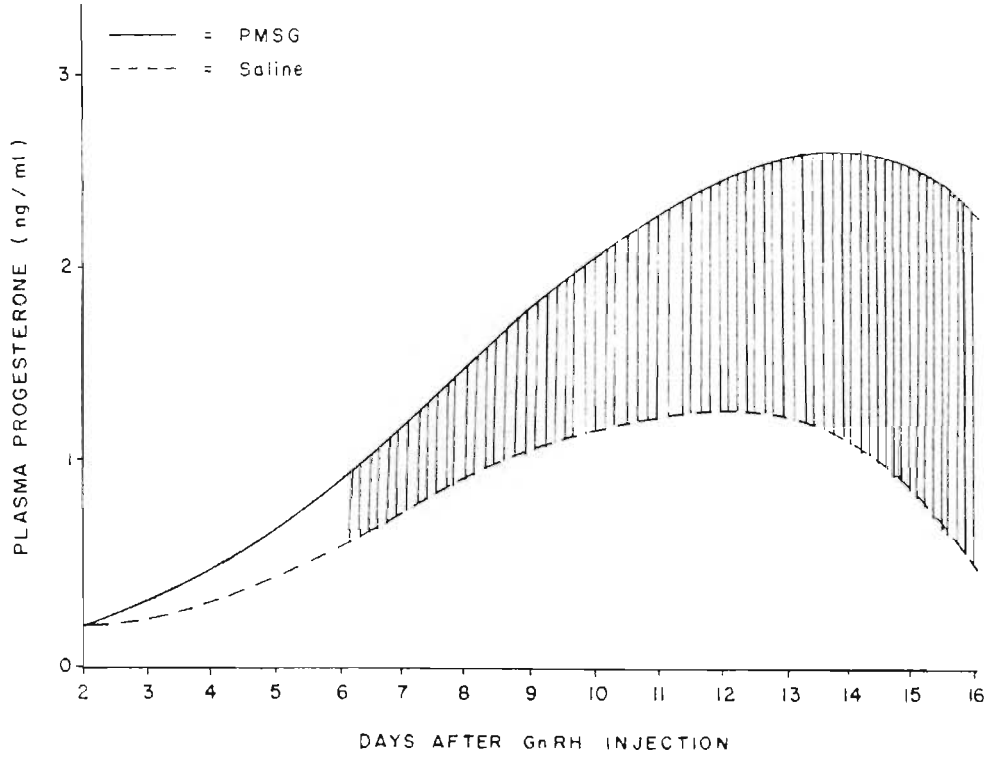


FIG. 3 : Estimated regression of the mean plasma progesterone concentration (ng/ml) of lactating ewes treated with saline or PMSG twice/24 h after GnRH administration 30 days post partum. The shaded areas indicating significant differences between the means ($p < 0,05$).

that lamb birth masses were reduced by 14% over a 4 week period and ewe mass by 16%, the level of protein intake during the last 4 weeks of pregnancy had no visible effect on the plasma progesterone level of the ewes. Under-nutrition of ewes had no marked influence on ovulation (Lishman, Stielau, Swart & Botha, 1974b) nor on total number of ewes served during a breeding period of six weeks (Lishman et al., 1974a). In post partum anoestrous beef cows the concentration of LH in the plasma after the administration of GnRH was reduced by a low level of feeding, but plasma progesterone secretion by corpora lutea induced with GnRH was not affected (Lishman et al., 1979). Artificial insemination after the GnRH-induced ovulation did not result in pregnancy as also reported by Segerson, Ulberg, Martin & Fellows (1974). If ewes are to be successfully rebred by 35 to 38 days post partum then it becomes necessary to induce ovulation and exogenous hormones are required (Ainsworth et al., 1982). Treatment with GnRH on Day 15 post partum has been found to reduce the period to first oestrus in Merino ewes during autumn from 49 to 40 days (Hamilton & Lishman, 1979), but the mean lambing interval was c. 197 days, indicating that the mean number of days from partus to conception was c. 50 days. It is possible that the corpora lutea induced by GnRH during that study (Hamilton & Lishman, 1979) regressed prematurely and that the ewes re-ovulated approximately 7 - 8 days later. The progesterone profiles (Fig. 2) shows that this apparently did not occur in the present study.

One of the factors that may adversely reduce the fertility of ewes soon after lambing is the stage of regression and receptivity of the uterus to the establishment of further pregnancy (Van Niekerk, 1979). In 6 of 16 ewes examined post partum the uteri were inflamed and enlarged on Day 26 post partum. This condition as well as in-

complete uterine involution may limit the number of ewes that can be successfully rebred so soon after lambing (Ainsworth et al., 1982). Lactation can also adversely affect reconception. The quantity of debris in the uterine lumen is greater in lactating than in non lactating ewes (Foote & Call, 1969). This is apart from the fact that the induction of oestrus is less successful in lactating ewes (Restall, Kearins, Hendegen & Carberry, 1978; Rhind, Robinson, Chesworth & Phillippo, 1980). The conditions under which the ewes in this study were inseminated are identical to those which Mauleon (1976) identified as depressing many endocrine parameters, namely the interaction between post partum x lactation x presence of the lambs.

The reasons for the complete failure of the ewes in this study to conceive can be only speculated on. The mean plasma progesterone concentration on Day 21 in the ewes where oestrous was detected within 20 days of A.I. was identical to the mean level in the ewes where no sign of overt oestrus could be detected. This implies that fertilization did not take place in the ewes where no oestrus was detected. These ewes could have undergone a silent ovulation. Poor ovum quality cannot be ruled out as ovulation in GnRH-treated ewes can take place in immature follicles (Segerson et al., 1974; Mauleon, 1976) which will impair fertilization. It is also possible that lack of fertilization is related to the paradoxical antifertility effects of GnRH demonstrated in both animals and humans (Kledzik, Cusan, Auclair, Kelly & Labrie, 1978; Labrie, Auclair, Cusan, Lemay, Belanger, Kelly, Ferland, Azadian-Belanger & Raynaud, 1979).

A positive aspect of this study was the evidence that when PMSG is administered twice daily after releasing hormone this can exert a trophic effect. The plasma progesterone levels of the PMSG group were clearly elevated above those of the control group (Fig. 2).

The area under the secretional curve was significantly larger for both Days 2 - 8 and 2 - 16 in favour of the ewes treated with PMSG (Table 2). The repeated measures analysis (Table 3) as well as the estimated regression curves and the comparisons between the daily means at each point (Fig. 3) support the conclusion. Using the same type of ewes and twice daily injections of PMSG Fletcher et al. (1980) could not rescue the CL from premature regression after GnRH-induced ovulations. As discussed earlier the dose of PMSG (60 I.U. per 24 h) was probably too low and a different synthetic releasing hormone was used. Since PMSG was administered after GnRH injection the greater quantity of progesterone secreted for the ewes treated with PMSG (Table 2) probably was not due to a higher ovulation rate. The ewes were not laparotomized, but as shown earlier in this study PMSG infusion after GnRH injection did not result in a higher ovulation rate in comparison to ewes infused with saline only. During both experiments the ewes received 200 I.U. PMSG per 24 h and although the earlier work was conducted during Spring (Chapter I) and this study during Summer the ewes were from the same flock and comparable as regards stage of reproduction, viz. early post partum.

The mean plasma progesterone levels during this study (Fig. 2) reached a peak of 3 and 1,5 ng/ml plasma on Day 12 of the cycle for the PMSG treated and control ewes, respectively. Both these levels are within the benchmark set for normal luteal function (1,5 ng/ml and an elevation within 4 days of GnRH) by McLeod et al. (1982b). Earlier, during this study (Chapter I, Fig. 3), cyclic ewes had a mean maximum level of 2,5 ng/ml. If this concentration is taken as an indication of normal luteal function, then the progesterone secretion of the ewes receiving GnRH only was subnormal and the PMSG administration via s.c. injections can be said to have resulted in functional corpora lutea.

The failure of the ewes to conceive resulted in the question as to whether a growing embryo can prevent a GnRH-induced corpus luteum to function abnormally, remaining unanswered. A technique involving embryo transfer, which was not possible during this study, will have to be employed to provide a definite answer.

With the role of PMSG as a luteotrophin established unequivocally this poses the question as to whether the action of progesterone on luteal function is mediated via E2 release prior to the pre-ovulatory LH surge or whether the route is a more direct one on the ovary.

C H A P T E R I I I

THE EFFECT OF PROGESTAGEN AND OESTRADIOL PRIMING ON LUTEAL FUNCTION IN SEASONALLY ANOESTRUS GnRH-TREATED EWES

1. INTRODUCTION

It was realized as long ago as 1950 that progesterone plays an important role in hormonally induced ovulations (Robinson, 1950), but progesterone alone given during anoestrus does not necessarily lead to ovulation (Pelletier & Thimonier, 1975). If progesterone is combined with PMSG during post partum anoestrus an oestrous cycle of normal duration is experienced (Oldham & Martin, 1979). In ewes pretreated with progestagen impregnated intravaginal sponges, a single injection of GnRH does not always lead to enhanced luteal function (Lewis et al., 1981), but available evidence suggests that GnRH treatment combined with progesterone more often that not increases luteal activity (Webb et al., 1977; Ainsworth et al., 1982; McLeod et al., 1982b). A progestational phase also prevents the premature regression of ram-induced CL (Oldham & Martin, 1979).

Poor luteal function was reported by Hamilton et al. (1979) after an i.m. injection of oestrogen followed by GnRH, but in beef cows pretreatment with subcutaneous oestradiol implants eliminated the problem of short oestrous cycles when a multiple GnRH injection treatment was applied (Walters et al., 1982). The object of this study was to better describe the effect of progestagen priming on the luteal function of GnRH-induced corpora lutea. The purpose was also to determine whether the action of progesterone pre-treatment on luteal function was direct on the ovary or mediated via E2 release prior to LH release.

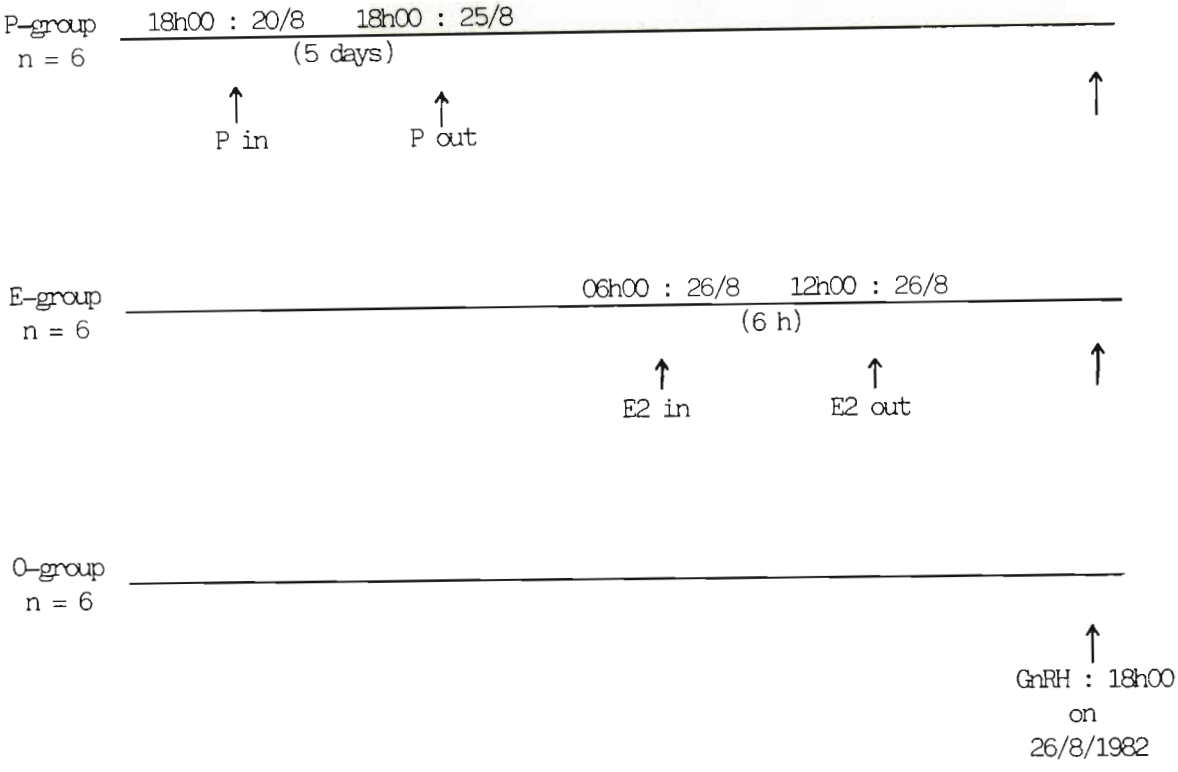
2. PROCEDURE

During early Spring (August, 1982) 18 multiparous dry Corriedale ewes were checked with vasectomized rams to ensure that they were in a state of seasonal anoestrus. The ewes were then randomly allocated to 3 groups of 6 ewes each, the P-group, E-group and the O-group. Prior to injection of all ewes with 1 ml GnRH (0,0042 mg buserelin acetate; Receptal Hoechts) one group (P-group) was pretreated with intra-vaginal progesterone impregnated sponges (Repromap, Tuco 60 mg) for 5 days. The second group of ewes (E-group) received 8,5 mm silicone rubber implants previously found to produce blood levels of 12 pg E2/ml plasma within 2 hours after insertion in ovariectomized S.A. Mutton merino ewes (Liebenberg, 1983). After having been incubated for 48 h at 37°C in 5% BSA in 0,01 M PBS, the implants were introduced s.c. per axilla immediately lateral to the front leg. The third group (O-group) served as a control with no pretreatments. The ewes had all lambed during the preceding Autumn (May, 1982). During the experiment the ewes were housed in individual pens on slattered floors. Each ewe received 1 200 g/day of a ration consisting of 80% lucerne and 20% maize.

2.1 Treatment protocol and blood sampling:

Commencing at sponge withdrawal (24 h prior to GnRH) blood samples to be assayed for E2 were taken from the P-group every 6 h until 18h00 on 26/8/1982, with the last sample being obtained immediately prior to GnRH injection. The assay of Butcher, Collins & Fugo (1974) was used to determine the E2 concentration of the plasma. The E2 implants were inserted at 06h00 on 26/8/1982 (12 h prior to GnRH) and removed at 12h00 of the same day (6 h prior to GnRH). Blood samples were taken at 06h00, 12h00 and 18h00 for assay of the E2 concentration. The ewes of the O-group (Controls) received only

a GnRH injection at 18h00 on 26/8/1982 and in these ewes blood samples for the determination of E2 were taken at 06h00, 12h00 and 18h00 on the same day. Commencing on the day following GnRH injection blood samples (for progesterone assay) from all 18 ewes were taken at 48 hour intervals until 15 days after the administration of releasing hormone. The experimental layout can be illustrated as follows:



3. RESULTS

3.1 Plasma oestradiol

By 6 hours prior to the GnRH injection the mean E2 plasma concentration in the ewes of both the P and E groups had risen to more than 8 pg/ml (Fig. 1). In contrast the concentration for the ewes in the O-group remained at less than 1 pg/ml plasma (Table 1).

TABLE 1 : Mean plasma E2 concentration (pg/ml) ± Sem of ewes pretreated with progestagen for 5 days (P-group), E2 silicone rubber implants for 6 h (E-group) and the control ewes (O-group).

Hours prior to GnRH	Plasma E2 concentration (pg/ml)		
	P-group	E-group	O-group
-24	1< Sponges out	-	-
-18	1<	-	-
-12	0,88 ± 0,11	1<	1<
- 6	8,18 ± 1,52	8,03 ± 1,74	1<
0	3,08 ± 0,66	2,19 ± 0,39	1<

3.2 Plasma progesterone

Progestagen pretreatment for 5 days and sponge removal 24 h prior to GnRH resulted in levels of progesterone markedly higher than those from E2 pretreatment and the control ewes.

TABLE 2 : Areas under the progesterone curve, of anoestrus ewes injected with GnRH after primed for 5 days with intra-vaginal progestagen sponges (P), for 6 h with E2 silicone rubber implants (E) and control (O) ewes (Days 1 - 7).

Group	n	Treatment mean ± SeM	As % of O mean
P	6	2,28 ± 0,14	108,6
E	6	2,18 ± 0,70	103,8
O	6	2,10 ± 0,44	100,0

$\bar{x}$ = 2,17 ± 0,28

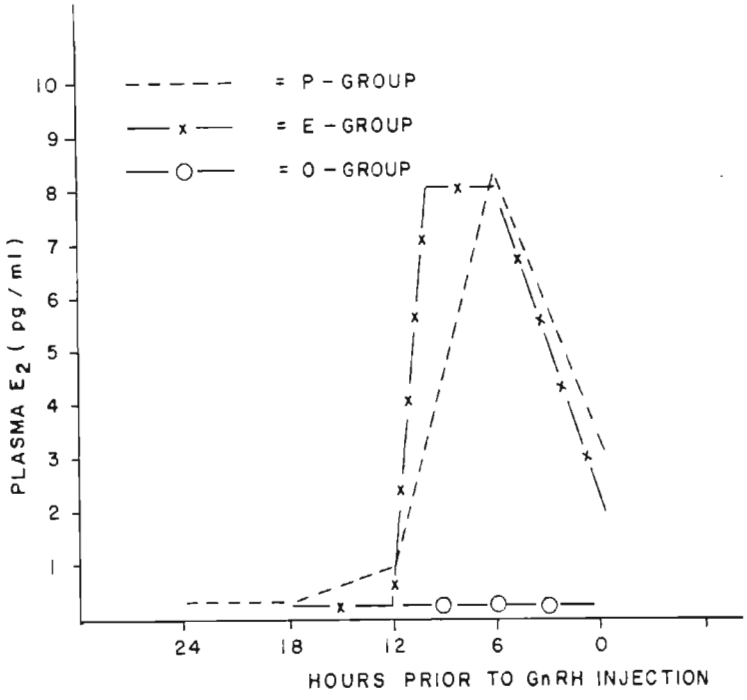


FIG. 1 : The mean plasma E2 concentration (pg/ml) of anoestrus ewes treated with intra-vaginal progestagen sponges for 5 days (P-group), E2 silicone rubber implants for 6 h (E-group) and control ewes (O-group).

As could be expected from the results in Fig. 2, the differences in progesterone secretion between the treatments (area under the progesterone secretional curves) during the first 7 days were small and non significant (Table 2).

The area under the secretional curve for the entire oestrous cycle (Table 3) was significantly greater for the P-group than for the O-group, indicating that the steroid was secreted in much larger quantities in ewes primed with progestagen.

TABLE 3 : Areas under the progesterone curve of anoestrus ewes injected with GnRH after primed for 5 days with intravaginal progestagen sponges (P), for 6 h with E2 silicone rubber implants (E) and control (O) ewes (Days 1 - 15).

Group	n	Treatment mean ± SeM	As % of O mean
P	6	11,07 ± 0,77	194,2
E	6	7,16 ± 1,98	125,6
O	6	5,70 ± 0,41	100,0

$\bar{x} = 7,98 \pm 0,85$

$P > 0 \quad (p < 0,01)$

3.3 Repeated measures analyses

Using the conservative F-test (Winer, 1962), the effect of time on concentration of progesterone was found to be highly significant (Table 4, $p < 0,01$). However, the significant ($p < 0,05$ conservative test) interaction between treatment and days, was of greater importance and indicated a difference in the response of the ewes to the different treatments which manifested itself during the luteal phase.

In order to examine the response curves of treatment over time the orthogonal components of the response curves of treatments over days

were determined.

TABLE 4 : Mixed model analysis of variance for plasma progesterone concentration with days as repeated measures.

Source	df	SS	MS	F	Prob	Tab F^2
Treatment	2	3,74	1,87	4,38*	0,03	-
Ewe x Trt	15	6,40	0,43	6,58	0,0001	-
Days	7	7,76	1,11	17,10**	-	8,68
Trt x Days	14	3,47	0,25	3,82*	-	3,69
Error (b)	105	6,81	0,06			

Tab F^2 : Tabulated F for conservative F-tests, Winer (1962).

* $p < 0,05$

** $p < 0,01$

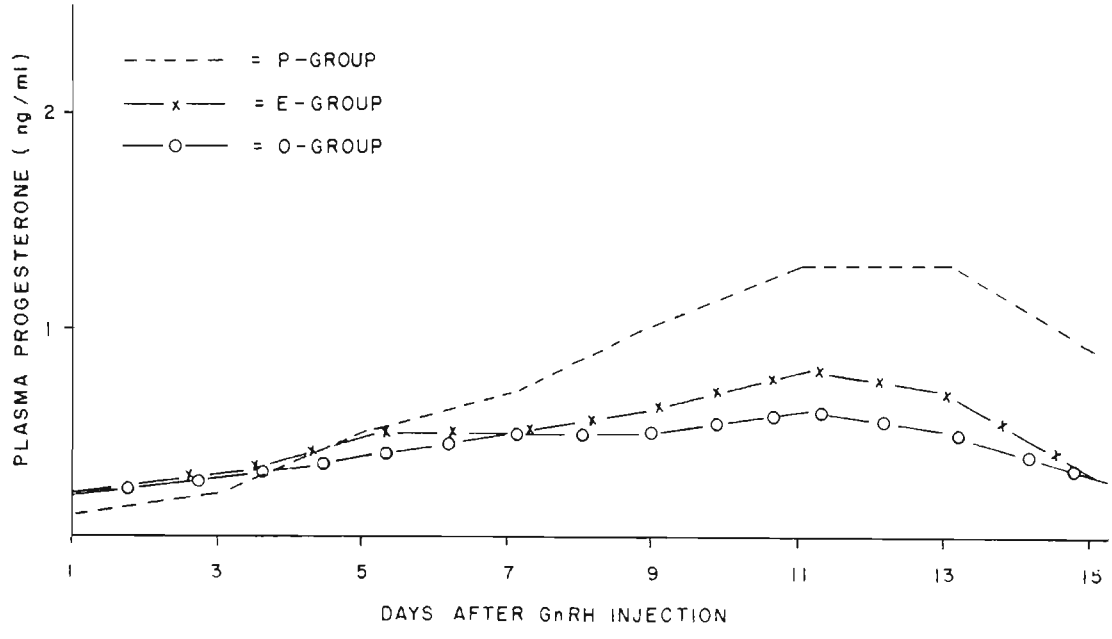


FIG. 2 : The mean plasma progesterone concentration (ng/ml) of anoestrus ewes injected with GnRH after primed for 5 days with intra-vaginal progestagen sponges (P), for 6 h with E2 silicone rubber implants (E) and control (O) ewes.

The tests of significance for each of the treatments were also conducted (Table 5). Analyses dividing the sums of squares for days up to the 5th degree were carried out, showing that the sums of squares up to and including the cubic term accounted for 89 - 99% of the variance between days. The conservative F-test of Winer (1962) was used.

TABLE 5 : The orthogonal components of time and tests of significance of each treatment for plasma progesterone concentration.

	Degree of polynomial							
Group	Sums of squares (days)							% Var[2] 1-3
	Total	Linear	Quad	Cub	Quard	Quin	Higher Order	Degree
P	8,42	7,05**	0,64**	0,65**	0,01	0,01	0,05	99,05
E	1,64	0,92*	0,45	0,09	0,07	0,00	0,11	89,02
O	0,99	0,05	0,80*	0,11	0,22	0,00	0,01	96,97

[2] % Variance accounted for by the first to the third order polinomial degrees

* $p < 0,05$

** $p < 0,01$

The estimated regression curves for progesterone concentration on days (Fig. 3) were tested for parallelism as suggested by Deaver (personal communication). From the preplanned contrasts the following results were obtained:

(a) P-group vs. E- and O-groups:

Remainder	df	SS	MS	F
E & O-groups (pooled)	81	6,48		
P-group	39	0,92		
Total	120	7,40	0.06	
P, E & O-groups (pooled)	123	10,41		
Difference	3	3,01	1,00	16,67**

∴ Response curve of ewes pretreated for 5 days with progestagen (P-group) is not parallel to the pooled response curves of the E and O-groups.

(b) E vs. O-group:

Remainder	df	SS	MS	F
E-group	39	4,28		
O-group	39	1,89		
Total	78	6,17	0,08	
E and O-groups (pooled)	81	6,48		
Difference	3	0,31	0,10	1,29 N.S.

∴ Response curve of ewes pretreated for 6 h (E-group) is parallel to the response curve of the control ewes (O-group).

In order to establish during which intervals the points on the response curve differed significantly, simultaneous inferences on the means at each sampling point were made for the progestagen-treated and control ewes (Fig. 4) according to the method of Bonferroni (Millar, 1966).

4. DISCUSSION

The development and maturation of ovarian follicles is intimately dependant on the sequential action and interaction of pituitary and ovarian steroid hormones on follicular cell differentiation (Richards,

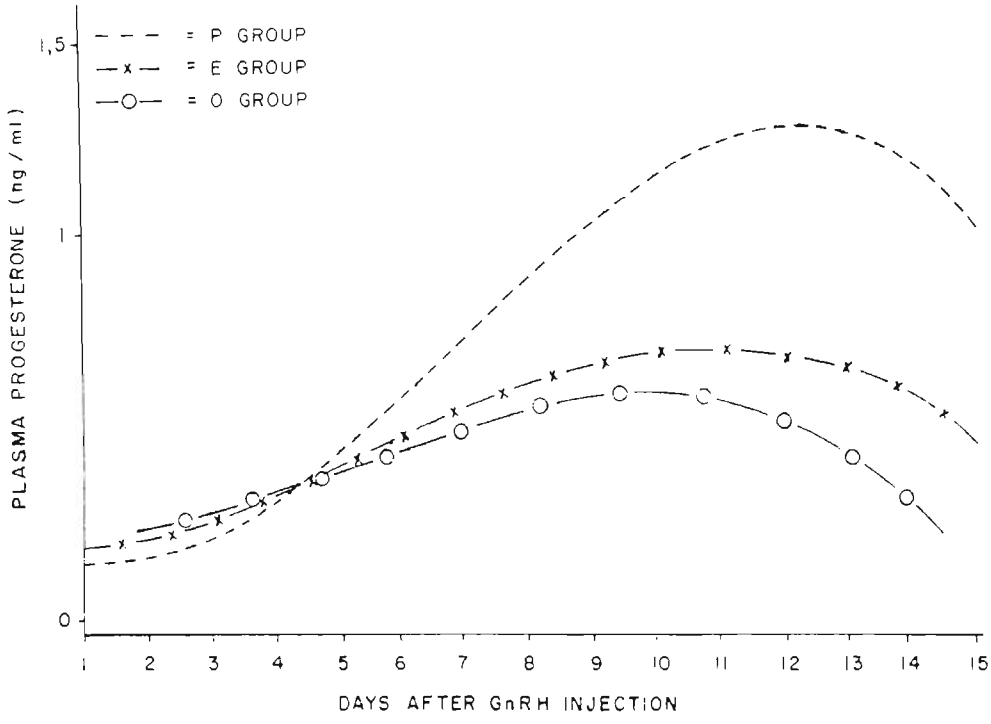


FIG. 3 : Estimated regression of mean plasma progesterone concentration on days of anoestrus ewes injected with GnRH after primed for 5 days with progestagen (P) for 6 h with E2 implants (E) and control ewes (O).

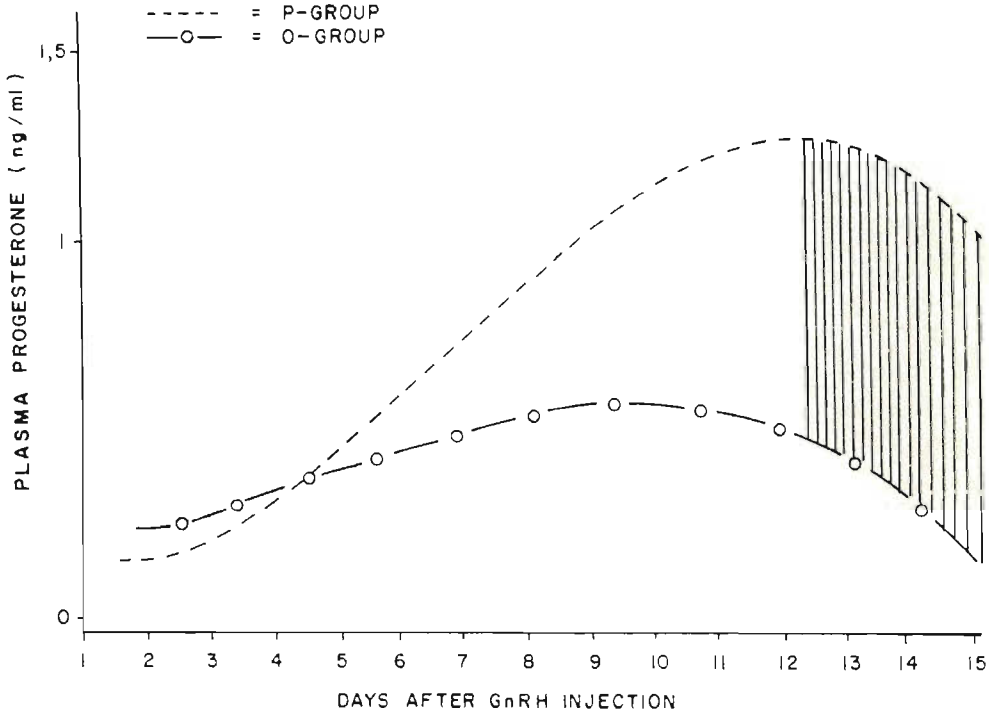


FIG. 4 : Estimated regression of mean plasma progesterone concentration on days of ewes pretreated with progesterone and the control ewes, with the shaded areas indicating significant differences between the means ($p < 0,05$)

1980). Patterns of gonadotrophin secretion during the follicular phase of the oestrous cycle indicate that follicular growth and development is controlled by the episodic mode of tonic LH secretion (Yuthasastrakosol et al., 1977; Baird, 1978). It is possible then that the failure to consistently produce normal functioning corpora lutea in response to GnRH treatment can be related to inadequate follicle development before the induction of ovulation (Haresign & Lamming, 1978), or to the lack of suitable steroid (and gonadotrophin) priming prior to GnRH treatment (Ainsworth et al., 1982).

The pituitary response to releasing hormone (in terms of LH secretion) after progesterone and oestradiol pretreatment is of a variable nature (McLeod et al., 1982b). It depends on the level and duration of steroid treatment, GnRH dose and mode of administration, and season and state of sexual activity of the recipient (Crighton, 1977; Jenkin et al., 1977; Webb et al., 1977). There is evidence suggesting breed differences as well. The pituitary response can be increased (Roche, Foster, Karsch, Cook & Dzuik, 1970; Cumming et al., 1972; Lewis et al., 1981), if physiological amounts of steroid are administered. A reduction in the amount of LH secreted in response to GnRH was encountered when progesterone was administered at levels exceeding physiological amounts (Jenkin & Heap, 1974; Wright et al., 1978) or when progesterone and oestradiol was administered as a daily dose at a rate similar to that of endogenous production in late pregnancy (Bedford, Harrison & Heap, 1972; Challis, Harrison & Heap, 1973). In some cases progesterone treatment (implants for 14 days containing 375 mg progesterone) had no significant effect on pituitary response to GnRH treatment (McLeod et al., 1982b). Oestradiol pretreatment resulting in 4 - 8 pg/ml plasma for 8 days selectively increased FSH levels in comparison to LH in response

to GnRH injections (Wheaton et al., 1982), but oestradiol pretreatment did not augment LH response, nor did progesterone diminish it. In an earlier study, Wheaton & Mullet (1982) found that a higher progesterone pretreatment (50 µg vs. 25 µg) diminished the LH-response to GnRH. A single injection of 50 µg oestradiol prior to GnRH increases the release of LH (Haresign & Lamming, 1978). The best response in terms of LH release was obtained when both oestrogen priming (30 µg) and GnRH were administered as divided doses. Significantly more LH was released in comparison to a divided dose of GnRH only (Hamilton et al., 1979). As reported during the earlier part of this study, LH release in response to GnRH was suppressed following the infusion of 50 µg oestradiol, together with 200 I.U. PMSG during a 24 h period preceding GnRH injection.

It has long been realised that progesterone plays an important role in hormonally induced ovulations. Priming of the central nervous system by progesterone is necessary to elicit behavioural oestrus at the first ovulation of the breeding season (Robinson, 1959), but progesterone alone given during anoestrus does not necessarily lead to LH release or ovulation (Pelletier & Thimonier, 1975). During post partum anoestrus progesterone acts to produce a cycle of normal duration if combined with PMSG (Oldham & Martin, 1979). These concepts are supported by the view (Karsch et al., 1978), that progesterone is the "organiser" of the oestrous cycle in sheep. It acts upon the systems which govern both the tonic and surge modes of gonadotrophin secretion, its presence in high levels inhibits oestradiol secretion and ovulation and its absence promotes both these events.

In the current study, using non-lactating ewes in seasonal anoestrus, progestagen priming for 5 days prior to GnRH resulted in a significantly enhanced luteal function in terms of progesterone secretion, while

E2 priming failed to have any noticeable effect. Ramirez-Godinez, Kiracofe, McKee, Schalles & Kittok (1981), clearly demonstrated that the majority of anoestrous cows that exhibit short cycles after weaning do not have elevated progesterone levels in their serum before the first detected post weaning oestrus, but those that have a normal cycle do have elevated pre-oestrus levels of progesterone. During the same study, progestagen implants reduced the incidence of short cycles. Lewis et al. (1981) could not demonstrate a luteotrophic effect of progestagen in lactating or non lactating ewes during early post partum in autumn. In fact the duration of the short luteal phases and the concentration of progesterone in ewes with "normal" luteal phases after GnRH were reduced in ewes treated with progestagen. The results of the current study are in consort with those of McLeod et al. (1982b) where seasonally anoestrous ewes were studied. These workers recorded a highly significant luteotrophic effect of progestagen priming followed by a multiple injection regime of GnRH. Normal luteal function was defined by McLeod et al. (1982b) as maximum plasma progesterone concentrations of at least 1,5 ng/ml, with the elevation in plasma progesterone starting within 4 days of GnRH injection. The life span of corpora lutea induced by HCG was prolonged during post partum anoestrus in cows pretreated with progesterone implants, but not in cows primed with oestradiol (Pratt, Berardinelli, Stevens & Inskeep, 1982) and the results pertaining to progesterone priming were confirmed by Sheffel et al. (1982). They concluded that the mechanism by which progesterone increased the level of luteal function remains unknown.

Oestradiol implants which produced E2 plasma concentrations similar to those resulting from progesterone priming in seasonally anoestrous ewes (Fig. 1), failed to induce a luteotrophic effect (Fig. 3).

These results provide some answer to the question as to whether the effect of progesterone is a direct one at the ovarian level, or an indirect effect via oestradiol secretion, which in turn acts upon gonadotrophin secretion from the hypothalamic-pituitary unit. It can be speculated that the effect is more direct than indirect. The near perfect mimicking of the oestrogen surge following progestagen withdrawal, that was accomplished in this study through E2 implants, did not have a luteotrophic effect as was the case with progestagen priming. It is interesting to draw a parallel between the current results and those of Ramirez-Godinez, Kiracofe, Schalles & Niswender (1982) regarding the effect of elevated progesterone levels prior to ovulation. In this study, the plasma progesterone concentrations for the three treatment groups were nearly identical until Day 5, after which the values in the ewes of the P-group became elevated (Fig. 2). Ramirez-Godinez et al. (1982) showed that serum progesterone levels in short cycle cows started to decline after Day 5 and that the short cycles were not preceded by elevated serum progesterone levels. Progesterone could be having a direct effect on the hypothalamo-pituitary axis to alter the pattern of LH and/or FSH secretion to one that is more beneficial to priming the pre-ovulatory follicle to become a secretor or progesterone.

It can be concluded then that without progesterone priming a corpus luteum, either naturally occurring or induced, does not appear to produce sufficient progesterone for a long enough period to always lead to normal luteal function (Sheffel et al., 1982). The next step would be to investigate whether the enhanced luteal function established in this study where ewes received PMSG after GnRH is due to corpora lutea of a higher mass, a higher activity per unit mass or a combination of these factors.

C H A P T E R I V

IN VITRO PROGESTERONE PRODUCTION OF CORPORA LUTEA FROM EWES TREATED WITH GnRH AND INFUSED WITH SALINE OR PMSG

1. INTRODUCTION

The inadequate luteal function of ewes treated with GnRH was clearly demonstrated earlier, the results being in consort with those of Haresign et al. (1975); Lishman et al. (1979), Fletcher et al. (1980); Van der Westhuysen et al. (1980) and others. Also, during the present study infusion of GnRH-treated ewes with PMSG during the luteal phase resulted in enhanced luteal function; manifested as a higher plasma progesterone concentration, equal to or higher than the levels in cyclic ewes. Stimulation of the corpus luteum in vivo with gonadotrophins (LH) does not only lead to higher plasma progesterone concentration (Piper & Wells, 1974), but also to heavier corpora lutea (Piper & Loucks, 1974).

The rate of passage of progesterone from luteal slices in vitro is increased by PMSG (Legault-Demare, Mauleon & Suarez-Soto, 1960, quoted by Kaltenbach et al., 1967). LH also consistantly increases progesterone secretion from luteal tissue in vitro (Kaltenbach et al., 1967) and in dose related quantities (Hansel, 1971). Gonadotrophins can thus be used to evaluate luteal function in vitro by incubating luteal slices or cells in a medium to which gonadotrophins were added to (McNeilly et al., 1981; Rhodes III, Randel & Long, 1982).

This study was planned with the object to determine whether:

(i) PMSG infusion affects luteal mass

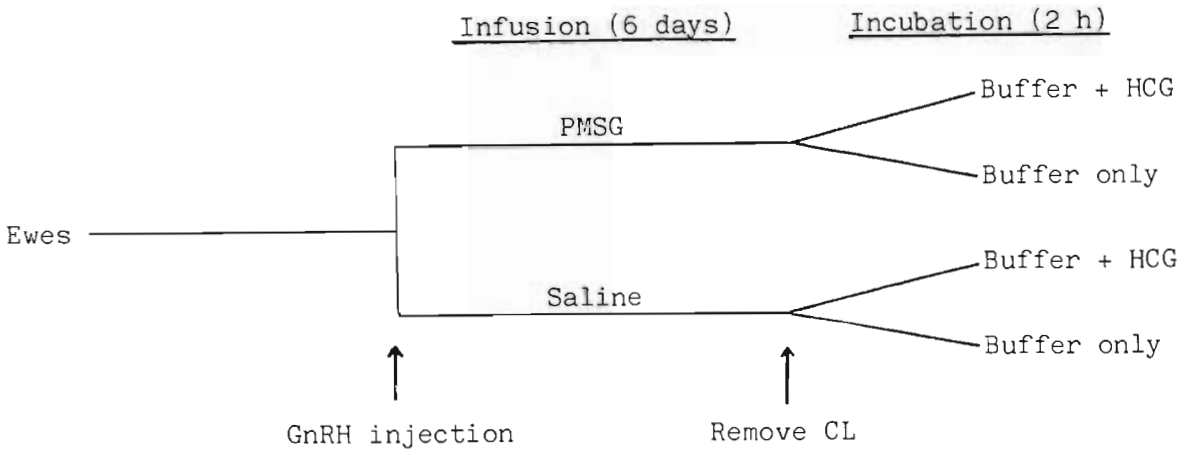
(ii) Inadequate luteal function is due to insensitivity to gonadotrophins.

2. GENERAL PROCEDURE

During early Summer (November, 1982) 9 multiparous non-lactating Corriedale ewes housed in individual pens on slattered floors were used as donors of corpora lutea to be cultured *in vitro*. The ewes were fed 1 200 g/day of a 80 : 20 lucerne-maize ration. After the ewes had been given an i.m. injection of 0,3 ml Cloprostenol (Estrumate, ICI) an intra-vaginal progesterone impregnated sponge (Repromap 60 mg - Tuco) was inserted in each ewe for 5 days. As the ewes were not tested for cyclic activity prior to commencing with the experiment the above procedure was followed to ensure that the CL was induced by releasing hormone. Until 1978 the Corriedale flock from which the ewes originated was mated from 1 December onwards (D. Yeates, personal communication) and some ewes could have been sexually active at the beginning of the experiment.

Eighteen hours after sponge withdrawal the ewes were injected i.m. with 1 ml GnRH (Receptal, Hoechts = 0,0042 mg buserelin acetate). After releasing hormone administration the ewes were infused directly into the jugular vein for 6 days with either normal saline (0,9% NaCl) or with PMSG in saline. Rate of infusion was 500 ml/24 h and 200 I.U. PMSG was administered over a 24 h period. On the 7th day after releasing hormone the corpora lutea of all ewes were removed surgically under anaesthesia (Rompun-Bayer), cleaned, weighed, sliced and incubated in a buffered medium with or without 5 I.U. HCG per ml of medium. After 2 h incubation the media plus luteal tissue were snap frozen by immersion in liquid nitrogen and kept frozen until assayed for progesterone concentration. The concentration was expressed as ng progesterone/ml medium/mg CL tissue. It was necessary to dilute the medium 200 - 500 times before it could be assayed. Prior to removal of the ovary on Day 7 (after GnRH)

a blood sample was taken from every ewe and later assayed for progesterone. A schematic representation of the experiment is as follows:



2.1 Preparation of luteal tissue for incubation

A modification of the method described by Hansel (1971) to collect and slice the luteal tissue was applied. After surgical removal of the ovary containing the active CL the ovary was immediately rinsed and placed in chilled saline (4°C), and transported approximately 1 km to the laboratory. Here the CL was dissected from the ovarian tissue and trimmed of connective tissue. The intact CL was rinsed in chilled saline to remove all traces of blood, blotted dry on towelling paper and weighed to the nearest milligram. Subsequently, the CL's were chopped into pieces no larger than 0,5 mm using a scalpel blade. The chopped luteal pieces were transferred to a 100 ml beaker containing c. 10 ml of chilled saline, swirled and the saline decanted. The latter step was repeated, (three to four times), until the saline remained clear. The sliced tissue was blotted dry and two portions weighed directly into separate 25 ml erhlenmayer flasks. Care was taken to ensure that the two portions did not differ by more than 15% in mass with a mean of 70 mg per portion.

2.2 Preparation of the incubation medium

A modified Krebs-Ringer solution (Umbreit, Burris & Stauffer, 1957) was used as incubation medium. The following stock solutions were prepared with de-ionized-distilled water and stored at 4°C.

(1) NaCl : 45 g/500 ml

(2) K Cl : 2,3 g/100 ml

(3) $CaCl_2$: 1,21 g/100 ml

(4) $K H_2PO_4$: 2,1 g/100 ml

(5) $Mg SO_4.7H_2O$: 3,82 g/100 ml

(6) $Na HCO_3$: 6,5 g/100 ml freshly prepared on the morning of incubation and a mixture of 95% O_2 : 5% CO_2 gas was bubbled through this solution for 40 minutes.

The incubation medium was made up immediately prior to incubation and gas (95% O_2 : 5% CO_2) bubbled through the medium for 10 minutes The medium consisted of:

75,25 ml of (1)

822,5 ml of H_2O (de-ionized-distilled)

15,0 ml of (2)

22,5 ml of (3)

7,5 ml of (4)

7,5 ml of (5)

31,5 ml of (6)

2 g of glucose and 3,582 g of nicotin amide was added, the latter to maintain the integrity of the pyridine nucleotides (Hansel, 1971). To prepare a medium containing 5 I.U. HCG/ml, 500 I.U. HCG was added to 100 ml incubation medium. The two media were retained at 4°C in tightly sealed volumetric flasks.

2.3 Incubation of sliced luteal tissue

Five ml of the incubation media was pipetted into pregassed

(95% O_2 : 5% CO_2) and sealed 25 ml erhlenmayer flasks. One portion of the sliced luteal tissue was weighed into a flask containing buffer plus HCG and the other portion into a flask with buffer, but without HCG. The flasks were again gassed and sealed with a double layer of parafilm. The flasks were then placed on a shaker in a water bath at 37,5° C and incubated for exactly 2 h. Special care was taken that the flasks were all sealed, since the buffering capacity of the medium was dependant on the presence of CO_2. After incubation the flasks were snap frozen in liquid nitrogen and kept frozen until assayed for progesterone.

The entire procedure from the removal of the ovary to the onset of incubation took less than 10 minutes.

3. RESULTS

3.1 Corpus luteum mass

The mean mass of the CL's obtained from the 5 ewes infused with PMSG after GnRH (0,345 $\pm$ 0,066 g) was significantly greater ($p < 0,01$) than that of the CL's contained in the ovaries of the 4 ewes after saline infusion (0,168 $\pm$ 0,042 g).

3.2 Progesterone concentration of incubation media and plasma

The progesterone content of the luteal tissue could not be determined even after diluting the extracted steroid 5 000 times and more, in order to attain a concentration within the required range for the assay. The resulting margin of error was too high and could have led to biased results. The determination was complicated by the fact that the total mass of incubated luteal tissue had to be extracted with ether as the mass of a subsample could not be accurately related to the original luteal mass due to possible differences in moisture

content. In order to establish the progesterone concentration of the incubation medium the latter had to be diluted 200 - 500 times to attain a concentration that fell within the range of the assay (Table 1).

TABLE 1 : Progesterone concentration (in duplicate) of the incubation media (ng/ml/mg CL incubated) for ewes infused with either PMSG or saline and their luteal tissue incubated without or with HCG.

Infusion					
PMSG (n = 5)			Saline (n = 4)		
No.	Buffer only	Buffer + HCG	No.	Buffer only	Buffer + HCG
1	8,62 10,00	11,67 12,50	1	16,94 15,28	15,82 15,27
2	5,90 5,64	7,95 8,68	2	11,93 11,58	16,73 17,31
3	5,48 5,48	8,57 8,88	3	6,25 6,25	6,83 6,66
4	7,55 8,98	11,33 12,17	4	13,49 14,29	15,61 15,61
5	7,40 7,10	8,18 7,58			
$\bar{x} = 7,22^{a} \pm 0,51$	$\bar{x} = 9,75^{b} \pm 0,61$		$\bar{x} = 12,00^{c} \pm 1,39$	$\bar{x} = 13,73^{c} \pm 1,44$	

Means with the same superscript do not differ significantly

It is evident (Table 1) that PMSG infusion resulted in the formation of luteal tissue with a lower secretional activity per unit mass than the saline infusion. Although the corpora lutea from the saline treated ewes secreted more progesterone per unit mass, the corpora lutea were smaller and a lower concentration of the steroid was present in the plasma of these ewes.

An estimation of total luteal progesterone content (mean luteal mass x mean progesterone concentration of incubation medium) revealed that the corpora lutea of ewes infused with PMSG contained more progesterone (Table 2).

TABLE 2 : Estimated total progesterone content of GnRH induced corpora lutea derived from ewes infused with normal saline or 200 I.U. PMSG per 24 h. CL's were removed on Day 7 and incubated in buffer only or buffer plus 5 I.U. HCG/ml buffer.

	Saline	PMSG
Buffer only	2,01	2,49
Buffer + HCG	2,31	3,36

The mean plasma progesterone concentration immediately prior to removal of the CL's was 0,93 $\pm$ 0,17 ng/ml for the PMSG group and 0,73 $\pm$ 0,06 ng/ml for the saline group. The response to HCG in terms of increase in P concentration of the media was 35% for the PMSG group, which is significantly more ($p < 0,05$) than the 14% of the saline group.

3.3 Correlation between luteal and plasma progesterone

The progesterone concentration of the incubation medium containing only buffer for all the ewes was multiplied by the luteal mass as determined after removal from the ovary. These values were correlated with the plasma progesterone concentration for the ewe from which the CL was removed. A positive coefficient of $r = 0,77$ ($p < 0,05$) existed between progesterone concentration of the media and of the plasma.

4. DISCUSSION

The experiment was conducted to provide answers to the important questions arising out of the earlier work viz., were the higher progesterone concentrations in the plasma after GnRH injection, followed by PMSG treatment, due to either the production of larger CL's, or to more active CL's, or a combination of the two factors? The results of the current study clearly show that PMSG infusion stimulated the CL mass. As long ago as 1960 it was claimed (Legault-Demare, Mauleon & Saurez-Soto, quoted by Short et al., 1963) that PMSG had a luteotrophic action. Generally, PMSG is used to induce ovulation or to stimulate multiple ovulations (Stabenfeldt, Edqvist, Kindahl, Gustafsson & Bane, 1978) and this procedure usually gives rise to a greater number of smaller CL'S (Stormshak, Inskeep, Lynn, Pope & Casida, 1963). The luteotrophic effect of PMSG infusion after GnRH, in terms of higher plasma progesterone levels, was clearly evident from the early experiments in the investigation reported here. The larger corpora lutea (as a result of the PMSG treatment) gave rise to higher plasma progesterone concentrations. This is in agreement with the conclusion that ewes with larger CL's show higher plasma progesterone concentrations throughout the oestrous cycle (Stormshak et al., 1963; Diekman et al., 1978). In the present study a correlation coefficient of $r = 0,77^{*}$ existed between the plasma progesterone concentration of the ewe and the progesterone concentration of the incubation medium, in spite of reported within day and even diurnal changes in plasma progesterone concentration (McNatty, Revfiem & Young, 1973).

When GnRH is used to induce corpora lutea during anoestrus the resultant CL's are of a lower mass and have a reduced progesterone content in comparison with corpora lutea of a normal oestrous cycle (McNeilly et al., 1981). These workers incubated 16 mg of luteal tissue

(70 mg in this study) and after homogenizing with absolute ethanol, succeeded in assaying the progesterone content of the luteal tissue, but refrained from stating the dilution used. In the present study a dilution factor of c. 5 000 had to be applied, leading to an unacceptable error margin. Kesler et al. (1981) using beef cows, treated with GnRH or ovulating naturally, reported lower plasma progesterone levels for cows with smaller corpora lutea on both Days 5 and 7 of the oestrous cycle. However, Rhodes III et al., (1982) could not demonstrate higher systemic serum progesterone concentrations in beef cows (Brahman) during winter than in summer in spite of an increase in luteal mass and luteal progesterone content from summer to winter. A possible explanation could be a higher metabolic clearance rate during winter in the Brahman which is inclined towards seasonality in oestrous activity (Harrison & Randel, 1981). In ewes the metabolic clearance rate of progesterone is higher in anoestrous than in normal cycling ewes (Bedford et al., 1972).

Incubation of the PMSG-stimulated luteal tissue in buffer, suprizingly yielded lower progesterone values per unit mass than the corpora lutea derived from saline infused ewes, a phenomenon, as far as could be ascertained, not described previously. One could be tempted to expect that the heavier more active corpora lutea, if plasma progesterone concentration is taken as a criterion for luteal activity, should yield more progesterone per unit mass during incubation. However, Stormshak et al. (1963) did observe that the total amount of luteal tissue present was the major factor in determining the total amount of progesterone in circulation. Kesler et al. (1981) reported a higher progesterone accumulation per mℓ of incubation medium for the heavier corpora lutea of beef cows on both Days 5 and 7 after ovulation. In sheep, GnRH-induced corpora lutea were

of lower mass than normal CL's, and had a reduced ability to secrete progesterone *in vitro*, but the binding of HCG was equivalent to that of normal corpora lutea (McNeilly *et al.*, 1981). Rhodes III *et al.* (1982) established both a breedtype (Brahman and Hereford) and seasonal (summer and winter) effect on the *in vitro* capacity of the luteal cells to release progesterone. The difference in the *in vitro* response between the corpora lutea in this study can be possibly explained in terms of the difference in the gonadotrophic status during the peri-ovulatory period. Harwood, Conti, Conn, Dafau & Catt (1978) reported that a lower concentration of gonadotrophin alters receptor numbers in the corpus luteum. Multiple injections of GnRH resulted in greater amounts of LH released, which appeared to reduce the incidence of abnormal corpora lutea (Restall *et al.*, 1977). In beef cows the duration of the GnRH-induced pre-ovulatory LH surge is approximately half the duration of the normal pre-ovulatory surge (Troxel, Kesler, Noble & Carlin, 1980) and this could lead to a dimunition in the *in vitro* release of progesterone in the luteal tissue derived from GnRH-induced ovulations (Kesler *et al.*, 1981). Short-term treatment with PMSG and FSH prior to HCG-induced ovulations, did not affect the life span, but did increase the level of function of corpora lutea (Sheffel *et al.*, 1982), but the authors refrained from characterizing the induced pre-ovulatory gonadotrophin surge. In Brahman heifers the LH surge is lower during winter than during spring (Harrison & Randel, 1981) and progesterone release *in vitro* from luteal cells of Brahman heifers increased from winter (low LH and large CL'S) to summer (Rhodes III *et al.*, 1982). The results presented here suggest that the difference between the treatment groups (PMSG and saline) in the secretory activity of luteal tissue per unit mass may be related to the reduced pre-ovulatory LH release

which results from PMSG administration. This was observed in the ewes infused with PMSG (Table 19, Fig. 14, Chapter I). A lower number of receptor cells per unit mass of luteal tissue could have resulted. The number of receptors for LH in the gonads of rats is affected by a variety of factors, but the primary factor appears to be LH itself (Richards & Midgley, 1976; Zipf, Payne & Kelch, 1978). Exposure to high concentrations of gonadotrophins leads to a loss of receptors for LH in luteal tissue (Conti, Harwood, Dufau & Catt, 1977a; Conti, Harwood, Dufau & Catt, 1977b). High concentrations of LH in the blood of ewes during the luteal phase are followed by decreased numbers of luteal receptors for LH, as observed for rats (Suter et al., 1980). The possibility arises that this was the case in the current study due to the infusion of PMSG with its LH-like activity.

Although a higher response of luteal tissue derived from PMSG-treated ewes (35% response vs. 14%) to HCG in vitro was clearly demonstrated, the mean concentration of progesterone accumulation in the medium per unit mass of luteal tissue was however still higher for the saline treated ewes (Table 1, 13,73 ng vs. 9,75 ng). These results are in harmony with the results of Kesler et al. (1981), who demonstrated that corpora lutea which resulted in higher plasma progesterone concentrations showed a higher response in terms of an increase in the accumulation of progesterone in vitro if challenged with LH, which in turn is related to a higher response of secretory cells to LH. In sheep, receptors for LH first appear in the thecal cells of small follicles and as the follicle enlarges there is a slight decrease in the capacity of thecal cells to bind LH concomitant with a dramatic increase in binding capacity of LH to granulosa cells (Carson et al., 1979). As the corpus luteum develops, the concentration of

progesterone in the CL increases and the concentration is highly correlated with the total number of LH receptors (Diekman et al., 1978).

The corpus luteum of the ewe contains both small and large luteal cells derived respectively from the theca and granulosa cells of the follicle (O'Shea, Cran & Hay, 1980). There are 3 - 5 times more small luteal cells than large cells in the CL of ewes (O'Shea, Cran & Hay, 1979). The small and large luteal cells differ in their response in vitro to stimulation by LH and other hormones and the small cells may be the principle source of progesterone production in the ewe (Rodgers, O'Shea & Findlay, 1983). Earlier, Rodgers, O'Shea & Findlay (1982), concluded that the large cells produce more progesterone per cell than the small cells. It would be extremely interesting to compare PMSG primed, GnRH-derived corpora lutea with unprimed CL's in this regard, and also with "normal" corpora lutea.

It can thus be concluded that the higher plasma progesterone levels as a result of PMSG treatment found throughout this study are the result of large corpora lutea capable of secreting progesterone in larger quantities, which is indicative of a larger number of LH receptors per CL. The data also suggest that the concentration of LH receptors per unit mass of PMSG-treated luteal tissue might be lower, resulting in a lower concentration of progesterone accumulation per unit mass during in vitro incubation in relation to saline treated corpora lutea. The higher progesterone concentration in the incubation media per unit mass of corpora lutea derived from saline treated ewes (Table 1), and the response to HCG suggest that the lower activity of subnormal corpora lutea is inherent to the CL itself and partly due to a lack of response to LH after releasing hormone treatment.

G E N E R A L D I S C U S S I O N

The dramatic absence of corpora lutea or the high frequency of subnormal luteal function manifested as low progesterone levels or short-lived corpora lutea as a result of GnRH administration (Crighton et al., 1975; Haresign et al., 1975; Webb et al., 1977; Lishman et al., 1979; Fletcher et al., 1980; Van der Westhuysen et al., 1980) was not apparent during the present study. The plasma progesterone concentrations recorded for the control treatment groups were albeit higher than those reported by other workers (Haresign et al., 1975); Ainsworth et al., 1982), but still lower than the values recorded for the cyclic ewes (Chapter I, Fig. 3 and 5 ; Chapter II, Fig. 2). The discrepancy between progesterone values in the current study and those of, for instance, Haresign et al. (1975) and Haresign & Lamming (1978) can inter alia be ascribed to breed differences. Those workers used British breeds, generally accepted to have short breeding seasons and not unrelated to the Corriedale used during the latter part of this study (Chapter III). Although not directly comparable as regards production status there is also a marked difference between the level of luteal function recorded for the S.A. Mutton merino ewes and Corriedale ewes (S.A. Mutton merinos: Chapter I, Fig. 3 and 5; Chapter II, Fig. 2 and Corriedales: Chapter III, Fig. 2). The plasma progesterone level in the Corriedales used as controls (GnRH only) were less than 1,5 ng/ml plasma throughout the luteal phase. This level could be described as "subnormal" by any standard (Chapter II, Fig. 2) and is very much the same as the 1,3 ng/ml recorded during Experiment I and II (Chapter I) for ewes having short-lived corpora lutea or where a CL was not observed during laparotomy. The values reported by Wright et al. (1983b) for ewes showing subnormal luteal function were similar. It would appear, judging by the available

evidence, that GnRH did in fact give rise to subnormal corpora lutea, in S.A. Mutton merinos and Corriedales, but the effect was more pronounced in the latter breed.

The higher progesterone levels in GnRH-treated ewes primed with PMSG is ascribed to suitable follicular development (McGovern & Lang, 1976; Haresign & Lamming, 1978). This was not unexpected as follicular development prior to ovulation is a direct function of gonadotrophin stimulation (Dufour et al., 1979) and ovarian acyclicity is due to a failure of follicle development (Wright et al., 1981b). In the present study PMSG priming in GnRH treated ewes resulted in luteal function slightly lower as in cyclic ewes (Chapter I, Fig. 3). However, on closer examination it appears that this luteotrophic effect is temporary and lasts until Day 7 after GnRH administration (Chapter I, Fig. 10 and 11). This was not evident from the work of Haresign & Lamming (1978). Further evidence in support of the "two phased" level of luteal function is demonstrated by the trend in the results (Chapter I, Table 3 and 4), indicating a higher progesterone secretion from Day 1 to Day 7 after GnRH administration by the ewes receiving PMSG prior to releasing hormone (EPOS & POS). The order of magnitude is reversed for progesterone secretion from Day 1 - 11 (Chapter I, Table 7) and total progesterone secretion during the luteal phase was higher (Chapter I, Table 9) for ewes infused with PMSG after GnRH. The priming with PMSG, which has both LH and FSH like properties (Lamond, 1960) could have increased the number of luteal cells, as the receptors for LH first appear in the thecal cells of small follicles (Carson et al., 1979) and after ovulation the number of receptors for LH and the peripheral concentration of progesterone are highly correlated (Diekman et al., 1978). The above evidence seems to suggest that the administration of PMSG after GnRH

is a prerequisite for continued luteal function at normal levels in a regime where PMSG is used as a gonadotrophic stimulus. Both treatment groups infused with PMSG after GnRH (Chapter I, Fig. 6 and 10) as well as ewes injected with PMSG twice daily (Chapter II, Fig. 3) provides evidence in this regard. Evidence for "two phased" luteal support is not unique to a regime where PMSG is utilized to provide luteal support. In the ewes pretreated with progesterone or E2 during the present study (Chapter III, Table 2 and Fig. 2) progesterone secretion during the first 7 days after GnRH was nearly identical for the 2 treatments whereas total secretion of the steroid (Chapter III, Table 3) was far superior for the progesterone-primed ewes. Furthermore, in cylic cows, progesterone levels start to decline by Day 5 in those cows where the oestrous cycle was not preceded by elevated progesterone levels (Ramirez-Godinez et al., 1982). The results relating to secretion of progesterone during the entire oestrous cycle seem to suggest that PMSG infusion both before and after GnRH is not necessary (Chapter I, Fig. 2 and 3). Administration of PMSG after GnRH always resulted in similar or superior luteal function for total progesterone secretion (Chapter I, Table 9), maximum secretion (Chapter I, Table 13) and continued secretion of the steroid at appreciable levels (Chapter I, Table 14) than for the ewes receiving PMSG before and after GnRH.

The results of this study, as discussed above, seem to indicate that gonadotrophin administration after GnRH (both as infusions and injections) is superior to gonadotrophin priming before GnRH. The work of Wright et al. (1983b) indicates that the major deficiency as regards GnRH-induced corpora lutea is contained in the events prior to ovulation, and this view is shared by other workers (Haresign et al., 1975; Haresign & Lamming, 1978; Wright et al., 1981a;

McNatty et al., 1982b; Wright et al., 1983a. Very few, if any of these workers successfully induced ovulations, by administering releasing hormone, that resulted in corpora lutea functioning normally and secreting to the same extent as reported on in this study by using pre-ovulatory gonadotrophin treatment regimes.

Not only gonadotrophin priming has been implicated as being necessary for normal luteal function after GnRH administration (Haresign & Lamming, 1978), but also suitable steroid priming is required (Ainsworth et al., 1982). This is because the development and maturation of follicles is intimately dependant on the sequential action and interaction of pituitary and ovarian steroid hormones (Richards, 1980). In the present study progesterone pretreatment resulted in significantly improved luteal function (Chapter III, Table 3) in response to GnRH-induced ovulations. In the ewe, as long as progesterone secretion is elevated, ovulation cannot occur naturally (O'Mary, Pope & Casida, 1950). Progesterone not only blocks the pre-ovulatory LH surge, but also has the ability to prevent the oestradiol trigger for this event (Karsch et al., 1978), and progesterone appears to play a critically important role in the inhibition of the tonic mode of LH secretion which regulates the pre-ovulatory oestrogen rise (Baird & Scaramuzzi, 1976; Hauger, Karsch & Foster, 1977; Karsch, Legan, Hauger & Foster, 1977). The withdrawal of progesterone in the ewe during the breeding season immediately initiates the events which culminate in ovulation and the onset of a new oestrous cycle (Robinson, 1959). It has long been realised that progesterone plays an important role in hormonally induced breeding cycles (O'Mary et al., 1950). Priming of the central nervous system by progesterone is necessary for behavioural oestrus at the first ovulation of the breeding season (Robinson, 1959), but progesterone treatment must

be combined with PMSG to induce ovulation during seasonal anoestrus (Pelletier & Thimonier, 1975) and also during post partum anoestrus. A near perfect mimicking of the progestagen-induced oestrogen surge through the use of E2 implants (Chapter III, Fig. 1), failed to promote luteal function to the same extent in GnRH-induced corpora lutea as progesterone pretreatment (Chapter III, Table 3; Fig. 2). It thus seems as if the effect of progesterone on luteal function is exerted prior to the pre-ovulatory oestrogen peak. Progesterone could also exert a direct effect on the hypothalamo-pituitary axis. This could alter the pattern and ratio of gonadotrophin secretion to more closely resemble the naturally occurring sequential action and interaction of the pituitary hormones. These hormones, which together with the ovarian steroids regulate the sequence of events leading to ovulation.

The present study demonstrated when GnRH was used to induce corpora lutea during early post partum such corpora lutea are capable of normal luteal function. This can be achieved by gonadotrophin and steroid priming. These results were encouraging, but failure of the ewes to conceive to artificial insemination, again emphasised the delicate hormonal balance that exists in the female and which must be satisfied in all respects before early rebreeding can be accomplished on large scale.

S U M M A R Y

CHAPTER I

Two similar experiments were conducted to

(i) determine the luteotrophic effect of PMSG and E2 i.v. infusion on GnRH-induced corpora lutea in early post partum ewes during Spring.

(ii) characterize the LH surge in response to GnRH and measure tonic levels of LH.

1. At laparotomy 81,4% of the ewes had macroscopically active corpora lutea. PMSG prior to GnRH stimulated more ewes to ovulate (95,8% vs. 71,4%) in response to GnRH, but did not result in a higher ovulation rate (1,65 vs. 1,45).

2. Although PMSG markedly stimulated progesterone production the greater response was obtained where this exogenous scource of luteotrophin was supplied after ovulation. PMSG administration both before and after GnRH did not result in an added advantage.

3. In those ewes not receiving PMSG the maximum progesterone level was 1,5 ng/ml, whereas the level in cyclic ewes was 2,3 ng/ml, indicating subnormal luteal function in the first group.

4. Promotion of LH receptors within the ovary by prior treatment with oestrogen was not beneficial in terms of luteal function.

5. Evidence of two phases of luteal support existed. In ewes primed with PMSG prior to GnRH luteal function seemed to decrease after Day 7 and from Day 10 onwards values recorded were similar to those for ewes not receiving PMSG. Where the luteotrophin was

administered after GnRH this effect was not evident.

6. Exogenous hormone administration decreased pituitary responsiveness to GnRH and resulted in higher tonic LH levels.

CHAPTER II

An experiment was conducted to establish the effect of a protein deficiency during late pregnancy on corpora lutea induced with GnRH 30 days post partum. The ewes were all subjected to A.I. in order to determine whether a developing embryo could rescue the corpus luteum from premature failure. This was followed by twice daily injection of PMSG or saline for 16 days.

1. At lambing the difference in body mass of the ewes receiving different protein levels was 16,2% and the mass of the lambs in the restricted protein group (30% of NRC recommendations) was 4,2 $\pm$ 0,17 kg as for 4,8 $\pm$ 0,12 kg for the ewes which received 100% of NRC recommendations.

2. Nineteen of the 40 ewes exhibited oestrus between Day 16 and 20 after GnRH injection. No association between protein intake and GnRH treatment existed. Plasma progesterone levels on Day 21 were similar for all ewes, irrespective of oestrus exhibition. None of the ewes subsequently lambed.

3. Levels of protein intake did not influence mean plasma progesterone levels after GnRH.

4. The luteotrophic effect of 100 I.U. PMSG injected s.c. twice daily was manifested in a total progesterone secretion of 100% more than for the ewes injected with saline after GnRH.

CHAPTER III

Seasonal anoestrous ewes were pretreated with either intra-vaginal progestagen sponges, subcutaneous E2 implants or served as controls in an experiment conducted to clarify the role of progestagen priming in GnRH-treated ewes.

1. Within 18 hours of sponge withdrawal and 6 hours after implant insertion E2 levels of 8 pg/ml plasma were recorded in the ewes. A near perfect mimicking of the endogenous E2 rise after progestagen removal was accomplished through the use of E2 implants.

2. Mean plasma progesterone secretion in the ewes were similar during the first 7 days, thereafter progestagen pretreated ewes secreted the steroid in significantly higher levels than the ewes in the E2 primed and control groups. This pattern of secretion suggested a two phased luteal support.

3. A short period of progestagen priming appears to be of vital importance to ensure normal luteal function of the induced corpus luteum.

CHAPTER IV

Following the successful trophic stimulation by PMSG on GnRH-induced corpora lutea in vivo, an experiment was conducted to determine whether PMSG affected luteal mass and to establish, in vitro, whether inadequate luteal function was due to insensitivity of the corpus luteum to gonadotrophins.

1. PMSG infusion after GnRH gave rise to corpora lutea significantly heavier than in ewes infused with saline.

2. Per unit mass, incubated luteal tissue derived from saline

treated ewes, produced more progesterone than luteal tissue derived from PMSG-treated ewes. However, the estimated luteal progesterone production and plasma progesterone concentration was higher in ewes treated with PMSG. Response to HCG was higher in luteal tissue derived from the latter ewes.

3. Higher plasma progesterone levels as a result of PMSG treatment are the result of large corpora lutea capable of secreting progesterone in large quantities.

4. The concentration of LH receptors seems to be lower in PMSG primed luteal tissue and the data suggests that the lower activity of subnormal corpora lutea lies within the CL itself, which is partly due to a lack in response to LH.

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