*S. Afr. J. Anim. Sci. 9, 197–203 (1979)*

# EFFECT IN EWES OF OESTROGEN PRIMING AND GnRH ON LH RELEASE AND LUTEAL FUNCTION DURING EARLY LACTATION IN SPRING

*Receipt of MS 22-03-1979*

**C.D. Hamilton*, A.W. Lishman and P.A. Lamb**
*Department of Animal Science, University of Natal, Pietermaritzburg, 3200*

**(Key words:** *Oestrogen, GnRH, LH, Progesterone, Ewe)*
**(Sleutelwoorde:** *Estrogeen, GnVH, LH, Progesteroon, Ooi)*

*OPSOMMING:* DIE INVLOED BY OOIE VAN ESTROGEEN VOORBEHANDELING EN GnVH OF LH VRYSTELLING EN LUTEALE FUNKSIE GEDURENDE VROEË LAKTASIE IN DIE LENTE

Die invloed van estrogeen voorbehandeling en van GnVH toediening op die vrystelling van LH en luteale funksie is ondersoek by 66 S.A. Vleismerino ooie wat gedurende die lente in vroeë laktasie was. Sewe ewekansig gekose groepe ooie is behandel met (i) 30 µg estradiol bensoaat (ODB) verdeel in drie inspuitings gevolg deur 50 µg GnVH in twee gelyke dosisse (ii) 30 µg ODB as drie inspuitings maar nie deur GnVH gevolg nie, (iii) 50 µg GnVH as twee toedienings sonder ODB voorbehandeling, (iv) 'n enkele dosis 30 µg ODB as voorbehandeling tot twee inspuitings van 25 µg GnVH elk, (v) 30 µg ODB sonder GnVH (vi)30 µg ODB as een inspuiting gevolg deur een toediening van 50 µg GnVh en (vii) wat geen behandeling ontvang het nie en as kontrole gedien het. Bloedmonsters is elke 30 minute vir 'n periode van 12 uur na die eerste GnVH toediening vanuit die venajugularis getrek en die LH konsentrasie daarvan is bepaal. Die beste LH reaksie in terme van die persentasie ooie wat 'n LH vrystelling getoon het, die hoogste konsentrasie, totale hoeveelheid afgeskei asook duur van vrystelling is verkry waar beide estrogeen voorbehandeling en vrystellingshormoon as verdeelde dosisse toegedien is (Behandeling (i)). In teenstelling hiermee was al hierdie eienskappe van LH-vrystelling beduidend laer by die ooie wat net drie inspuitings van ODB ontvang het (Behandeling (iii)) en ook waar 'n enkele toediening van GnVH die een toediening van 30 µg ODB (Behandeling (vi)) gevolg het. By laasgenoemde behandeling was die piek LH konsentrasie nie beduidend laer as by behandeling (i) nie. 'n Verdere betekenisvolle verlaging van LH afskeiding is waargeneem by die oorblywende behandelings (iii), (iv) en (v). Die vrystelling van LH by die ooie wat net 30 µg ODB (Behandeling (v)) ontvang het, is so vertraag dat net 'n gedeelte van die vrystellingskurwe beskryf kon word en ook net by 55,6 persent van die behandelde ooie.

Die plasma progesteroon konsentrasies, met tussenposes van 2 tot 4 dae na die LH-vrystelling, het aangetoon dat al die behandelings onverwagte lae luteale funksie tot gevolg gehad het. Die behandelings wat toegedien is, was nie daartoe in staat om die aanvang van bronstigheid by ooie wat gedurende lente in vroeë laktasie was, te vervroeg nie.

*SUMMARY:*

The effects of oestrogen priming and GnRH administration on the release of LH and on luteal function were investigated using 66 spring-lambing S.A. Mutton Merino ewes in early lactation. Seven randomly selected groups of ewes received either (i) 30 µg oestradiol benzoate (ODB) divided into three injections followed by 50 µg GnRH injected in two equal doses, (ii) 30 µg ODB as three injections, but not followed by GnRH, (iii) 50 µg GnRH as two injections without ODB pre-treatment, (iv) a single injection of 30 µg ODB as a prelude to 50 µg GnRH in two injections, (v) 30 µg ODB not followed by GnRH, (vi) 30 µg ODB in one administration followed by a single dose of 50 µg GnRH or (vii) remained untreated and served as controls. The LH concentration of jugular blood-samples obtained every 30 min for 12 hours after the time of the first GnRH injection was determined. The best response in terms of, the proportion of ewes exhibiting an LH release, the greatest concentration, the total quantity released and the duration of LH release, was obtained where both the oestrogen priming and releasing hormone stimulation were administered as divided doses (Treatment (i)). In contrast, all these characteristics of the LH release were significantly lower in the ewes treated only with three doses of oestrogen (Treatment (ii)) and where a single injection of GnRH followed 30 µg ODB, also as a single dose (Treatment (vi)). In the latter treatment the peak LH level was not significantly lower than in treatment (i). A further significant reduction in the characteristics of the LH surge was noted in the remaining treatment groups (Treatments (iii), (iv) and (v)). The LH release in the ewes which received only a single 30 µg dose of ODB (Treatment v) was so delayed that only part of the release curve could be quantitated and then only in 55,6 per cent of the ewes treated. Plasma concentrations of progesterone at 2–4 day intervals after the LH release demonstrated that all the treatments resulted in unexpectedly poor luteal function. The treatments applied were not capable of precipitating the onset of oestrous cycles in ewes which were in early lactation during spring.

**Present address: Cedara Research Institute, Private Bag X9059, Pietermaritzburg, 3200*

## Introduction

Attempts to induce the resumption of oestrous cycles by administering GnRH to ewes during seasonal anoestrus (Crighton, Foster & Haresign, Hayes & Lamming, 1973; Haresign, Foster, Haynes, Crighton & Lamming, 1975; Shareha, Ward & Birchall, 1976) have produced evidence of sub-normal luteal phases in the ewes which ovulated (Haresign *et al.*, 1975; Shareha *et al.*, 1976). The possibility that increasing the release of LH (both peak concentrations and duration of release) would improve the response to GnRH, both in terms of ewes ovulating and lifespan of the corpus luteum appeared to warrant investigation. In this context the augmentation effect of oestrogen on the release of LH, when elicited by exogenous GnRH, has been documented by Arimura and Schally (1971), Reeves, Arimura and Schally (1971) and Poultney, Lishman, Louw, Botha and Arangie (1977). All reported greater quantities of LH being released when compared to no oestrogen priming. Although oestrogen sensitization resulted in peak LH levels which equalled those observed during the pre-ovulatory surge in cycling ewes, the duration of the release was only about half that encountered prior to spontaneous ovulation (Lishman, Stielau, Dreosti, Botha, Stewart & Swart, 1974; Poultney *et al.*, 1977).

Bonnar (1973) reported that a single s.c. injection of GnRH was more effective in achieving a sustained release of LH and FSH than either i.v. or i.m. administration. Attempts to mimic the natural pattern of GnRH secretion by administering a divided dose have given rise to conflicting results. Rippel, Johnson and White (1974) reported a refractory period of 96 hours after a single administration of GnRH, while Symons, Cunningham and Saba (1974) found that the LH response decreased when GnRH was injected at 24-hour intervals. However, when two injections were spaced three hours apart, they observed a heightened response to the second GnRH injection. Reeves, Arimura, Schally, Kragt, Beck and Casey (1972) reported a significant change in pituitary responsiveness when two consecutive injections were spaced four hours apart. In all cases, however, the duration of the elevated LH levels were still shorter than those normally found in cyclic ewes.

The purpose of the experiment described here was to evaluate the effect of (i) oestrogen priming prior to the injection of GnRH and (ii) administering GnRH in more than one injection, on the release of LH during early lactation and on luteal function.

## Procedure

### *Experimental plan*

Sixty-six lactating S.A. Mutton Merino ewes, aged between 2 and 7 years were randomly assigned to seven treatments, according to age and lambing status. One group served as controls, while the remaining ewes received ODB and/or GnRH according to the schedule shown in Table 1.

**Table 1**

*Quantities and times of hormonal treatments administered at approximately 21 days post partum to ewes lactating in spring*

| Treatment group | n | Hormonal Treatment | | | | |
|---|---|---|---|---|---|---|
| | | µg Oestradiol benzoate injected (i.m.) at: | | | µg of GnRH injected (s.c.) at: | |
| | | TIME | | | | |
| | | 08h00 | 16h00 | 24h00 | 08h00 | 12h00 |
| (i) | 10 | 10 | 10 | 10 | 25 | 25 |
| (ii) | 9 | 10 | 10 | 10 | – | – |
| (iii) | 9 | – | – | – | 25 | 25 |
| (iv) | 10 | – | – | 30 | 25 | 25 |
| (v) | 9 | – | – | 30 | – | – |
| (vi) | 9 | – | – | 30 | 50 | – |
| (vii) | 10 | – | – | – | – | – |

As lambing was spread over a four-week period commencing on 17 October, the ewes were treated in three groups, with a maximum difference of nine days in the date of lambing within each group. Hormonal therapy was initiated on the average 21 days after lambing.

Indwelling silastic jugular catheters (Portex) were inserted into all ewes and at intervals of 30 min 5,0 $cm^3$ blood samples were withdrawn into heparinized syringes. Sampling commenced immediately prior to the first injection of GnRH and ceased 12 hours later.

After centrifugation, the plasma samples were stored at −15 °C until their LH content was quantitated by the technique of Niswender, Reichert, Midgley and Nalbandov (1969). Assay variation was monitored by incorporating a plasma pool in each assay. The between assay coefficient of variation was 10,8 per cent.

In order to assess plasma progesterone levels after hormonal therapy every 2 to 4 days blood samples were taken by venipuncture for 20 days. The assay procedure was that of Butcher, Collins and Fugo (1974) and the between assay coefficient of variation of a plasma pool was 13,5 per cent. Teaser rams with marking raddles were introduced on the first day of sampling, being replaced 14 days later by fertile rams. The latter remained with the ewes for 51 days.

Until lactation was terminated 6 weeks post partum, the ewes were housed indoors on concrete and fed *Eragrostis curvula* hay *ad lib.*, plus 1,0 kg of a 2 : 1 maize meal/broiler litter mixture.

After weaning the ewes grazed on a Stargrass pasture, supplemented by 0,5 kg of the concrete mixture.

## Results

### *LH response to hormonal therapy*

Where the ewes received only oestrogen, either as three or as a single dose, two and five ewes, respectively, did not exhibit an LH surge. Only one ewe in each of the remaining treatments apparently did not respond.

### *Oestrogen priming*

The response to a divided dose of GnRH, as measured by the maximum LH level, the total LH released and the duration of the release, was significantly improved (Table 2; Fig. 1) by oestrogen priming only where the ODB was administered in three doses (Treatment (iii) vs Treatments (i) and (iv). Thus the split dose of GnRH gave virtually the same LH release, whether preceded by a single injection of ODB or not (Treatment (iii) vs (iv); Table 2; Fig. 1).

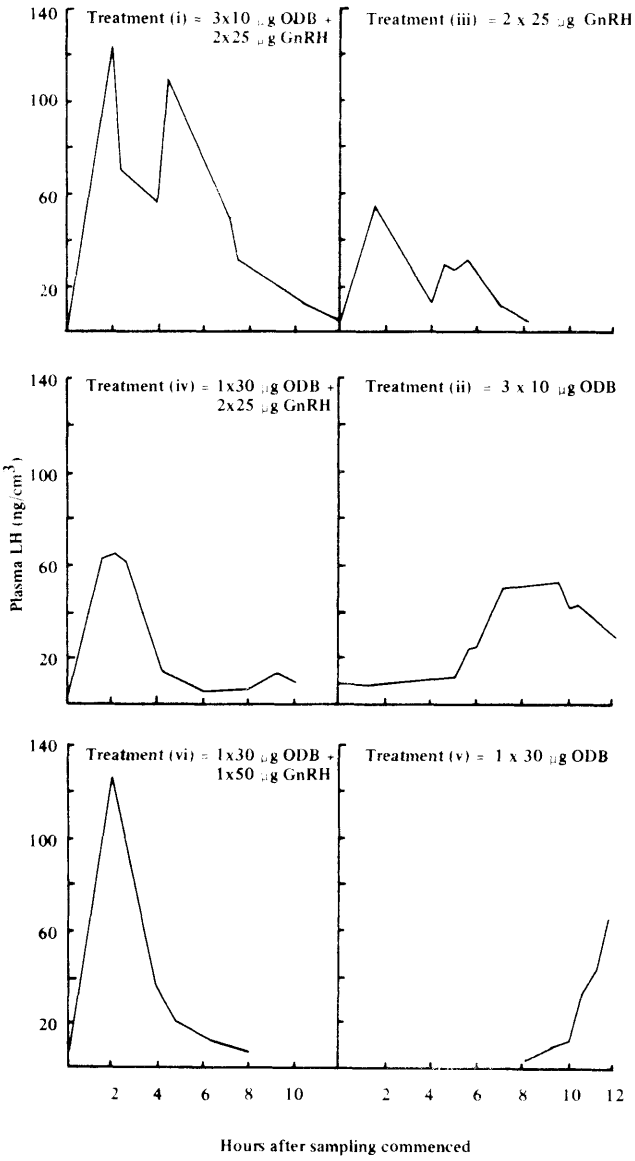


*Fig. 1 LH release in lactating ewes following treatment with oestradiol benzoate and GnRH. (See Table 1 for time of, and interval between injections.)*

### *Subdivision of GnRH*

When ODB was limited to a single injection the LH response (Table 2; Fig. 1) could be significantly improved by not sub-dividing the GnRH therapy, although the proportion of ewes responding was reduced in this instance (Table 2).

**Table 2**

*LH release following oestrogen (ODB) and/or GnRH administration in lactating ewes*

| Parameter | Treatment (i) | (ii) | (iii) | (iv) | (v) | (vi) |
|---|---|---|---|---|---|---|
| | 3 x 10 µg ODB + 2 x 25 µg GnRH | 3 x 10 µg ODB + – | – + 2 x 25 µg GnRH | 1 x 30 µg ODB + 2 x 25 µg GnRH | 1 x 30 µg ODB + – | 1 x 30 µg ODB + 1 x 50 µg GnRH |
| Ewes exhibiting an LH release (%) | $100,0^{a}$ | $77,8^{b}$ | $100,0^{a}$ | $100,0^{a}$ | $*55,6^{c}$ | $88,9^{b}$ |
| Mean maximum LH peak (ng/ml) | $141,1^{a}$ ± 17,4 | $100,0^{a}$ ± 19,7 | $74,4^{b}$ ± 16,5 | $82,6^{b}$ ± 16,5 | $82,8^{b}$ ± 26,1 | $136,5^{a}$ ± 18,5 |
| Total LH release (area under curve) | $587,4^{a}$ ± 48,0 | $*377,2^{b}$ ± 46,1 | $210,1^{c}$ ± 43,8 | $213,5^{c}$ ± 43,8 | $*108,8^{c}$ ± 69,3 | $363,5^{b}$ ± 48,0 |
| Mean duration of release (hours) | $10,7^{a}$ ± 1,4 | $*7,5^{b}$ ± 1,5 | $6,4^{c}$ ± 1,3 | $6,0^{c}$ ± 1,3 | $*2,7^{c}$ ± 2,4 | $7,1^{b}$ ± 1,4 |

a > b > c  Values with the same superscript do not differ significantly

*  indicates sampling ceased before LH release was completed.

From the appearance of the release curves (Fig. 1) and quantitation of the release (Table 3) it is evident that the second dose of GnRH administered 4 hours after the first resulted in a further release of LH. In Treatment (i) (3 x 10 µg ODB plus 2 x 25 µg GnRH) all the ewes responded to both the initial and final GnRH administrations with the LH release being similar at each stage (Table 3). Where GnRH was administered as two doses, but no ODB pre-treatment was applied (Treatment (iii)) two ewes failed to respond to the second GnRH injection and one ewe exhibited a second LH surge which exceeded the initial response. However, on the average the total LH release was significantly smaller after the final GnRH dose (Table 3). Where 2 GnRH injections were preceded by a single dose of oestrogen (Treatment (iv)) the response was more variable in that only three ewes exhibited LH peaks in the second 4-hour sampling period. However, 8 ewes had LH surges which commenced only about 4 hours after the second GnRH injection.

**Table 3**

*LH release induced by a divided dose of GnRH with or without oestrogen priming*

| Treatment | Group | LH release during 4-hour periods after: First administration Max. peak ng/ml | First administration Total * release | Second administration Max. peak | Second administration Total * release |
|---|---|---|---|---|---|
| 3 x 10 µg ODB + 2 x 25 µg GnRH | (i) | $139,0^{a}$ ± 14,1 | $226,1^{a}$ ± 23,0 | $115,1^{a}$ ± 14,1 | $241,1^{a}$ ± 23,0 |
| – + 2 x 25 µg GnRH | (ii) | $70,2^{a}$ ± 12,6 | $145,0^{a}$ ± 20,6 | $32,3^{b}$ ± 12,6 | $69,7^{b}$ ± 20,6 |
| 1 x 30 µg ODB + 2 x 25 µg GnRH | (iii) | $82,6^{a}$ ± 12,6 | $155,3^{a}$ ± 20,6 | $18,3^{b}$ ± 12,6 | $28,0^{b}$ ± 20,6 |

*  Arbitrary units

a,b: Values relating to the same characteristic which bear the same superscript do not differ significantly.

*Oestrogen without GnRH*

Since the LH release appeared to commence approximately 14 hours after the final injection of 10 µg ODB (Fig. 1) whereas it was delayed for a further 12 hours when only 30 µg ODB was administered, any comparison between these two treatments is invalid under the sampling procedure followed.

**Table 4**

*Luteal function in lactating ewes following administration of exogenous hormones*

| Treatment | | | | Group | Ewes exhibiting luteal activity* | Occurrence of luteal activity after first GnRH injection | | Ewes in which progesterone concentration exceeded 1,0 ng/ml in at least one sample |
|---|---|---|---|---|---|---|---|---|
| Oestrogen | | GnRH | | | | | | |
| No. of injections | Dose/ injection | No. of injections | Dose/ injection | | | Before 9th day | After 9th day | |
| 3 | 10 µg | 2 | 25 µg | 1 | 1/10 | 0 | 1 | 0 |
| 3 | 10 µg | 0 | | 2 | 3/9 | 0 | 3 | 1 |
| 0 | - | 2 | 25 µg | 3 | 3/9 | 3 | 0 | 1 |
| 1 | 30 µg | 2 | 25 µg | 4 | 3/10 | 2 | 1 | 2 |
| 1 | 30 µg | 0 | | 5 | 3/9 | 3 | 0 | 2 |
| 1 | 30 µg | 1 | 50 µg | 6 | 4/9 | 4 | 0 | 1 |
| | | | | 7 | 0/9 | 0 | 0 | 0 |

* Progesterone concentration exceeded 0,60 ng/ml plasma in at least two consecutive samples.

*Occurrence of oestrus and lambing*

The data on the incidence of oestrus and the resultant lambing are incomplete and have therefore been omitted. The marking crayons produced doubtful results because of the unusually wet weather. An outbreak of Rift Valley fever resulted in the death of five ewes and an unknown number of abortions.

*Luteal function*

The interpretation of the results obtained from assay of plasma samples for progesterone (Table 4) is complicated by the long interval between some samplings (up to four days) and the failure of marking crayons. Due to the latter, little oestrus data was available to verify the patterns of luteal activity detected.

According to the data in Table 4 none of the treatments applied, resulted in conditions which favoured a reasonable level of luteal function.

## Discussion

*Pituitary depletion and oestrogen priming*

The results obtained in the study described here suggest that the pituitary depletion need not necessarily be a limiting factor as regards a pre-ovulatory LH surge during the early post-partum period. The combination of oestrogen priming for some 24 hours, together with the application of a 4-hour interval between GnRH stimulations, elicited an LH release which can be considered virtually equal to that of cycling ewes, with regard to peak values, duration and total amount of LH released (Lishman *et. al.*, 1974). The relatively poor response to GnRH in the absence of oestrogen priming would support the contention that the priming effect of oestrogen might include increased pituitary synthesis of LH via hypothalamic stimulation (Poultney *et. al.*, 1977).

Neither the earlier work by Jackson, Thurmon and Nelson (1975) and Poultney *et al.* (1977) nor the present investigation both of which incorporated repeat injections of oestrogen, provide a conclusive indication of whether oestrogen sensitization is time or dose dependent. The studies based on continuous infusion (Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969) and implants (Karsch & Foster, 1975) of oestrogen also do not settle the issue, although Foster & Karsch (1975) apparently believe that at least a threshold level needs to be exceeded. Certainly, both duration and magnitude of the priming treatment could be as important in the ewe as in the rhesus monkey (Karsch, Weick, Butler, Dierschke, Krey, Weiss, Hotchkiss, Yamaji & Knobil, 1973).

The sampling procedure that was followed in this investigation was based on earlier studies (Reeves, Beck & Nett, 1974; Jackson, 1975) which suggested that oestrogen would trigger an LH release within approximately 12–15 hours. This assumption proved correct where ODB was administered in 3 doses at 8-hour intervals, but the LH release was not detected until

18 hours after a single injection of 30 μg ODB, and then in only 4 of 9 ewes treated. This contrasts with the finding that in ovariectomized ewes the LH surge commenced as early as 9 hours after a single injection of 50 μg ODB (Jackson, 1975; Jackson, *et al.*, 1975). Clearly, a more protracted sampling period than that employed here, would have been informative.

In view of the foregoing and the failure to demonstrate an improvement in LH release when oestrogen priming was limited to eight hours before GnRH administration, it would appear that the timing of the first GnRH application, in relation to oestrogen priming, could possibly have been delayed by a further 4 hours. This would have allowed the oestrogen sensitization (Beck & Convey, 1974; Coppings & Malven, 1976) to be more fully exploited. The conclusion that pituitary sensitization occurs within six hours after oestrogen administration (Poultney *et al.*, 1977) would thus appear to be not entirely correct, although their data referred to non-lactating ewes that exhibited regular oestrous cycles. The suggestion that the stimulatory effect of oestrogen on LH release is mainly exerted on the brain (12 to 20 hours after oestrogen) but is accompanied by a transient action on the pituitary (Copping & Malven, 1976) and the conclusion that oestrogen increases the duration of the LH response but not peak LH values (Beck & Convey, 1974) warrant careful interpretation.

*GnRH autosensitization*

The priming effect of GnRH on the subsequent response of the pituitary to further GnRH stimulation has been demonstrated in rats (Aiyer, Chiappa & Fink, 1974; Castro-Vasquez & McCann, 1975), ewes (Crighton & Foster, 1977) and rams (Stelmasiak & Galloway, 1977). A refractory period after an initial GnRH stimulation has also been reported (Rippel, Johnson & White, 1974; Symons, Cunningham & Saba, 1974).

The data presented here demonstrate that unless oestrogen is administered, autosensitization by repeated GnRH treatment is transient, lasting less than 4 hours. Even with oestrogen priming no enhanced response was observed when the second GnRH application occurred 4 hours after the first. Clearly, if autosensitization is to be exploited, the interval between GnRH injections should be short and continuous infusion would be preferable.

*Corpus luteum function*

The transient release of progesterone recorded in this experiment is in agreement with that reported by Shareha *et al.* (1976). However, the duration of elevated progesterone levels was inferior to that recorded for anoestrous ewes (Haresign *et al.*, 1975) and ewes lactating in autum (Lewis, Lishman & Inskeep, 1977).

Progesterone secretion is dependent on luteotropic stimulation which has been suggested to consist of both LH and prolactin (Denamur, Martinet & Short, 1973). Prolactin levels are elevated during lactation and only small quantities appear to be required for normal cyclic activity (Louw, Lishman, Botha & Baumgartner, 1974; Niswender, 1974). The reasonable success rate realised when exogenous hormones were used during early lactation in autumn (Hamilton & Lishman, 1979) was in contrast to the poor initiation of cycles when similar techniques were used during spring.

A study of the basal LH levels after the pre-ovulatory surge and their influence on luteal function could be informative. The modifying influence of factors such as season of the year, suckling and presence of rams would also require clarification.

**Acknowledgements**

The generous donation of GnRH by Dr H. Hardie of Abbott Laboratories, of LH standard by the National Institute of Arthritis, Metabolic and Digestive Diseases, of purified LH by Dr Harold Papkoff and of progesterone antiserum by Dr Roy L. Butcher are gratefully acknowledged.

**References**

AIYER, M.S., CHIAPPA, S.A. & FINK, G., 1974. Effect of ovarian steroids on pituitary sensitivity to luteinizing hormone releasing factor in the rat. *J. Endocr.* 61, xii.

ARIMURA, A. & SCHALLY, A.V., 1971. Augmentation of pituitary responsiveness to LH-releasing hormone (LH-RH) by oestrogen. *Proc. Soc. Exp. Biol. Med.* 136, 290.

BECK, T.W. & CONVEY, E.M., 1974. LH after oestradiol and GnRH in spayed heifers. *J. Anim. Sci.* 39, 198.

BONNAR, J., 1973. Studies in the use of luteinizing hormone - follicle stimulating hormone - releasing hormone to induce ovulation in women. *J. Endocr.* 58, xxxiv.

BUTCHER, R.L., COLLINS, W.E. & FUGO, N.W., 1974. Plasma concentrations of LH, FSH, prolactin, progesterone and oestradiol-17 β throughout the 4-day oestrous cycle of the rat. *Endocrinology* 94, 1704.

CA STRO-VAZQUEZ, A. & McCANN, S.M., 1975. Cyclic variations in the increased responsiveness of the pituitary to luteinizing hormone-releasing hormone (LH RH) induced by LHRH. *Endocrinology* 97, 13.

COPPINGS, R.J. & MALVEN, P.V., 1976. Biphasic effect of oestradiol on LH release mechanisms. *J. Anim. Sci.* 43, 279.

18 hours after a single injection of 30 μg ODB, and then in only 4 of 9 ewes treated. This contrasts with the finding that in ovariectomized ewes the LH surge commenced as early as 9 hours after a single injection of 50 μg ODB (Jackson, 1975; Jackson, *et al.*, 1975). Clearly, a more protracted sampling period than that employed here, would have been informative.

In view of the foregoing and the failure to demonstrate an improvement in LH release when oestrogen priming was limited to eight hours before GnRH administration, it would appear that the timing of the first GnRH application, in relation to oestrogen priming, could possibly have been delayed by a further 4 hours. This would have allowed the oestrogen sensitization (Beck & Convey, 1974; Coppings & Malven, 1976) to be more fully exploited. The conclusion that pituitary sensitization occurs within six hours after oestrogen administration (Poultney *et al.*, 1977) would thus appear to be not entirely correct, although their data referred to non-lactating ewes that exhibited regular oestrous cycles. The suggestion that the stimulatory effect of oestrogen on LH release is mainly exerted on the brain (12 to 20 hours after oestrogen) but is accompanied by a transient action on the pituitary (Copping & Malven, 1976) and the conclusion that oestrogen increases the duration of the LH response but not peak LH values (Beck & Convey, 1974) warrant careful interpretation.

*GnRH autosensitization*

The priming effect of GnRH on the subsequent response of the pituitary to further GnRH stimulation has been demonstrated in rats (Aiyer, Chiappa & Fink, 1974; Castro-Vasquez & McCann, 1975), ewes (Crighton & Foster, 1977) and rams (Stelmasiak & Galloway, 1977). A refractory period after an initial GnRH stimulation has also been reported (Rippel, Johnson & White, 1974; Symons, Cunningham & Saba, 1974).

The data presented here demonstrate that unless oestrogen is administered, autosensitization by repeated GnRH treatment is transient, lasting less than 4 hours. Even with oestrogen priming no enhanced response was observed when the second GnRH application occurred 4 hours after the first. Clearly, if autosensitization is to be exploited, the interval between GnRH injections should be short and continuous infusion would be preferable.

*Corpus luteum function*

The transient release of progesterone recorded in this experiment is in agreement with that reported by Shareha *et al.* (1976). However, the duration of elevated progesterone levels was inferior to that recorded for anoestrous ewes (Haresign *et al.*, 1975) and ewes lactating in autum (Lewis, Lishman & Inskeep, 1977).

Progesterone secretion is dependent on luteotropic stimulation which has been suggested to consist of both LH and prolactin (Denamur, Martinet & Short, 1973). Prolactin levels are elevated during lactation and only small quantities appear to be required for normal cyclic activity (Louw, Lishman, Botha & Baumgartner, 1974; Niswender, 1974). The reasonable success rate realised when exogenous hormones were used during early lactation in autumn (Hamilton & Lishman, 1979) was in contrast to the poor initiation of cycles when similar techniques were used during spring.

A study of the basal LH levels after the pre-ovulatory surge and their influence on luteal function could be informative. The modifying influence of factors such as season of the year, suckling and presence of rams would also require clarification.

**Acknowledgements**

The generous donation of GnRH by Dr H. Hardie of Abbott Laboratories, of LH standard by the National Institute of Arthritis, Metabolic and Digestive Diseases, of purified LH by Dr Harold Papkoff and of progesterone antiserum by Dr Roy L. Butcher are gratefully acknowledged.

**References**

AIYER, M.S., CHIAPPA, S.A. & FINK, G., 1974. Effect of ovarian steroids on pituitary sensitivity to luteinizing hormone releasing factor in the rat. *J. Endocr.* 61, xii.

ARIMURA, A. & SCHALLY, A.V., 1971. Augmentation of pituitary responsiveness to LH-releasing hormone (LH-RH) by oestrogen. *Proc. Soc. Exp. Biol. Med.* 136, 290.

BECK, T.W. & CONVEY, E.M., 1974. LH after oestradiol and GnRH in spayed heifers. *J. Anim. Sci.* 39, 198.

BONNAR, J., 1973. Studies in the use of luteinizing hormone - follicle stimulating hormone - releasing hormone to induce ovulation in women. *J. Endocr.* 58, xxxiv.

BUTCHER, R.L., COLLINS, W.E. & FUGO, N.W., 1974. Plasma concentrations of LH, FSH, prolactin, progesterone and oestradiol-17 β throughout the 4-day oestrous cycle of the rat. *Endocrinology* 94, 1704.

CA STRO-VAZQUEZ, A. & McCANN, S.M., 1975. Cyclic variations in the increased responsiveness of the pituitary to luteinizing hormone-releasing hormone (LH RH) induced by LHRH. *Endocrinology* 97, 13.

COPPINGS, R.J. & MALVEN, P.V., 1976. Biphasic effect of oestradiol on LH release mechanisms. *J. Anim. Sci.* 43, 279.

CRIGHTON, D.B. & FOSTER, J.P., 1977. Luteinizing hormone release after two injections of synthetic luteinizing hormone releasing hormone in the ewe. *J. Endocr.* 72, 59.

CRIGHTON, D.B., FOSTER, J.P., HARESIGN, W., HAYNES, N.B. & LAMMING, G.E. 1973. The effects of a synthetic preparation of gonadotrophin releasing factor on pituitary and ovarian function in anoestrous ewes. *J. Physiol.* 231, 98.

DENAMUR, R., MARTINET, J. & SHORT, R.V. 1973. Pituitary control of the ovine corpus luteum. *J. Reprod. Fert.* 32, 207.

FOSTER, D.L. & KARSCH, F.J., 1975. Development of the mechanism regulating the preovulatory surge of luteinizing hormone in sheep. *Endocrinology* 97, 1205.

GODING, J.R., CATT, K.J., BROWN, J.M., KALTENBACH, C.C., CUMMING, I.A. & MOLE, B.J., 1969. Radioimmunoassay for ovine luteinizing hormone. Secretion of luteinizing hormone during oestrus and following oestrogen administration in the sheep. *Endocrinology* 85, 133.

HAMILTON, C.D. & LISHMAN, A.W., 1979. Reducing the partum-to-mating period in autumn lactating ewes through the use of exogenous hormones. *S. Afr. J. Anim. Sci.* 9, 59.

HARESIGN, W., FOSTER, J.P., HAYNES, N.B., CRIGHTON, D.B. & LAMMING, G.E., 1975. Progesterone levels following treatment of seasonally anoestrous ewes with synthetic LH-releasing hormone. *J. Reprod. Fert.* 43, 269.

JACKSON, G.L., 1975. Blockage of oestrogen-induced release of luteinizing hormone by reserpine and potentiation of synthetic gonadotropin-releasing hormone-induced release of luteinizing hormone by oestrogen in the ovariectomized ewe. *Endocrinology* 97, 1300.

JACKSON, G.L., THURMON, J. & NELSON, D., 1975. Oestrogen-induced release of LH in the ovariectomized ewe: Independence of time of day. *Biol. Reprod.* 13, 358.

KARSCH, F.J. & FOSTER, D.L., 1975. Sexual differentiation of the mechanism controlling the preovulatory discharge of luteinizing hormone in sheep. *Endocrinology* 97, 373.

KARSCH, F.J., WEICK, R.F., BUTLER, W.R., DIERSCHKE, D.J., KREY, L.C., WEISS, G., HOTCHKISS, J., YAMAJI, T. & KNOBIL, E., 1973. Induced LH surges in the rhesus monkey: Strength-duration characteristics of the oestrogen stimulus. *Endocrinology* 92, 1740.

LEWIS, G.S., LISHMAN, A.W. & INSKEEP, E.K., 1977. Corpus luteum function in post partum anoestrous ewes after treatment with gonadotropin-releasing hormone (GnRH). *Abstracts, N.E. Section Meeting Amer. Soc. Anim. Sci.*

LISHMAN, A.W., STIELAU, W.J., DREOSTI, I.E., BOTHA, W.A., STEWART, A.M. & SWART, C.E., 1974. The release of LH at oestrus in ewes on two planes of nutrition during lactation. *J. Reprod. Fert.* 41, 227.

LOUW, B.P., LISHMAN, A.W., BOTHA, W.A. & BAUMGARTNER, J.P., 1974. Failure to demonstrate a role for the acute release of prolactin at oestrus in the ewe. *J. Reprod. Fert.* 40, 455.

NISWENDER, G.D., 1974. Influence of 2-Br-α-ergocryptine on serum levels of prolactin and the oestrous cycle in sheep. *Endocrinology* 94, 612.

NISWENDER, G.D., REICHERT, L.E., MIDGLEY, A.R. & NALBANDOV, A.V., 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. *Endocrinology* 84, 1166.

POULTNEY, B.G., LISHMAN, A.W., LOUW, B.P., BOTHA, W.A. & ARANGIE, P.A.R., 1977. The effects of feed restriction, oestrogen priming and stage of the oestrous cycle on Gn-RH-induced release of LH in ewes. *S. Afr. J. Anim. Sci.* 7, 141.

REEVES, J.J., ARIMURA, A. & SCHALLY, A.V., 1971. Pituitary responsiveness to luteinizing hormone-releasing hormone in ewes pretreated with oestradiol benzoate. *J. Anim. Sci.* 33, 266.

REEVES, J.J., ARIMURA, A., SCHALLY, A.V., KRAGT, C.L., BECK, T.W. & CASEY, J.M., 1972. Effects of synthetic luteinizing hormone-releasing hormone/follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH) on serum LH, serum FSH and ovulation in anoestrous ewes. *J. Anim. Sci.* 38, 84.

REEVES, J.J., BECK, T.W. & NETT, T.M., 1974. Serum FSH in anoestrous ewes treated with 17 β -oestradiol. *J. Anim. Sci.* 38, 374.

RIPPEL, R.H., JOHNSON, E.S. & WHITE, W.F., 1974. Effect of consecutive injections of synthetic gonadotropin releasing hormone on LH release in the anoestrous and ovariectomized ewe. *J. Anim. Sci.* 39, 907.

SHAREHA, A.M., WARD, W.R. & BIRCHALL, K., 1976. Effect of continuous infusion of gonadotropin-releasing hormone in ewes at different times of the year. *J. Reprod. Fert.* 46, 331.

SYMONS, A.M., CUNNINGHAM, N.F. & SABA, N., 1974. The gonadotropic hormone response of anoestrous and cyclic ewes to synthetic LH-RH. *J. Reprod. Fert.* 39, 11.

STELMASIAK, T., & GALLOWAY, D.B., 1977. A priming effect of LH-RH on the pituitary in rams. *J. Reprod. Fert.* 51, 491.