

The Effect of Oestradiol-17 β and Progesterone on the Pituitary Response to Gonadotrophin-Releasing Hormone (Gn-RH) in Anoestrous Ewes

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ABSTRACT

The effect of progesterone (P) injection (2×12.5 mg/day) for 4 days and/or oestradiol-17 β (E₂-17 β) infusion at a rate of 3 μ g/hr for 4 hours on the concentration of luteinizing hormone (LH) released by Gn-RH infusion (3 μ g/hr for 8 hours) was investigated in anoestrous ewes. The concentration of the LH released by Gn-RH was similar to that observed at oestrus. Sequential (P) and (E₂-17 β) administration had no effect on LH release by Gn-RH, however, (E₂-17 β) alone increased and (P) alone decreased the response.

INTRODUCTION

Peripheral plasma concentrations of luteinizing hormone (LH) in the ewe can be increased by oestrogen administration (4,9,30). Although progesterone has been shown to suppress LH release by either oestrogen or Gn-RH (14,20,27). Other investigators have reported no effect of Gn-RH in ewes (5,6). It has been suggested by Hooley *et al.* (14) that a progesterone stimulus for more than 24 hours may be required to suppress LH release. Inhibition of the induction of ovulation by Gn-RH was overcome in the rabbit either by increasing the dose of Gn-RH or by treatment with oestrogen (12). Thus the duration of stimulus and dose may both be important factors regulating anterior-pituitary response.

The purpose of this study was to observe the effect of exogenous steroids, in doses producing plasma concentrations similar to those found in normal oestrous cycle, on LH release by Gn-RH in Scottish Blackface ewes during the anoestrous season. Normal values for concentrations of steroids and LH during the oestrous cycle, which have been used for comparative purposes in this study, are taken from previous investigations (28,29).

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MATERIALS AND METHODS

Twenty nine anoestrous ewes were randomly assigned to seven groups:

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| I. Gn-RH | II. Oestradiol-17 β B, (E ₂), Gn-RH |
| III. Progesterone (P), Gn-RH | IV. P, E ₂ , Gn-RH, |
| V. E ₂ , | VI. P, E ₂ and |
| | VII. P |

Treatment was as follows:

Progesterone (Intervet), 12.5 mg in oil, was injected interamuscularly twice daily for 4 days. Eighty four hours after the last progesterone injection, intracarotid infusions were started at a rate of 3 μ g/hr of either oestradiol-17 β in 5% ethanolic saline (for 4 hours), or Gn-RH (synthetic decapeptide ICI 82, 286, obtained from ICI Alderley Park, Macclesfield Cheshire U.K.) made up in saline immediately before use (for 8 hours). When oestradiol-17 β and Gn-RH treatments were preceded by progesterone (Group IV), infusion of Gn-RH was started at 88 hours after the progesterone treatment. Jugular blood samples were obtained once daily from the beginning of progesterone treatment until the infusion, then every 2 hours (with an additional sample at 1 hr after the start of Gn-RH) up to 36 hr. Total oestrogens, progesterone and LH were measured in plasma, using radioimmunoassay (Shareha *et al.*, 1976). Results are expressed as mean $\pm$ standard deviation with N = number of determinations and were analyzed using student's 't' test or analysis of variance.

RESULTS AND DISCUSSION

In Groups I, II, III, and IV (Fig. 1), there was no significant difference between the maximum concentration of LH at oestrus (138.8 ± 83.4 N = 8) and that following Gn-RH infusion in the anoestrous season (138.8 ± 22.5 ng/ml N = 4, Fig. 1 I). When ewes were infused with oestradiol-17 β (Fig. 1 II) peak concentrations of total-oestrogens (66.9 ± 14.7 pg/ml, N = 5) were three times those at oestrus (21.1 ± 3.1 pg/ml, N = 11). A similar infusion used by Pant (19) in anoestrous Clun Forest ewes gave plasma concentrations of LH within the upper range of those observed at oestrus. Since total oestrogen concentration was the same when assayed with the two different antisera used in these studies, it is suggested that the two breeds may metabolize oestrogen at different rates. A difference in the number of ovulations induced by Gn-RH between Clun Forest and Scottish Blackface ewes during the anoestrous season has recently been reported (11).

Pre-treatment with oestradiol-17 β resulted in a significant increase ($P > 0.01$) in LH released by Gn-RH (200.0 ± 15.8 ng/ml N = 5) when compared with that released by Gn-RH alone (138.0 ± 22.5 ng/ml, N = 4). These findings are in agreement with previous reports on an increase in the sensitivity of the pituitary to synthetic Gn-RH during oestrus and following treatment with oestradiol benzoate (13,24,25).

Investigations of events culminating in the LH surge at oestrus in rats indicate that first, as a result of the increase in oestrogen, there is augmentation of pituitary sensitivity (1) and this is then followed by a self-potentiating effect of Gn-RH on LH release (2). However, one hour after the start of Gn-RH infusion, in ewes pretreated with oestradiol-17 β , the LH concentration was significantly lower ($P > 0.001$) than in ewes

receiving Gn-RH alone (13.2 ± 5.0 ng/ml, $N = 5$; vs. 46.8 ± 16.1 ng/ml, $N = 4$). Evidence for a negative feedback effect of oestrogen on LH release has been obtained in the ovariectomized ewe (9,23,27), as well as, in other ovariectomized animals (13,17,18), also in intact rat (13) and in prepubertal intact gilt (21). In ovariectomized and intact rats, maximal inhibitory effect, of oestrogen on LH release has been observed at 2 and 3 hr respectively after the start of treatment, but in ovariectomized rats this effect had disappeared by 4 hr (7). Similarly, in the heifer and ewe, the inhibitory effect has been observed at 2 or 3 hr and in the heifer a positive response could not be elicited by Gn-RH until 4–6 hr after oestrogen. Thus, in the present investigations it is suggested that the Gn-RH infusion was started at a time when the inhibitory effect of oestrogen on LH release was still prevailing, therefore, the system was unable to respond fully to the Gn-RH stimulus. Peak concentrations of LH were observed 4 hr later. Since maximal concentrations of LH are attained 2–4 hr after the start of an infusion of Gn-RH without previous treatment with oestrogen (18), it is assumed that the pituitary would be capable of responding to the Gn-RH after 6–8 hr from the start of oestrogen infusion. However, in Group V there was no change in LH concentration for 16 hrs, at which time only 2/4 ewes responded to the oestradiol-17 β infusion with LH peaks of 36 and 80 ng/ml. The time lapse between oestrogen treatment and LH release is similar to that reported by Beck and Reeves (3), who also observed that there was no dose response relationship between oestradiol-17 β and LH and that 33% anoestrous ewes failed to respond to administration of 12.5 μ g oestradiol-17 β . In the present study, 1 of the 2 ewes which did not respond had a relatively high plasma concentration of progesterone (2.3 ng/ml).

The progesterone treatment schedule in Group III, IV, VI and VII resulted in maximum concentrations (5.8 ± 2.0 , $N = 4$; 5.0 ± 0.9 , $N = 4$; 56.1 ± 2.2 , $N = 3$ and 3.8 ± 1.2 ng/ml, $N = 5$ respectively), which were not significantly different from each other or from those found in a normal luteal phase (5.1 ± 1.4 ng/ml, $N = 11$). In a preliminary experiment, ewes were treated with progesterone and the plasma concentrations were measured before, during and up to 96 hr after the treatment. The concentrations returned to pre-treatment values 72–84 hr after the last progesterone injection; therefore an interval of 84 hr was scheduled before starting infusions. Nevertheless, the progesterone concentration for Group III at the start of the infusion was 1.1 ± 0.3 ng/ml, $N = 4$, which was significantly higher ($P > 0.05$) than that found in the normal cycle prior to oestrus (0.6 ± 0.4 , $N = 10$), while that for each of Groups IV, VI, and VII was significantly lower ($P > 0.25$) being (0.4 ± 0.4 , $N = 0.5 \pm 0.2$, $N = 3$ and 0.4 ± 0.4 , $N = 5$ respectively at 84 hr. The maximum concentration of LH following treatment with Gn-RH in ewes pretreated with progesterone (Fig. 1 III) being 95.0 ± 19.2 mg/ml, $N = 4$ significantly less ($P > 0.5$) than that released by Gn-RH alone and concurs with the findings of Pant & Ward (20) and Hooley *et al.* (14). Since Jaume (16) and Pretorius (22) have reported an increase in pituitary content of LH during the luteal phase, also in a previous work Roche *et al.* (26) had shown in ewes, that the pituitary content of LH was high during anoestrus. The inhibitory effect of progesterone is apparently not due to a lack of LH, but may be a result of the stored acting at the pituitary to cause either a reduction in the pool of LH available for release (14) or an impairment in the mechanisms of release. There was no change in the LH concentration in 5 ewes following P pretreatment with progesterone alone (Group VII) or 2/3 ewes receiving progesterone and oestrogen (Group VI). A small amount of LH (20 ng/ml maximum concentration) was released in the third ewe in Group VI, 16 hr after the start of oestrogen infusion.

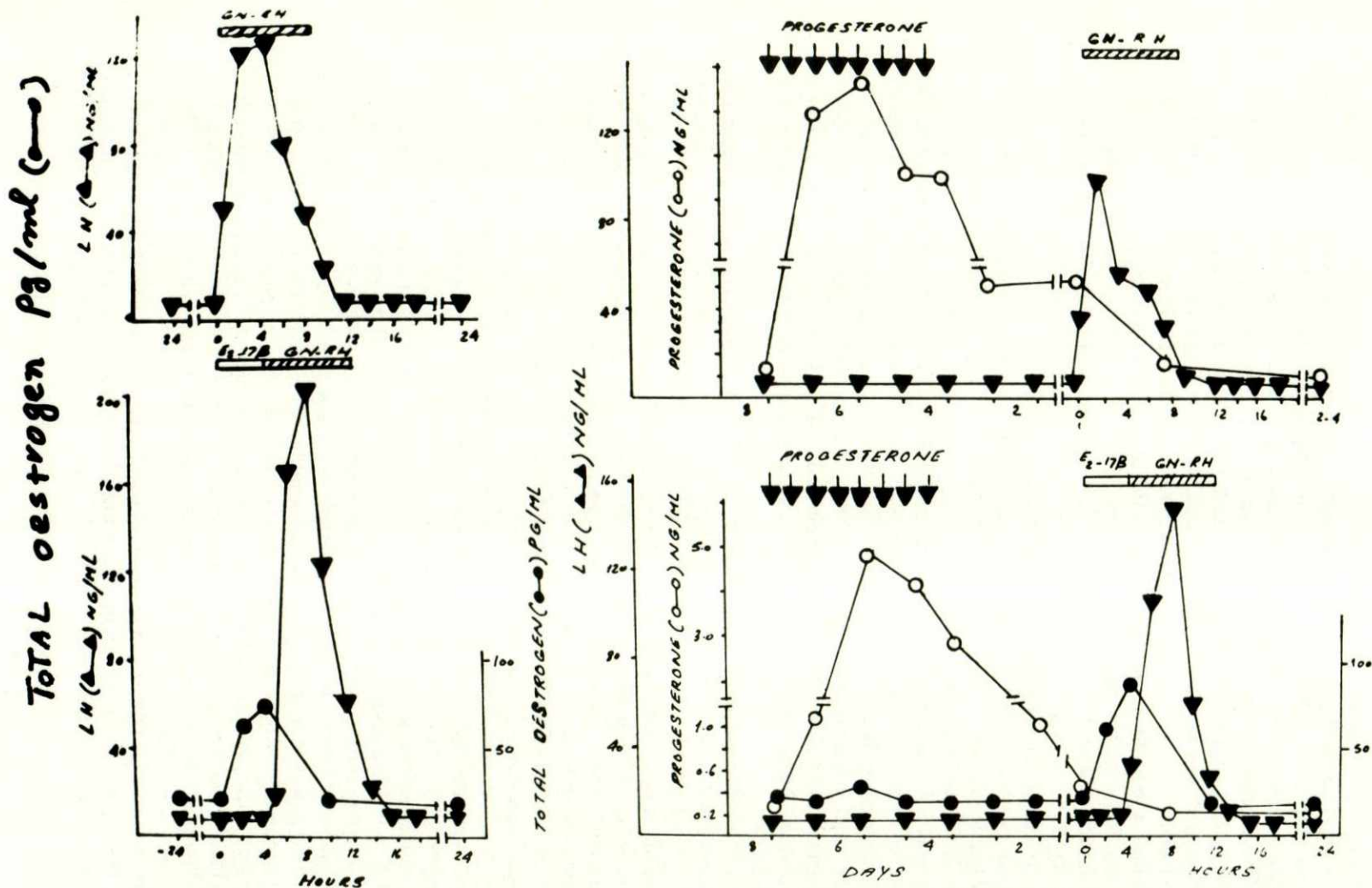


Fig. 1. Plasma concentrations of LH (▲—▲), total oestrogen (●—●) and progesterone (○) following different treatment schedules. Arrows indicate progesterone injections. Horizontal bars indicate infusion open- E_2 ; crosshatched-Gn-RH. (I) Gn-RH. (II) Gn-RH, (II) P, Gn-RH, (VI) P, E_2 , Gn-RH (see text).

Thus, despite treatments simulating a normal cycle, the pituitary response in terms of LH released was inadequate. Since Gn-RH activity of the hypothalamus is greater during anoestrus than in the breeding season (15), it is possible that in anoestrus there are alterations in the potency of the neurohumoral signal required to trigger a possible 'activation' or release of endogenous Gn-RH.

The concentration of LH after progesterone and oestrogen treatment (Group IV), being 148.8 ± 41.7 ng/ml, $N = 4$, (Fig. 1 IV) was not significantly different from that released by Gn-RH alone, but was more than that released in Group III ($P > 0.05$) and less than that released in Group II ($P > 0.05$). The different results of Debeljuk (7), who reduced the LH release by Gn-RH by pre-treatment with a combination of progesterone and oestrogen can be related to differences in route of administration, timing of treatments and amounts of steroids and Gn-RH administered. Inhibition of the positive feedback effect of oestrogen on LH release by progesterone has been previously demonstrated in the intact ewes, in the present investigation. Also, has been reported in the intact Rhesus Monkey (8).

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تأثير هرمون الاستروجين والبروجسترون على حساسية الغدة اللمفاوية للهرمون المسبب (Gn-RH)

في غير موسم الاخصاب عند الاغنام

د . عاشور مسعود شريجه

المستخلص

تأثير حقن هرمون البروجسترون (١٢٥٥ مللي جرام مرتين في اليوم لمدة اربعة ايام) و / أو حقن مستمر في الاوعية الدموية للاستروجين (٣ ميكروجرام / الساعة لمدة اربعة ساعات) على تركيز هرمون (LH) الذي ارتفع في الدم بعد حقن مستمر للهرمون المسبب (Gn-RH) (٣ ميكروجرام / الساعة / لمدة ٨ ساعات) . قد اكتشف في غير موسم الاخصاب عند الاغنام .

١ - تركيز هرمون المبيض (LH) في الدم كنتيجة لحقن الهرمون المسبب (Gn-RH) كان مماثلاً للتركيز الملحوظ عند عملية الشبق .

٢ - تعاقب حقن الهرمونين البروجسترون والاستروجين ليس له تأثير ملحوظ على ارتفاع هرمون المبيض (LH) كنتيجة لحقن الهرمون المسبب (Gn-RH) غير أن حقن مستمر لهرمون الاستروجين لوحده زاد من حساسية لغدة اللمفاوية للهرمون المسبب (Gn-RH) وحقن البروجسترون لوحده سبب في انخفاض لهذه الحساسية .