Assiut Vet. Med. J. Vol. 41 No. 81 April 1999.

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RESPONSE OF LH TO LONG-TERM PULSATILE AND CONTINUOUS INFUSIONS OF GNRH IN OVARIECTOMIZED, ESTRADIOL –17 B IMPLANTED EWES DURING SEASONAL ANOESTROUS

(With 3 Figures)

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(Received at 16/3/1999)

استجابة الهرمون المنبه للجسم الأصفر للاعطاء المتذبذب والمستمر للجونادوتروفين في نعاج منزوعة المبيض خلال فصل انعدام الشبق

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تم دراسة استجابة الجونادوترفين بعد الاعطاء الطويل والمتذبذب للهرمون المحفز لافراز الجونادوترفين من خلال حقنة في نعاج (لوهي-باكستان) منزوعة المبيض ومزروعة في اذانها بالاستراديول ١٧-ب خلال موسم عدم الشياع لها. تم إجراء التجربة علي مجموعتين. كل مجموعة تتكون من ٨ نعاج. تم حقن نصف عدد النعاج في كل مجموعة بالهرمون المحفز لافراز الجونادوترفين (١٧٥ نانوجرام/ساعة) والنصف الآخر تم حقنها حقنا متذبذباً ب ٣٥٠ نانوجرام من الهرمون المحفز لافراز الجونادوترفين كل ساعتين ولمدة ٢٠ يوماً. وقد تم إعطاء الهرمون المحفز لافراز الجونادوترفين عن طريق قسطرة داخل الوريد الوداجي. تم تجميع عينات الدم لتحديد الهرمون المنبه للجسم الأصفر كل ١٥ دقيقة بداية من ٦ ساعات قبل وحتى ٢٤ ساعة بعد بداية العلاج وبعد ذلك كل ٨ ساعات في اليوم الثالث والسادس والعاشر والخامس عشر والعشرين من بداية العلاج. بعد أخذ عينة الساعة الثامنة في اليوم العشرين، تم أخذ عينة اخرى بعد ١٢ ساعة من نهاية العلاج ماشرة. وجدت زيادة في تركيز الهرمون المنبه للجسم الأصفر في الحيوانات المحقونة بالهرمون المحفز لافراز الجونادوترفين قبل بدء العلاج. بينما بعد الزيادة المبدئية في اليوم الأول، لم يكن هناك زيادة في الهرمون المنبه للجسم الأصفر عنه قبل العلاج في خلال باقي فترة العلاج. في المقابل وجد أن النعاج المحقونة قد استجابت للهرمون المحفز لافراز الجونادوترفين خلال فترة العشرين يوم العلاجية. وأظهرت النتائج أن الغدة النخامية تظل تستجيب للاعطاء المتذبذب وليس للاعطاء المستمر للهرمون المحفز لافراز الجونادوترفين لمدة طويلة.

SUMMARY

Pituitary LH responses to long-term pulsatile and continuous infusion of GnRH were monitored in estradiol-17 B-implanted ovariectomized ewes during seasonal anoestrous (April and May). The experiment was performed in 2 replicates (8 ewes per replicate). Half of the animals in each replicate were infused continuously with GnRH (175 ng/h), while the other half were given a pulsatile injection of 350 ng GnRH every 2 h for a period of 20 days. GnRH administration was carried out via indwelling jugular vein catheter. Blood samples for LH determination were collected at 15-min intervals, from 6 h before until 24 h after the start of treatment, and then at 8- h intervals on days 3, 6, 10, 15, and 20 of the treatment. The 8-h bleed on day 20 was immediately followed by a 12-h bleed once the treatment had ended. Before the start of GnRH treatment, plasma LH concentrations rose immediately in infused animals. However, after an initial significant elevation on day 1, LH values were not different from mean pre-treatment concentrations for the rest of the treatment period. In contrast, injected ewes (350 ng GnRH) responded to each GnRH injection throughout the 20-day treatment period. The results suggested that the pituitary gland remains responsive to pulsatile but not to continuous GnRH administration for longer time periods.

Key Words: LH GNRH Ewes Anoestrous

INTRODUCTION

Seasonal anoestrous is characterized by an inadequate pattern of tonic LH release (Yuthasastrkosol et al, 1977; Baird, 1978) and correction of this inadequacy by pulsatile administration of low doses of GnRH into seasonally anoestrous ewes eventually leads to a preovulatory LH surge and ovulation (McLeod et al, 1982). Moreover, if pulsatile GnRH treatment is prolonged (40-80 days), ovarian cyclicity can be restored for longer periods in such animals (McNatty et al, 1982). While there is a consistency in response to pulsatile GnRH treatment, there is considerable controversy regarding the continuous administration of GnRH. Pituitary refractoriness has been reported to develop in seasonally anoestrous ewes when infused continuously with GnRH (2.3 ug/h) for 24 h (Chakraborty et al, 1974). On the other hand, Mcleod et al (1983) have shown that short-term continuous infusion of

low doses of GnRH (125 or 250 ng/h for 48 h) in progesterone-primed seasonally anoestrous ewes not only results in a sustained increase in LH secretion, but also induces ovulation followed by normal luteal function. Although pulsatile GnRH treatment can restore ovarian cyclicity in seasonally anoestrous ewes for as long as the treatment continues, this method of GnRH administration is impractical under field conditions. Ideally there is a need for a therapy which could not only maintain oestrous cycles in ewes throughout the anoestrous season but also to be practical on a commercial scale. Although it has been shown that continuous infusion of GnRH will induce fertile ovulations in anoestrous ewes, these studies to date have involved only short period's (48 h) of GnRH administration. It has been suggested that protracted periods of GnRH infusion result in pituitary down-regulation at least in hypothalamic-lesioned, ovariectomized Rhesus monkeys (Knobil, 1980). The present study was, therefore, designed to investigate whether long-term (21 days) continuous infusion of GnRH is as effective as pulsatile administration in maintaining tonic gonadotrophin secretion in seasonally anoestrous ewes.

MATERIALS and METHODS

Ewes used in this study were of Lohi breed. They aged two to three years. This study was done in the premises of the College of Veterinary Sciences, University of Agriculture, Lahore, Pakistan during the months of April and May. All the ewes were housed under conditions of natural day length and temperature, with the exception that blood sampling at night was carried out under dim white light. The ewes were restrained in metabolism crates 15 throughout the treatment period. They were fed a diet consisting of "indoor" ewe concentrates and hay, with fresh water always available. Animals were divided into two replicates. For each replicate, eight ewes were allocated. At least 2 weeks prior to each trial, the ewes were bilaterally ovariectomized and immediately given an implant containing oestradiol-17 B which was inserted subcutaneously in the axillary region. These implants were designed to maintain plasma oestradiol concentrations at luteal phase levels of 3-5 pg/ml (Karsch, et al, 1973). Half of the animals in each replicate were infused continuously with GnRH 18 (Lutal: Fabwerke Hoechst, Frankfurt, West Germany) at the rate of 175 ng/h for 20 days, while the other half were given a pulsatile injection of 350 ng GnRH every 2 h for a period of 20 days. The infusions were given via an

indwelling jugular vein catheter by means of a peristaltic pump (Model CPP15, Chemlab Ltd., Hornchurch, Essex, England) at an infusion rate of 20 ml/h. Theinjections were also administered through an indwelling jugular vein catheter using a peristaltic pump (Model MC10, Watson Marlowe Ltd., Falmouth, England) fitted with a timer; the volume of each injection was 2 ml and was administered over a period of 20 seconds. Blood samples (2 ml) for LH determination were collected via a catheter inserted in the contralateral jugular vein at 15-min intervals from 6 h before until 24 h after the start of treatment and then for 8-h periods on days 3, 6, 10, 15 and 20. The 8-h bleed on day 20 was immediately followed by a 12-h bleed once the treatment had ended.

Plasma LH concentrations were measured according to the method of Foster and Crighton (1974) as modified by McLeod et al (1982). The limit of sensitivity of the assay was 0.37 ng NIH-LH-S19 equiv/ml. The intra- and inter-assay coefficient of variation was 11.2% and 9.9%, respectively. Because of the great variation observed in LH concentrations between animals and between days within animals, all the values were transformed into logarithmic form. The purpose was to normalize the distribution of the data before subjecting it to split-plot analysis of variance. However, for the sake of clarity, the hormone concentrations presented were back-transformed from the mean logarithmic values. Since the results of 2 replicates were not different from each other, the data were pooled before subjecting it to statistical analysis. Total LH release was estimated only during the first day of GnRH treatment by measuring the area under the LH profile using a machine designed to measure leaf areas and was expressed as cm^2.

RESULTS

Before the start of GnRH treatment, plasma LH concentrations were basal in all animals and characteristic of the seasonally anoestrous ewe. At the start of treatment, plasma LH concentrations rose immediately in infused animals (fig 1) and injected ewes responded to each GnRH injection with an episodic release of LH (fig 2). Whereas in infused animals LH response was short-lived, injected ewes remained responsive throughout the 20-day treatment period (fig 3 a, b). However, at the end of treatment, the mean plasma LH levels decreased rapidly to basal concentrations in both groups.

GnRH treatment resulted in considerable variation between the individual ewes in the pattern of LH release in both the infused and

injected animals. The maximum concentration of the initial LH release ranged from 14.5 to 64.6 ng/ml in infused animals, whereas the maximum amplitude of GnRH-induced LH episodes in injected animals ranged from 2.6 to 64.5 ng/ml during the first day of treatment.

The overall mean LH concentrations for before- and after - treatment periods and each of days 1, 3, 6, 10, 15 and 20 of the treatment for both infused and injected ewes are presented in figure 3. The overall mean LH release (throughout the experimental period) in response to injections was greater ($p<0.01$) than that in response to the infusion, although the amount of LH released during the first day of the GnRH treatment was similar (non-significant) for both infused ($78 + 11$ cm^2) and injected ($79 + 22$ cm^2) ewes. There was a difference ($p<0.001$) in LH concentrations between the different time periods after the start of GnRH treatment, but the significant ($p<0.001$) interaction between periods and methods of GnRH administration suggested that the difference between the experimental periods depended upon the method of GnRH administration (fig 3 a, b).

After the initial response on day 1 of GnRH treatment, the pattern of LH release varied both in infused and injected animals. In infused animals after an initial elevation on day 1, LH values were not different from mean before-treatment concentrations although they were slightly higher on days 15 and 20 before falling again when infusion ceased (fig 3a). In injected animals, mean plasma LH concentrations remained elevated ($p<0.001$) until day 20 of the treatment (fig 3b).

DISCUSSION

The data obtained in this study (fig 3 a, b) suggest that after an initial stimulation, the pituitary gland did not maintain a consistent response to GnRH infusion and increases observed beyond the initial 24 h of the infusion period were very small, and may not be important from a physiological point of view. The initial response of the pituitary gland was much greater than that observed previously (McLeod et al, 1982, 1983) using similar doses of GnRH in entire seasonally anoestrous ewes. In fact the magnitude of this response was closer to that observed after single large dose injections (Crighton et al, 1974; Haresign et al, 1975) or continuous infusion of large doses of GnRH (Shareha et al, 1976; McLeod and Haresign, 1984) in intact seasonally anoestrous ewes.

Previous studies have shown that initial high LH responses, resulting either from large dose continuous infusions (Chakraborty et al,

1974) or from large dose multiple injections (Crighton et al, 1975) of GnRH in seasonally anoestrous ewes, lead to desensitization of the pituitary gland. In this experiment, although the dose of GnRH used was not high, the initial LH response was similar to that observed after high dose infusion or injections of GnRH. This was most probably due to the hyper-stimulation of the pituitary gland, which resulted from the specific animal model used. However, it is also possible that the lack of pituitary responsiveness throughout the treatment period may be due to the continuous mode of administration of GnRH, as has been reported previously in the Rhesus monkey (Knobil, 1980). This is further supported by the fact that desensitization of the pituitary gland was observed only in infused animals. Had desensitization been due to the higher initial LH response and/or specific animal model used, it would have been observed in the injected ewes as well.

In contrast to responses to continuous infusion, each GnRH injection resulted in an episodic release of LH, and the pituitary gland remained responsive to pulsatile GnRH therapy throughout the 20-day experimental period. Whereas, continuous infusion of GnRH seemed to result in only immediate release of LH, pulsatile GnRH treatment not only increased the responsiveness in the short-term, but also appeared to cause long-term priming of the pituitary gland. As pulsatile GnRH is necessary for both LH secretion (Clarke et al, 1996) and synthesis (Hamernick et al, 1985), it may be speculated that the short-term priming of the pituitary gland may be due to the conversion of the non-release form to the releasable form of LH, whereas the long-term effect may have resulted either from an increase in de novo LH synthesis by the pituitary gland or alternatively from the release of already existing pituitary LH stores without any increase in LH synthesis.

GnRH has been reported to be secreted in a pulsatile manner at least in the ovariectomized ewe (Levine et al, 1982). If pulsatile mode of GnRH secretion is believed to be the physiological mode, then lack of pituitary responsiveness to continuous GnRH administration could be explained. If this would be the case, then the ability of continuous infusion of GnRH to maintain pituitary responsiveness for a short-term (as observed in this experiment) but enough to induce normal follicular phase pattern of LH secretion leading to fertile oestrous (Mcleod et al, 1983; Wright et al, 1993) remains an enigma. The fact that continuous infusion of a low dose of GnRH maintained LH secretion only for a short-term, precludes the possibility of the development of implants to

release GnRH at constant rates for long durations for the maintenance of cyclic ovarian activity throughout the anoestrous season.

ACKNOWLEDGEMENTS

We thank the National Institute of Health for purified LH, Professor L. E. Reichert for purified ovine LH for the production of radioligand and Hoechst Pharmaceutical for the GnRH.

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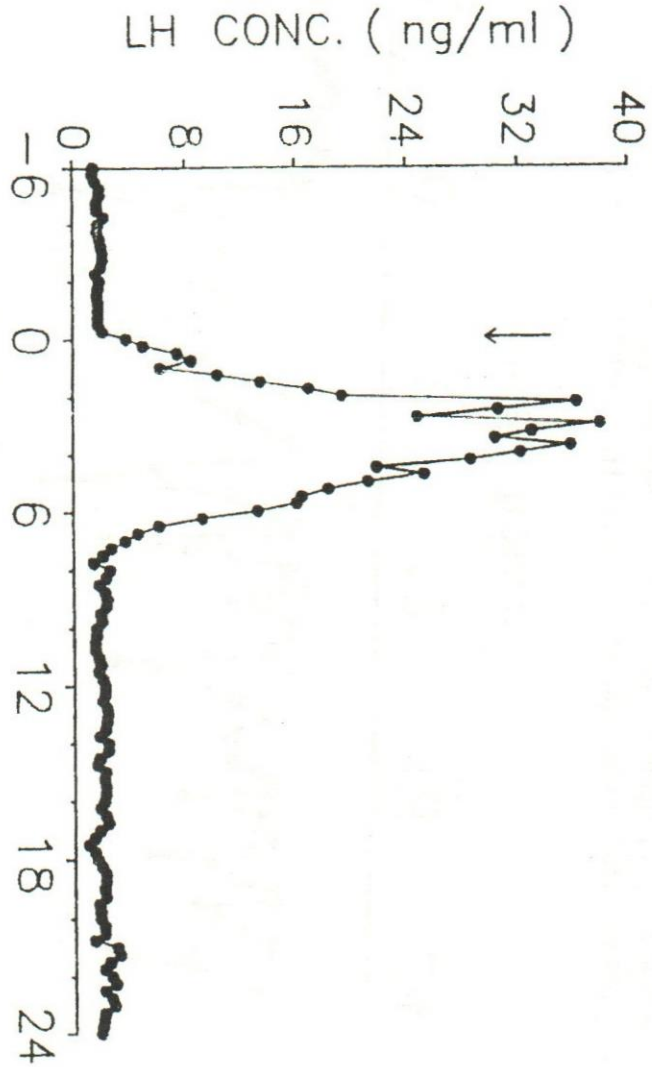

Figure 1. Mean plasma LH concentrations (NIH-LH-S19 equiv/ml) from 6 h before until 24 h after start of GnRH infusion (175 ng/h) in ewes (n = 8). The arrow indicates the start of GnRH infusion

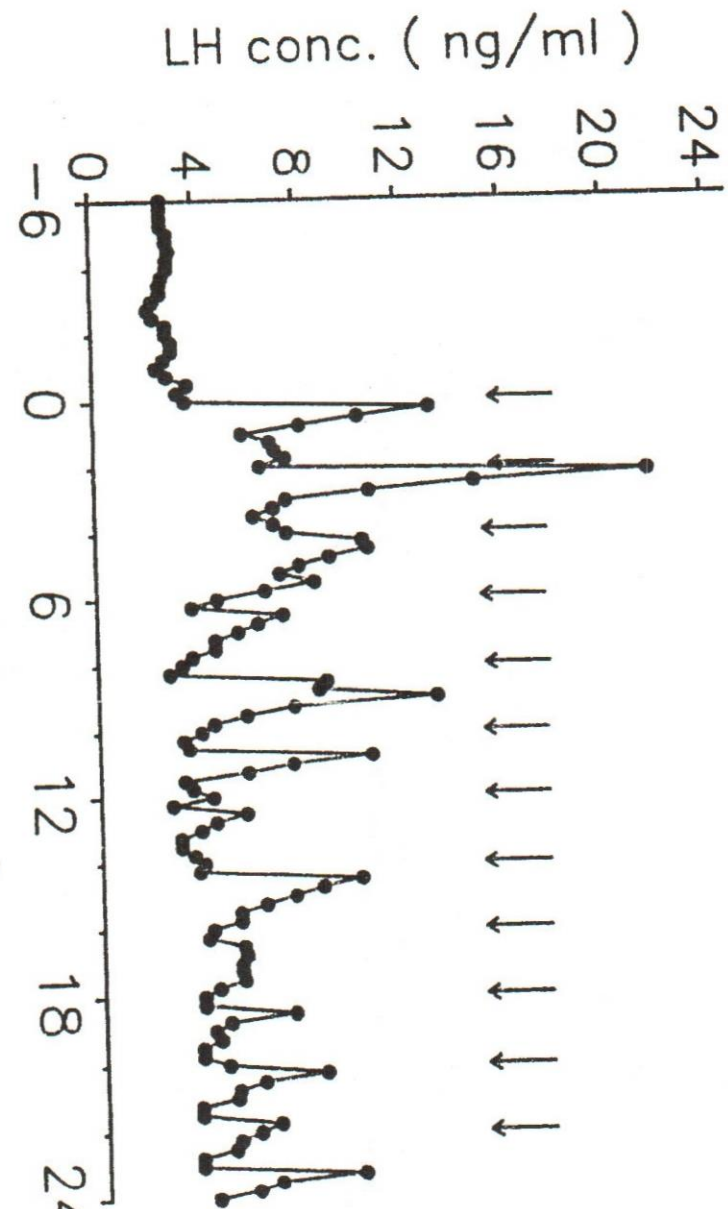

Figure 2. Mean plasma LH concentrations (NIH-LH-S19 equiv/ml) from 6 h before until 24 h after start of GnRH injections (350 ng/2 h) ewes (n = 8). The arrows indicate the time of GnRH injections

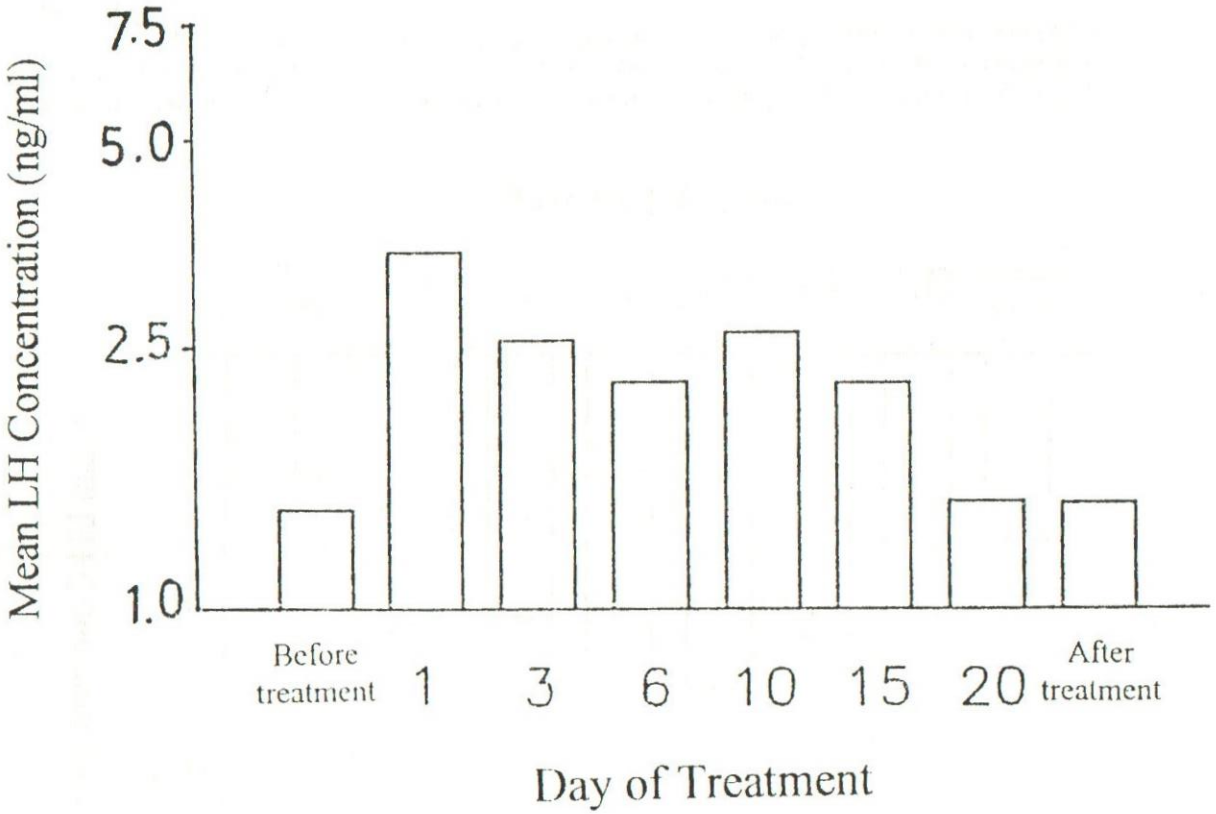

Figure 3a. Mean LH response of the ewes infused with 175 ng of GnRH from day -1 (6 h before treatment) until 24 h after the start of GnRH treatment (day 1), and then for 8 h periods on days 3, 6, 10, 15, and 20, and for a further 12 h period at the end of GnRH treatment (after treatment).

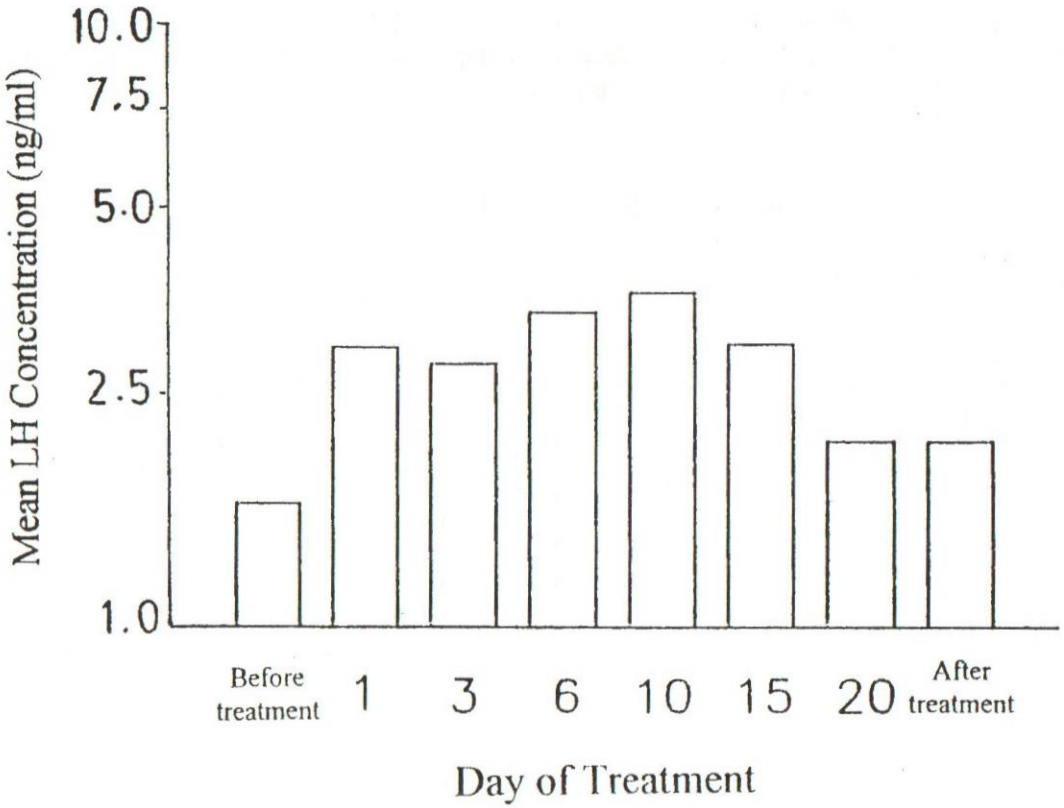

Figure 3b. Mean LH response of the ewes injected with 350 ng/2 h of GnRH from day -1 (6 h before treatment) until 24 h after the start of GnRH treatment (day 1), and then for 8 h periods on days 3, 6, 10, 15, and 20, and for a further 12 h period at the end of GnRH treatment (after treatment).