# THE RELEASE OF LUTEINIZING HORMONE IN EWES FOLLOWING THE WITHDRAWAL OF INTRAVAGINAL SPONGES CONTAINING PROGESTAGEN

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*Summary*

Intravaginal pessaries (hereafter termed "sponges"), impregnated with 9α-fluoro-11β-hydroxy-17α-acetoxy-progesterone were used for synchronization of oestrus in ewes during the breeding season. Luteinizing hormone (LH) concentration was measured in jugular venous plasma collected at one to four hour intervals following withdrawal of the sponges. Similar studies were made in ewes where oestrus was synchronized by treatment with progesterone, and also in untreated ewes about the time of oestrus.

In the 12 untreated ewes, a massive release of LH occurred, the magnitude of which was similar in all animals; the interval between the onset of oestrus and beginning of the LH pre-ovulatory release varied between 0 and 18 hours. In the six progesterone-treated ewes, an LH release of magnitude similar to the control ewes was observed; in this group, the time of commencement of the LH release ranged from 2 hours before to 21 hours after the onset of oestrus.

However, when "Cronolone"-impregnated sponges were used, although the magnitude of the LH release was similar to that found in the preceding groups, the onset of the LH release frequently occurred much earlier in relation to the onset of oestrus; in 7 of 12 ewes, the release began from 4 to 26 hours bfore oestrus.

These results afford a possible explanation for the lowered fertility of ewes mated at the first oestrus after removal of "Cronolone"-impregnated sponges.

## I. INTRODUCTION

Dutt and Casida (1948) first reported that intramuscular injections of progesterone suppressed ovulation and oestrus in sheep. Oestrus and ovulation occurred within five days of progesterone withdrawal. Fertility following natural or artificial insemination after this treatment may be normal (Lamond and Bindon 1962; Lamond 1963), but is sometimes reduced (Robinson 1956, 1958; Davies and Dun 1957; Davies 1960; Lamond 1960).

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Robinson and colleagues pioneered the use of sponges impregnated with a progestagen for synchronization of oestrus on a commercial basis in sheep flocks. Progesterone itself was not absorbed in sufficient quantity for adequate control of ovulation, but "Cronolone"† 9$\alpha$-fluoro-11$\beta$-hydroxy-17$\alpha$-acetoxy-progesterone, Searle), a more potent progestagen, was found to be effective. Ovulation and oestrus were suppressed while "Cronolone''-impregnated sponges were in position and usually occurred within five days after sponge withdrawal. Field trials revealed that fertility at this oestrus was frequently lower than normal (Clarke et *al.* 1966; Robinson et ***al.*** 1967).

No information was available concerning the release of Luteinizing Hormone (LH) in "Cronolone''-treated animals. With the development of a simple and effective solid phase method of radio-immunoassay of LH plasma (Goding et ***al.*** 1969), it was possible to make detailed observations of the pituitary response to progestagen withdrawal. The experiments described in this paper were undertaken to show a comparison between "Cronolone" and progesterone in both magnitude and time relationships of the LH release after withdrawal of progestagen. Normally cycling ewes were studied as controls.

## II. MATERIALS AND METHODS

Progesterone‡ in arachis oil (10 mg/ml) was administered by intramuscular injection.

The ewes were run continuously with two mature vasectomized Corriedale rams wearing harnesses and marking crayons (Radford, Watson and Wood 1960), and the ewes were checked for markings at least once every three hours. Jugular vein blood was collected from indwelling silastic cannulae into heparinized syringes, centrifuged and the plasma removed and stored at -10°C. The method for the determination of LH was reported previously (Goding et ***al.*** 1969).

Two experiments were conducted:—

### *(a) Experiment 1*

In July 1968, "Cronolone" sponges were withdrawn from eight Corriedale ewes (three years old), 14 to 16 days after insertion. Blood was sampled from these ewes every four hours after sponge withdrawal for a period of five days if mating did not take place. However, as soon as a ewe mated during this period, blood was collected hourly for the ensuing 24 hours. The ovaries of all ewes were examined at laparotomy eight days after withdrawal of the sponges.

### *(b) Experiment 2*

This experiment was conducted in April 1969. Twenty-five Merino ewes (five years old) were divided into three groups. Group A (seven ewes) was treated with sponges for 14 days and blood sampling was commenced as soon as the sponges were withdrawn. Group B (six ewes) was treated with intramuscular injection of 20 mg progesterone every second day for 12 days; blood

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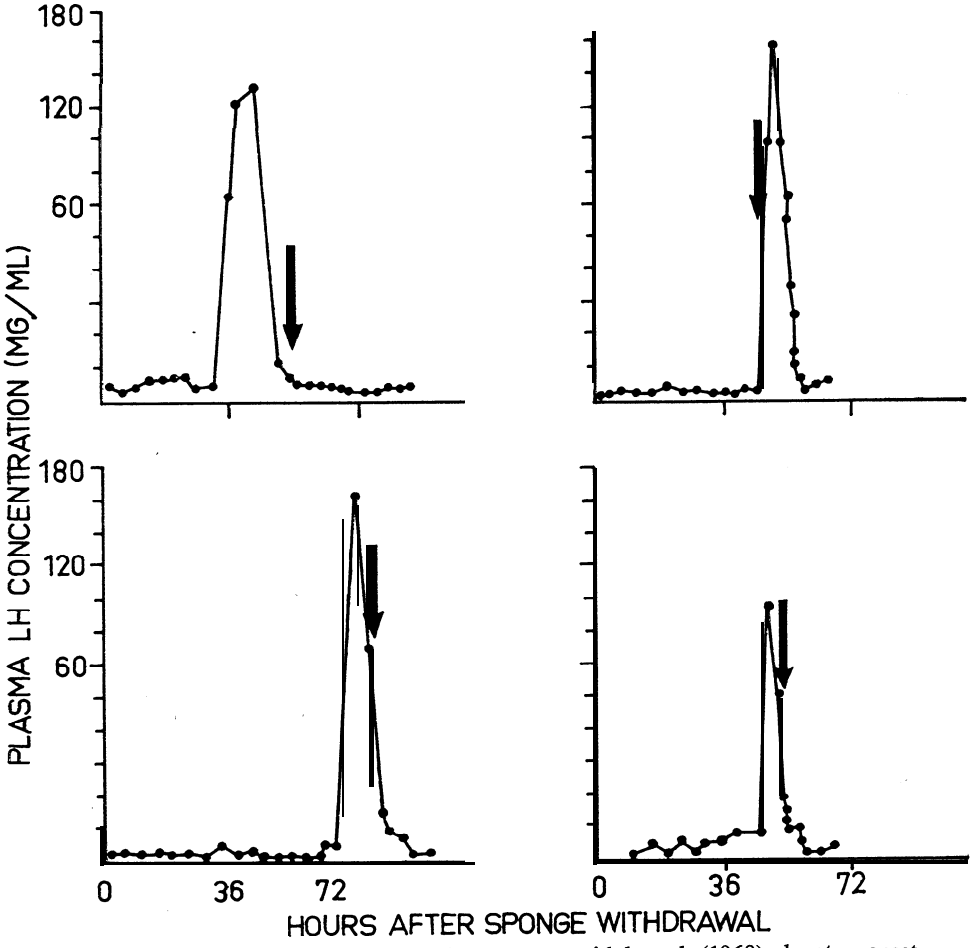


Fig. 1.—Plasma LH concentration after sponge withdrawal (1968) denotes onset of oestrus.

sampling commenced 48 hours after the last progesterone injection. In Group C (12 untreated controls), blood sampling began 24 hours before the earliest expected onset of oestrus. In all ewes, blood was collected every 3 hours until 24 hours after the onset of oestrus; the ovaries of all animals were observed at laparotomy two to three days later.

## III. RESULTS

### *(a) Experiment 1*

In four of the eight ewes, neither oestrus nor ovulation was observed during the five days following sponge withdrawal. None of these animals showed a plasma LH concentration of over 20 mg/ml at any time during this period.

All four ewes that exhibited oestrus showed evidence of recent ovulation as indicated by the presence of new corpora lutea. These ewes all had a LH peak similar in magnitude and duration to that seen at a natural oestrus (Goding *et al.* 1969). However, in three of these ewes, this surge began 4 to 16 hours before the onset of oestrus (Figure 1).

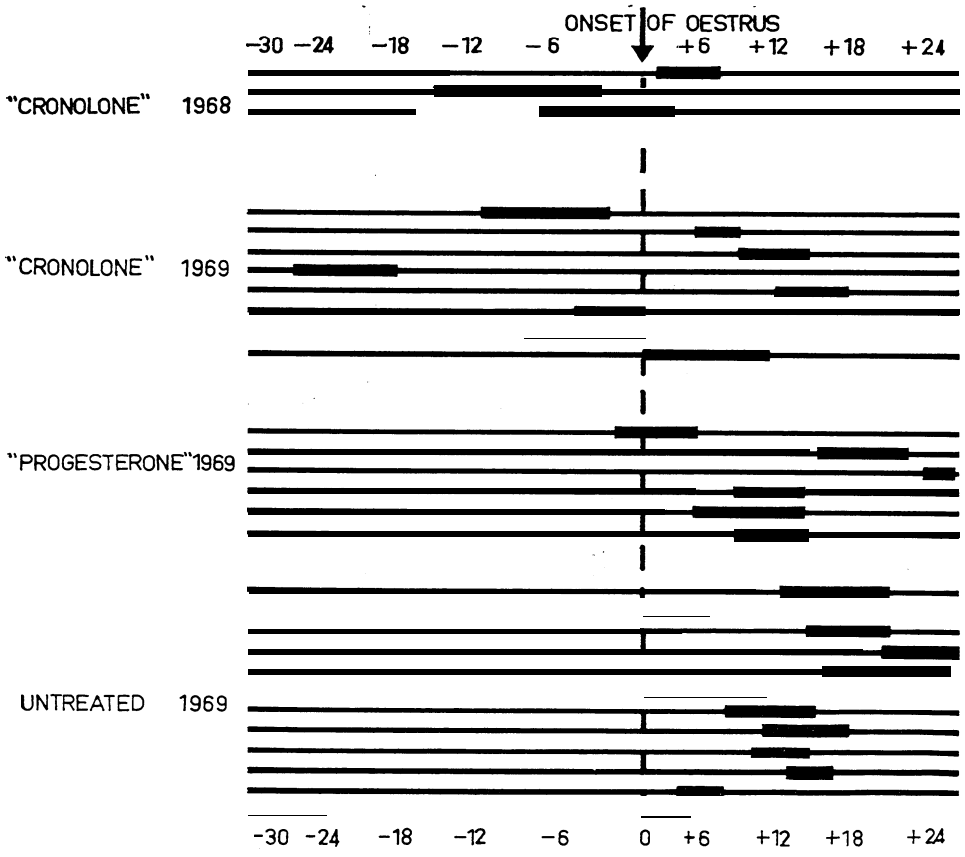


Fig. 2.—Time of pre-ovulatory LH release in relation to time of onset of oestrus (LH < 15 mg/ml—: LH ⩾ 15 mg/ml—)

*(b) Experiment 2*

. All ewes exhibited oestrus and had recently ovulated. In four of the seven ewes treated with "Cronolone", the pre-ovulatory LH release began before the first signs of oestrus. In all untreated control ewes, and in all but one of the progesterone-treated animals, the pre-ovulatory LH release began ator after the onset of oestrus (Figure 2).

## IV. DISCUSSION

Whenever a pre-ovulatory LH release was observed, its magnitude and duration was similar in all groups ("Cronolone"-treated, progesterone-treated and untreated controls). However, in over half the "Cronolone''-treated animals, the release of LH occurred early relative to the onset of oestrus. No evidence is currently available concerning the time interval between the pre-ovulatory release of LH and the time of ovulation in any of these experimental situations. However, it seems reasonable to assume that this interval may be more or less constant. If so, the implication of the timing of LH release after "Cronolone" treatment is that ovulation also may frequently occur early, relative to the onset of oestrus. The decreased fertility which has been observed at the first oestrus after sponge treatment could then be accounted for by virtue of a decrease in fertilizability of the ageing ovum.

A. similar situation was reported by Morrant and Dun (1960), also Restall (1961), in work on artificial insemination (AI) in normally cycling ewes. They found that highest fertility resulted when AI was performed at the middle of the oestrous period. The later AI was deferred after this time, the lower fertility became. Restall (1961) also suggested that the simplest explanation for this result was a relative infertility of the ageing egg.

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