PLASMA LH AND PROGESTERONE LEVELS AFTER SINGLE OR MULTIPLE INJECTIONS OF SYNTHETIC LH-RH IN ANOESTROUS EWES AND COMPARISON WITH LEVELS DURING THE OESTROUS CYCLE

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It has been shown that administration of a single intravenous injection of 150 or 300 μg synthetic LH-releasing hormone (LH-RH) to seasonally anoestrous Clun Forest ewes induced LH release in all animals and ovulation in the majority (Foster & Crighton, 1973, 1974). The induced LH peak was, however, much smaller than that observed at oestrus (Foster & Crighton, 1974). Luteal function, as assessed by peripheral plasma progesterone levels, was absent in the majority of treated animals (Crighton, Foster, Haresign, Haynes & Lamming, 1973; Haresign, Foster, Crighton, Haynes & Lamming, 1975), possibly because of an inadequate release of LH before ovulation. As an alternative means of administration to simulate more accurately the natural preovulatory LH peak, a single dose of 150 μg LH-RH was administered subcutaneously or intramuscularly instead of intravenously, but a greater release of LH was not obtained (S. C. Barnett, J. P. Foster and D. B. Crighton, unpublished data). Similarly, increasing the dose of the single intravenous injection from 150 to 300 μg failed to increase the height of (110 ± 20 and 118 ± 16 ng/ml respectively) or area under (1839 ± 398 and 2097 ± 247 mm² respectively) the induced LH peak (Foster & Crighton, 1973).

The development of a radioimmunoassay for LH-RH-like activity (Jeffcoate, Fraser, Gunn & Holland, 1974) allowed its simultaneous determination with that of LH in the jugular venous plasma of the sheep at oestrus (Crighton, Foster, Holland & Jeffcoate, 1973). This work suggested that, in order to simulate the natural LH peak in anoestrous ewes, administration of synthetic LH-RH intravenously as a series of injections might be appropriate.

In the present work, two cyclic and nine anoestrous Clun Forest ewes were used during the summer and autumn of 1973, respectively. All ewes were placed with a colour-marked vasectomized ram in order to detect the occurrence of oestrus. The cyclic ewes were bled (2 ml) from an indwelling jugular vein cannula at 4-min intervals from the onset of oestrus for 18 hr for the purposes of two separate studies. Every fourth sample only was used for the present work.

The anoestrous ewes were allocated to two groups: (1) five animals received a

single injection of 150 μ g synthetic LH-RH through a jugular vein cannula; (2) four animals received four injections each of 30 μ g synthetic LH-RH at 90-min intervals followed by a fifth injection of 30 μ g 5 hr after the fourth. A total dose of 150 μ g was thus administered over a 9½-hr period. This sequence of injections was designed to mimic the pattern of natural discharge of LH-RH-like immunoreactivity previously observed at oestrus (Crighton, Foster, Holland & Jeffcoate, 1973). All treated animals were sampled (2 ml) from the jugular vein cannula at 15-min intervals for 1 hr before and up to 12 hr after the first or only injection of synthetic LH-RH. Plasma from all samples was stored at -20°C for later assay of the LH concentration by a double-antibody radioimmunoassay (Foster & Crighton, 1974). The standard used was NIH-LH-S17. In addition, blood samples (10 ml) were taken from the treated ewes daily for 3 days before and 24 days after treatment and progesterone levels were measured by radioimmunoassay (Haresign *et al.*, 1975).

Laparotomy was carried out on all treated ewes 4 days after treatment and the ovaries were examined.

The two ewes sampled at oestrus showed preovulatory LH peaks similar to those observed previously (Foster & Crighton, 1974).

In each of the five anoestrous ewes treated with a single injection of synthetic LH-RH, the pattern of release following treatment (Text-fig. 1) was similar to that observed in ewes given identical treatment in previous experiments (Foster & Crighton, 1974).

All four anoestrous ewes treated with the multiple-injection sequence responded to the initial dose of synthetic LH-RH with a rise in the LH level. There was an accelerated rate of rise following the second injection, followed

TEXT-FIG. 1. Mean LH concentrations in the jugular venous blood of five ewes given a single injection of synthetic LH-RH. The arrow indicates the time of injection of 150 μ g LH-RH. Vertical bars represent the S.E.M.

TEXT-FIG. 2. Mean LH concentrations in the jugular venous blood of four ewes given multiple injections of synthetic LH-RH. The arrows indicate the times of injection of 30 μ g LH-RH (total dose 150 μ g LH-RH). Vertical bars represent the S.E.M.

by a decline in the LH level. Another elevation in plasma LH occurred following the third injection. Thereafter, the level fell to baseline (Text-fig. 2). The height of the LH peak induced by multiple injections was significantly greater than that induced by single injection (172 ± 16 versus 98 ± 12 ng/ml, $P < 0.01$), as was the duration of (6.87 ± 0.87 versus 4.30 ± 0.22 hr, $P < 0.05$) and the area under (7442 ± 815 versus 2477 ± 279 mm², $P < 0.001$) the peak. Since the peak levels were not reached at exactly the same time after injection in the individual animals, the mean heights of the LH peaks are different from the maxima shown in Text-figs 1 and 2. The LH release induced by multiple injections of LH-RH resembled the natural preovulatory LH release (height 159 ± 24 ng/ml, duration 8.8 ± 0.62 hr, area $12,119 \pm 2719$ mm²) more closely than that induced by a single injection. The results provide evidence, therefore, that the natural pattern of LH-RH-like immunoreactivity observed previously in jugular venous blood at oestrus (Crichton, Foster, Holland & Jeffcoate, 1973) may indicate the natural discharge pattern of LH-RH from the hypothalamus, although it is also possible that the pattern observed could have been influenced by the pituitary. It is noteworthy that the lack of response to the fourth and fifth injections is in agreement with the finding that endogenous peaks of LH-RH-like immunoreactivity which occur after the natural LH peak at oestrus are not associated with further increases in the LH level (Crichton, Foster, Holland & Jeffcoate, 1973). The doses of synthetic LH-RH used, however, may have resulted in non-physiological levels at the pituitary gland, thus making comparison of the natural and artificial situations hazardous.

When laparotomy was carried out 4 days after treatment, fresh ovulation

points were visible in all five ewes given a single injection and in three of four ewes given multiple injections.

The plasma progesterone levels remained basal (<0.4 ng/ml) throughout the sampling period in seven of the nine treated ewes. In the remaining two ewes (single injection group), plasma progesterone levels began to rise 3 days after injection to reach maximum levels of 1.4 and 1.0 ng/ml, falling again 10 to 12 days later. These levels were similar to those reported previously following LH-RH administration and are lower than the levels found during the luteal phase of the oestrous cycle (Crighton, Foster, Haresign, Haynes & Lamming, 1973; Haresign *et al.*, 1975).

Thus, despite the much larger release of LH induced by multiple injections of synthetic LH-RH compared with that induced by a single injection, normal luteal function did not result. Work is in progress to study this further.

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