Induction of cyclical ovarian activity in seasonally anoestrous Romney ewes: studies with exogenous GnRH or LH

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ABSTRACT

The aim of the present study was to establish whether ovulation and normal corpus luteum function could be induced in anoestrous ewes by supplementing the endogenous level of luteinizing hormone (LH) and whether cyclical ovarian activity could be induced and maintained in seasonally anoestrous ewes by an unvarying long-term intravenous pulse regime of gonadotrophic releasing hormone (GnRH), 500 ng once per 2 h for 43-80 days.

The results demonstrated that a normal ovulation can be induced in anoestrous ewes with LH alone and that the aforementioned GnRH regime was successful in inducing cyclical progesterone activity. These findings suggest that normal breeding activity may be restored to anoestrous ewes when the frequency of hypothalamic GnRH is increased from an apparent endogenous level of 1 secretory episode per 3.5 h to 1 episode per 2 h.

INTRODUCTION

In New Zealand, most breeds of sheep are, for obscure reasons, anovulatory and anoestrous during the spring and summer months (September to February).

During the breeding season, cyclical ovarian (i.e., oestrogenic/progestational) activity is regulated by the pituitary hormones, luteinizing hormone (LH) and follicle-stimulating hormone, and in turn, the secretory activity of these 2 hormones is regulated by the secretion of gonadotrophic-releasing hormone from the hypothalamus (see Peters and McNatty, 1980 for review). From studies on anoestrous ewes, the collective evidence from several recent studies have led us (McNatty *et al.*, 1982) to suggest that seasonal infertility is due to inadequate hypothalamic activity rather than to refractoriness at the level of the ovary, anterior pituitary or pineal gland *per se* (Munro *et al.*, 1979; McNatty *et al.*, 1981, 1982; K. P. McNatty, unpublished data). For example, in Romney ewes during the breeding season the pattern of LH secretion during most of the oestrous cycle is characterised by discrete episodic discharges of the hormones occurring at a frequency of 10 to 15 in 24 h, whereas during anoestrus, the frequency is only 5 to 8 in 24 h. It is possible that this difference in episodic LH secretion reflects differences in the frequency of release of gonadotrophic-releasing hormone (GnRH) from the hypothalamus (Carmel *et al.*, 1976; Belchetz *et al.*, 1978) rather than to any major change in the functional status of the pituitary gland (Karsch *et al.*, 1980).

To test the hypothesis that seasonal anoestrus is a consequence of inadequate pituitary production of LH which in turn is due to a reduced output of GnRH, 2 experiments were performed: in the first, 3 anoestrous Romney ewes were injected with a purified preparation of ovine LH once every 2 h for 21 to 28 days; in the second 3 anoestrous Romney ewes were injected with GnRH once every 2 h for 43 to 80 days (McNatty *et al.*, 1982). In both experiments ovarian activity was assessed from the levels of progesterone in peripheral plasma.

MATERIALS AND METHODS

A total of 37 mixed-aged (2½ to 6 year) Romney ewes were studied between September and February over 2 seasons, 1979/80 and 1980/1. The animals to be injected with LH or GnRH (n = 6), were housed in adjacent wooden crates, in which they were free to lie or stand. The remaining animals (n = 31) which served as untreated controls were housed in pens adjacent to the treated animals. All animals were fed daily with lucerne hay, lucerne pellets, sheep nuts, grass clippings and water. Although indoors, the animals were in well-lit rooms and exposed to variations in lighting similar to those outdoors. Before the experiments began, the ovaries of each ewe were examined by laparoscopy to confirm the absence of corpora lutea and gross pathology. For each ewe to be 'pulsed' with LH or GnRH, cannulae were inserted into the left and right jugular veins as previously described (McNatty *et al.*, 1982). Subsequently, ovine LH (10 μg; NIH-LH-S21; National Pituitary Agency, U.S.A.) or GnRH (500-600 ng; Luliberin, Pierce Chemical Co., Rockford, Illinois, U.S.A.) was infused through 1 of the cannulae into each animal over a 110-sec period every 2 h for 21 to 28 days or 43 to 80 days respectively. The cannulae delivering the LH or GnRH were coupled to a Technicon autoanalyser pump which was activated every 2 h by a timing device developed at Wallaceville.

Blood samples (10 ml/sample) were collected at least once every 3 days from each animal treated with LH or GnRH. Thirty consecutive daily 10 ml blood samples were also collected from the 31 untreated anoestrous Romney ewes. All blood samples were centrifuged (4000g) at 18 to 20°C for 20 min within 30 min of collection. The plasmas were recovered and stored at −20°C until analysed for progesterone (Neal *et al.*, 1975). The minimum detectable level of progesterone was 0.3 ng/ml and the intra- and inter-assay coefficients of variation were < 14%.

RESULTS

The patterns of progesterone secretion in the 3 ewes 'pulsed' with either LH or GnRH are shown in Fig 1. In the 3 LH 'pulsed' ewes, a progestational ('luteal-like') phase was induced within 4 to 5 (1 ewe) or 12 to 13 (2 ewes) days of starting the LH treatment: during each progestational phase the progesterone levels exceeded 1 ng/ml for 10 to 12 days. In the 3 GnRH 'pulsed' ewes, biphasic progestational cycles were induced on 2 or 4 separate but consecutive occasions with the progesterone levels during each of these phases exceeding 1 ng/ml for 8 to 13 days. The mean progesterone value of control ewes was 0.39 ± 0.01 (s.e.m., n = 930): only 2 (0.22%) of the progesterone values (each from a different animal) were ≥ 1 ng/ml.

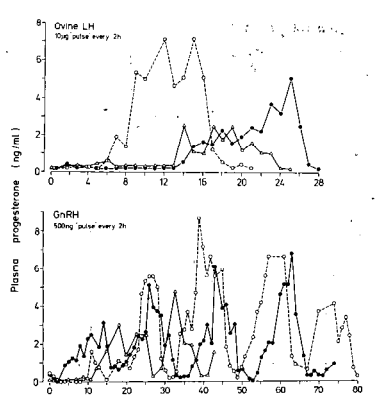

FIG. 1 Changes in the plasma concentrations of progesterone in 3 individual ewes throughout the period when LH or GnRH was administered once every 2 h. Data for GnRH treated ewes reproduced from McNatty *et al.* (1982).

Laparoscopic examination of the ovaries of the LH-treated and control ewes, 30 days after the experiments began, confirmed that cyclical progesterone output in the LH-treated ewes was due to the formation of at least 1 corpus luteum per animal.

DISCUSSION

These data show that progesterone secretion can be induced in anoestrous ewes when LH or GnRH is administered as a pulse once every 2 h on a long-term basis. In the absence of a sexually active ram and/or hormone treatment (see McNatty *et al.*, 1981), evidence of progesterone secretion consistent with corpus luteum function was not observed in the anoestrous control ewes.

The results of this study are consistent with the hypothesis that there is insufficient LH available to promote the final stages of ovarian follicular maturation in anoestrous ewes (McNatty *et al.*, 1981; McNatty, 1982) and that the reduced output of LH may be due to a lowered frequency of episodic GnRH release.

The indentification of LH as a critical determinant of preovulatory follicular maturation (see Karsch *et al.*, 1980; McNatty, 1982 for reviews) and of LH discharge frequency as an index of hypothalamic function suggests that short-term (e.g., 10 min) changes in LH secretion, over a period of 4 to 10 h, is a useful biochemical marker for testing the contribution of certain variables such as nutrition, light intensity or duration, a ram and pheromones on stimulating preovulatory follicular maturation in both breeding and seasonally anoestrous ewes.

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