THE HORMONAL RESPONSES TO TEASING

G.B. MARTIN*, Y. COGNIE**, F. GAYERIE**, C.M. OLDHAM*, P. POINDRON**, R.J. SCARAMUZZI*** and J.C. THIERY**

Some of the results presented here are from very recent experiments, unpublished when this manuscript was being prepared. Due to a shortage of space, all the experimental details, although available, cannot be included. This paper represents the state of our thinking on the endocrine mechanisms behind the teasing response.

In the normally cycling ewe, the sequence of endocrine events leading to ovulation is 1) decrease in progesterone secretion, 2) increase in basal LH secretion, 3) increase in oestradiol secretion and 4) positive feedback leading to the preovulatory surges of LH and FSH, and ovulation (Baird and Scaramuzzi 1976). Each of these is dependent on the event preceding it. In the seasonally anoestrous or anovular ewe, the sequence cannot begin because there is no corpus luteum, or cannot proceed because rising oestradiol levels exert a strong negative feedback on LH secretion, inhibiting the basal rise prior to the surge (Legan et *al.* 1977). However, introducing rams can induce ovulation in these ewes, so the mechanism of anoestrus can be by-passed or reversed.

The ram-stimulated ovulation is the result of an apparently normal preovulatory surge of LH, which can be very rapidly induced, e.g. half of the ewes in the study by Oldham et *al.* (1978) had their surge within 20h of them being introduced to rams. This rapid response led to the postulate that some, if not all, preovulatory surges are induced by a neural reflex (Oldham *et al.* 1978; Knight et *al.* 1978; Poindron *et al.* 1980). In this reflex, the ram stimulus, acting at hypothalamic level, would bypass the positive feedback action of oestradiol and directly elicit an LH surge. To investigate this possibility the most obvious avenue was to measure oestradiol levels prior to the LH surge. However, this is difficult due to the low levels of oestrogens in the non-pregnant ewe, and the consequent necessity for chromatographic steps in the assay (Scaramuzzi and Land 1976). Indeed, this probably explains the lack of any pattern in peripheral levels of oestradiol reported by Knight et *al.* (1978). In the absence of a sufficiently sensitive assay for peripheral oestradiol, we chose to investigate events closer to the introduction of rams. If there were a pre-ovulatory rise in oestradiol it would most likely be due to increased basal LH secretion as in the normal oestrous cycle (Baird and Scaramuzzi 1976). There was some evidence of an increase in mean levels of LH after the introduction of rams, though it apparently was not associated with ovulation (Chesworth and Tait 1974). Furthermore, LH secretion is pulsatile (Scaramuzzi and Martensz 1975) so there should be an increase in pulse frequency some time after the introduction of rams. We observed such an increase and found that ovulation does not result without it (Martin et *al.* 1980; Poindron et *al.* 1980). The ovary is able to secrete oestradiol after each LH pulse during anoestrus (Scaramuzzi and Baird 1976) so presumably there was a sustained increase in oestradiol levels leading to the initiation of the preovulatory surge of LH.

Further evidence of the necessity for positive feedback has come from studies on the effects of chronic and acute progesterone treatment: luteal phase levels of progesterone (from implants) begun four days prior to the introduction of rams and continued for a further four days (until laparoscopy) prevent

* Dept of Animal Science, University of Western Australia, Nedlands, W.A. 6009.
** INRA – Station de Physiologie de la Reproduction, Nouzilly 37380, France
*** CSIRO Division of Animal Production, Prospect, N.S.W. 2148.

ovulation; similar treatment begun six hours after the introduction of rams and after the rise in LH pulse frequency, and presumably oestradiol, also prevents ovulation; an injection of progesterone (10 mg in oil) concurrent with the introduction of rams, attenuates but does not prevent the increase in LH pulses, and delays, but does not prevent, the preovulatory surge (Fig. 1). These results indicate that progesterone can prevent ovulation by selectively blocking the LH surge, as in ovariectomized ewes (Scaramuzzi et *al.* 1971). Preovulatory surges resulting from 'reflex' neural action are therefore unlikely, so alternative explanations for the rapidity of the response are needed. Some of the increases in pulsatile LH secretion may themselves be sufficient to induce ovulation (Martin *et al.* 1980). Furthermore, the presence of rams advances the onset and extends the duration of the LH surge induced in ovariectomized ewes with an injection of oestradiol. The presence of rams has also been shown to advance the LH surge in normally cycling entire ewes (Lindsay et *al.* 1975b), so may increase the sensitivity of the ewe's hypothalamus to positive feedback.

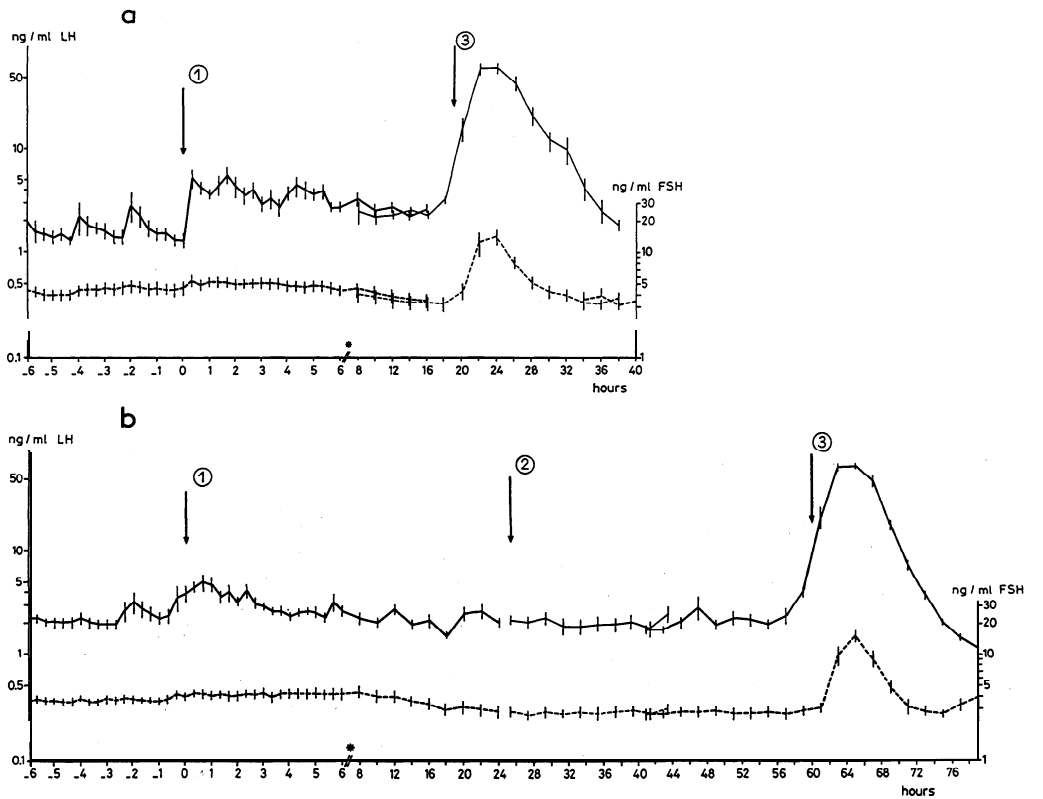

Fig. 1 Mean (± SE) levels of LH (——) and FSH (----) in serum of Préalpes ewes after a) teasing (n=8), or b) teasing plus 10 mg progesterone (n=10). The data are adjusted to 1) introduction of rams, 2) the mean time at which progesterone concentrations returned to < 0.2 ng/ml, or 3) the start of the preovulatory surge of LH. Samples were taken at 20 min intervals until + 6h, and two-hourly thereafter, and assayed according to Poindron *et al.* (1980).

Initially we interpreted the increase in the frequency of LH pulses entirely in terms of 'escape' from negative feedback, since heightened sensitivity to oestradiol has been proposed as the mechanism causing anovulation (Scaramuzzi and Baird 1976; Legan et *al.* 1977). Oestradiol certainly can reduce the frequency of LH pulses (Diekman and Malvern 1973) and a large dose (100 µg oestradiol-17β i.m.) just prior to the introduction of rams will depress and delay the ovulatory response (Martin 1980), while 10 µg oestradiol benzoate i.m. will prevent the rise in frequency of LH pulses. Both of these doses are capable of eliciting positive feedback in anoestrous ewes (Goding *et al.* 1969) making interpretation of the results for LH surges and ovulation difficult, but the effects on basal LH secretion and delayed ovulation were quite clear.

Changes in the frequency of LH pulses, independent of endocrine feedback systems, may be a component of the ram effect. In 3/10 ovariectomized ewes without steroid pretreatment, the frequency rose from 0.6 to 0.9 pulses/h, with a concomitant rise in basal levels, after the introduction of rams. Ovariectomized ewe lambs display a rise in frequency of LH pulses at about the expected time of puberty (D.L. Foster 1980, pers. comm.) and ovariectomized adult ewes demonstrate seasonal changes in pulse frequency without steroid pretreatment (P.J. Wright 1980, pers. comm.). The pulse frequency in ovariectomized animals has been considered maximal but, in the absence of any convincing evidence of the role of adrenal steroids in negative feedback, the data support the thesis that the brain has primary control of pituitary output - not the ovary.

Upon the introduction of rams there are no functionally related changes in the basal levels of FSH in either seasonally or lactationally anovular ewes (Poindron et *al.* 1980) or in ovariectomized ewes. Indeed, in ovariectomized ewes with or without rams, no significant changes were observed after the administration of oestrogen or progestagen, though both steroids had profound effects on basal LH secretion. Furthermore, ewes actively immunized against androstenedione, in which FSH levels are depressed while the frequency of LH pulses is increased (Martensz and Scaramuzzi 1979), show normal responses to the introduction of rams, in terms of ewes ovulating, though the ovulation rate is elevated. This result, the lack of any functionally-related changes in plasma levels, the non-pulsatile form of secretion, and the long half-life, indicate that the role of FSH is primarily permissive.

Prolactin has been implicated in the control of both seasonal and lactational anoestrus (Thimonier *et al.* 1978; Kann et *al.* 1977). However, ewes in either condition will ovulate after the introduction of rams with similar responses in pulsatile LH secretion and ovulation, despite large differences in prolactin levels. Depressing prolactin secretion with a dopamine agonist also has no effect on the response to rams. The frequency of LH pulses was lower in ewes suckling twin lambs than in ewes with single lambs (before the introduction of rams) but this also had no apparent effect on their response to the rams (Poindron et *al.* 1980). Although anovulation and elevated levels of prolactin are both caused by season and lactation, this does not mean that prolactin causes anovulation.

In-conclusion, it seems likely that the primary endocrine response induced in anovular ewes by the introduction of rams is the rapid rise in the frequency of LH pulses. This begins the normal sequence of events which lead to ovulation. The roles of prolactin and FSH are not clear, but changes in the levels of these gonadotrophins are probably not important in determining whether the ewe will ovulate.