

## Gonadotrophic control of follicular development and function during the oestrous cycle of the ewe

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**Summary.** In the adult non-pregnant ewe the secretion of FSH is sufficient to ensure a continuous growth and development of antral follicles to 3-5 mm size at all times. Further development and increased secretion of oestradiol through the final 72 h to ovulation depends on adequate stimulation by LH. During anoestrus and the luteal phase of the cycle LH pulses occur too infrequently to stimulate sufficient oestradiol to evoke an LH surge. Moreover, during the luteal phase progesterone secreted by the corpus luteum not only reduces the frequency of LH pulses but also inhibits the ability of oestrogen to evoke an LH surge. At the time of luteal regression the frequency of LH pulses increases to at least one per hour due to the fall in progesterone secretion. This change in pulse frequency of LH is associated with a decrease in the secretion of FSH, probably because of a direct inhibitory action of oestrogen on the anterior pituitary gland. The dominant follicle is probably relatively independent of circulating levels of FSH due to the high concentration of oestradiol and FSH within the microenvironment of the follicular cavity.

Once the oestrogen secretion achieves a certain level a preovulatory surge of LH (and FSH) occurs. Increased sensitivity of the anterior pituitary to LH-RH and increased secretion of LH-RH from the hypothalamus both play a part in producing the LH surge. The rise in prolactin at this time probably reflects a decrease in hypothalamic dopamine turnover which is necessary for maximum release of LH-RH.

The preovulatory LH surge initially stimulates and then totally inhibits further secretion of oestrogen and androgen from the ovulatory follicle. This suppression of steroid secretion is accompanied by a second peak of FSH at about the time of ovulation. The function of this second peak of FSH remains unknown although it may be responsible for the development of the large antral follicles which occur on Days 3 and 4. It is probably more important in those mammals like the rat and hamster which only form a functional corpus luteum if pregnancy occurs and in which oestrogen is necessary for implantation.

### Introduction

Although endocrinology is involved in the study of control mechanisms and the way physiological systems adapt to change, most of our knowledge has been pieced together from experiments involving cross-sectional measurements. For example, a study of the secretion of pituitary gonadotrophins throughout the ovarian cycle was only possible by measuring the pituitary content in groups of animals killed at various intervals (Greep, 1961). These cross-sectional studies established much of our basic knowledge but did not allow a study *in vivo* of the dynamics of the endocrine system.

In the past decade several advances in techniques have facilitated more detailed studies of endocrine control mechanisms. In particular, the development of relatively simple, sensitive and specific assays for the measurement of the major reproductive hormones in samples of blood and urine has been particularly important in expanding the scope of experimental design. It is only 26 years since the publication of the first chemical method for the measurement of oestrogens (Brown, 1955); and the first method able to measure oestrogens in the blood of non-pregnant women required 50 ml blood and had a productivity of 4 samples per week (Baird, 1968)!

The sheep has proved an ideal model with which to exploit the potential of these new experimental techniques. Of comparable size to man, it is large enough to withstand serial collections of blood from easily accessible veins for the measurement of hormones. It has even been possible to relocate structures such as the ovary, uterus and adrenal to more accessible subcutaneous sites where their venous effluent can be collected more easily (Goding, Baird, Cumming & McCracken, 1971). The sheep is tolerant of experimental procedures, including surgery, and is free from many of the health hazards of other species, e.g. rhesus monkey. It has been possible to exploit genetic differences between breeds, e.g. in ovulation rate, to test controlling physiological mechanisms. Of particular importance to the study of the reproductive system specific radioimmunoassays for all three pituitary gonadotrophins (FSH, LH and prolactin) have been available for at least 5 years (Cole & Cupps, 1977). These assays are sufficiently sensitive to detect small short-term changes in the concentrations of hormones and to allow study of the dynamic relationship between the anterior pituitary gland and the gonad.

This paper reviews our knowledge of the control of follicular development and function (i.e. hormone secretion) in cyclic sheep. Our knowledge of follicle growth and development is still based on cross-sectional studies (e.g. Brand & de Jong, 1973) although recent advances in biophysics, e.g. use of ultrasound scanning, make serial longitudinal measurements of at least large antral follicles a possibility (Hackeloer, 1977).

### Folliculogenesis

Follicular development or folliculogenesis is thought to start before birth and in most mammals to continue throughout life. Once recruited from the pool of primordial follicles development continues until either atresia or ovulation occurs (Peters, Byskov, Himelstein-Braw & Faber, 1975). However, the rate of division of cells (mitotic index) within a follicle varies depending on its stage of development and hence the rate of growth of a follicle is not constant. By incorporating labelled thymidine, it has been found that it takes approximately 19–20 days for the mouse follicle to proceed through to ovulation once it enters the growth phase (Peters & Levy, 1966). Using less direct methods involving a study of the mitotic index of granulosa cells, Turnbull, Braden & Mattner (1977) concluded that the smallest antral follicle (approximately 0.2–0.3 mm diameter) in the sheep takes 25–35 days to become a mature preovulatory Graafian follicle (about 8 mm). The number of follicles entering the growth phase is inversely related to the total number of oocytes in both ovaries (Mauléon & Mariana, 1977). Although the number of small pre-antral follicles entering the growth phase in the remaining ovary is increased after unilateral ovariectomy, the total number when compared to the combined total of both ovaries is reduced (Dufour, Cahill & Mauléon, 1979). However, the percentage of antral follicles that become atretic is lower and so the number of large antral follicles, and hence ovulation rate, remains the same.

#### *Gonadotrophin requirements for folliculogenesis*

Although initiation of follicle growth appears to continue following hypophysectomy, the number of non-atretic pre-antral and antral follicles is markedly reduced (Dufour *et al.*, 1979).

Thus at some point in their early development (probably 0.06–0.07 mm diameter) follicles require pituitary gonadotrophins without which further development is severely impaired or ceases altogether. Once an antrum is formed (in the sheep 0.2–0.3 mm diameter) withdrawal of gonadotrophins leads to immediate atresia.

Even in the presence of gonadotrophins most antral follicles become atretic and only a small minority survive to ovulate. Whether an individual follicle becomes atretic or not depends on its sensitivity to gonadotrophins as well as on the amount of gonadotrophins secreted by the pituitary. Administration of exogenous FSH or PMSG increases the ovulation rate by decreasing the proportion of large antral follicles which become atretic (Peters *et al.*, 1975; Mauléon & Mariana, 1977). It is a reasonable assumption, therefore, that follicles become atretic because they receive inadequate amounts of FSH or lack the ability to respond to FSH.

The sensitivity of individual follicles to gonadotrophins is probably determined by the number of receptors for LH, FSH and possibly prolactin (Richards & Midgley, 1976). In turn, the development of gonadotrophin receptors is influenced by the local concentration of steroid hormones within the follicle. For example, oestradiol enhances the sensitivity of the granulosa cells to FSH and together with FSH generates LH receptors. On the other hand, it is known, at least for rats, that androgens (particularly dihydrotestosterone) hasten atresia (Louvét, Harman, Schriber & Ross, 1975). The limited data which are available on the concentrations of androgens and oestrogens in follicular fluid of sheep suggest that, as in women, there is great variation in the concentration of steroids in follicular fluid (Moor, Hay, Dott & Cran, 1978). In healthy, non-atretic follicles the concentration of oestradiol is much higher than that of androgens while the reverse applies to atretic follicles. Thus, whether a follicle becomes atretic or not may depend on the oestrogen/androgen ratio in its microenvironment.

### Action of gonadotrophins

#### FSH

Classically, FSH stimulates the growth and development of egg-bearing follicles (Greep, 1961). Undoubtedly FSH plays a crucial role in stimulating cell division and the mitotic index of granulosa cells grown in monolayer is increased when FSH is added to the culture medium. In rats, receptors for FSH are present on the granulosa cells of the smallest antral follicles while LH receptors occur on theca cells and granulosa cells of the mature Graafian follicle (Richards & Midgley, 1976). Both oestradiol and FSH are important in generating LH receptors on granulosa cells. In the absence of LH receptors granulosa cells do not luteinize and secrete progesterone in response to an LH surge; instead those follicles without LH receptors on the granulosa cells become atretic.

Recent data confirm that a similar situation exists in the sheep (Carson, Findlay, Burger & Trounson, 1979). LH receptors are present on the theca cells of follicles at all stages of development (even in early atresia) but are confined to the granulosa cells of large (4–6 mm) non-atretic follicles. Granulosa cells from non-atretic follicles bind FSH irrespective of size. The increased binding of both FSH and LH to large non-atretic follicles is due to the increased number of receptors and increased number of cells rather than a change in the affinity of binding.

#### LH

The steroidogenic action of LH on the ovary is well established. Its main action is to increase steroid secretion by stimulating the conversion of cholesterol to pregnenolone (Marsh, 1976). The initial steps involve interaction with the receptor on the cell membrane, the activation of the adenyl cyclase enzyme and the production of cyclic AMP. Because this early step in steroid synthesis is stimulated by LH, it is hardly surprising that all steroids on the biosynthetic pathway

from pregnenolone to androgens and oestrogens are increased following administration of LH (McCracken, Uno, Goding, Ichikawa & Baird, 1969).

A certain basal level of LH is necessary to maintain steroid secretion by all cell types in the ovary. Infusion of LH antiserum causes swift cessation of progesterone secretion from the corpus luteum (Fuller & Hansel, 1970; McCracken, Baird & Goding, 1971). LH, like many other pituitary hormones, is released in pulses so that the concentration in blood is not constant (Yuthasastrakosol, Palmer & Howland, 1977). Each pulse of LH is followed within 10 min by a rapid increase in the secretion of oestradiol and androstenedione from the follicle (Baird, Swanston & Scaramuzzi, 1976a). The rapid increase in androgen in response to LH is to be expected for there are numerous LH receptors in the theca cells. However, the increase in oestradiol secretion implies that either the theca cell in the sheep can synthesize oestradiol or that there is a very rapid transfer of androgen precursor from theca to granulosa cell. If the latter is correct it seems likely that the oestradiol secreted into the ovarian vein is produced by those granulosa cells lining the basement membrane which are nearest the capillaries supplying the theca layer. In the rat these parabasal cells have in fact been shown histochemically to have a higher concentration of cytochrome P450 than those granulosa cells nearer the follicular antrum (Zoller & Weiss, 1978).

### *Prolactin*

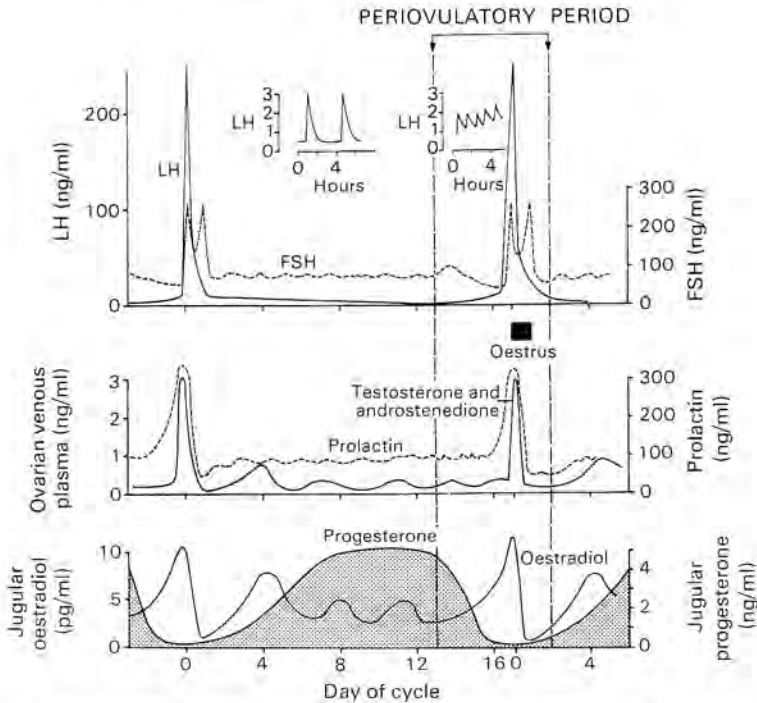
Serum levels of prolactin increase after luteal regression in the ewe and remain elevated until the end of the ovulatory surge of LH (Reeves, Arimura & Schally, 1970; Cumming, Brown, Goding, Bryant & Greenwood, 1972; Kann & Denamur, 1974; Text-fig. 1). This increase appears to be related both to the rise in the concentration of oestradiol and to a decrease in hypothalamic dopamine turnover necessary to allow the increase in pulse frequency of LH-RH and LH release occurring at this time (McNeilly, 1980). During the luteal phase prolactin levels remain low without significant variation.

The action, if any, of prolactin in development of the follicle in the sheep is not clear. Suppression of prolactin with ergocornine hydrogen maleinate or bromocriptine throughout the oestrous cycle does not affect the occurrence of ovulation, ovulation rate, or corpus luteum function (Louw, Lishman, Botha & Baumgartner, 1974; Niswender, 1974). It may therefore be presumed that if prolactin is necessary for normal follicular development in the ewe, the circulating levels required are considerably lower than occur spontaneously and at present no clear role for prolactin in follicular development in the ewe has been demonstrated. On the other hand prolactin is required together with LH for the maintenance of the corpus luteum (Denamur, Martinet & Short, 1973).

High circulating levels of prolactin are associated with the cessation of ovulation during lactational and seasonal anoestrus (Kann & Martinet, 1975; Walton, McNeilly, McNeilly & Cunningham, 1977) and with low levels of progesterone during early pregnancy induced by PMSG treatment at the end of the breeding season (Rhind, Chesworth & Robinson, 1978). Whether the increased levels of prolactin alone are directly implicated in the reduction of ovarian activity is unclear. Suppression of prolactin levels with bromocriptine during anoestrus does not result in (1) a return of oestrous cycles (B. P. Fitzgerald & F. J. Cunningham, personal communication), (2) an increase in the number of intact ewes showing positive feedback in response to oestrogen (Land, Carr, McNeilly & Preece, 1980), or (3) the number of ewes ovulating in response to LH-RH treatment or their subsequent luteal function (McNeilly & Land, 1979).

In contrast, ovulation and normal luteal function can be induced during anoestrus, when prolactin levels are elevated, by the injection of LH in amounts sufficient to mimic a normal LH pulse and given at a frequency similar to that seen during the preovulatory period of a normal oestrous cycle (A. S. McNeilly, M. A. O'Connell & D. T. Baird, unpublished). This suggests that high levels of prolactin do not significantly affect folliculogenesis provided the follicles receive





**Text-fig. 1.** Hormone changes throughout the oestrous cycle of the ewe. The gonadotrophins are the mean of several published series (Goding *et al.*, 1973; Salamonsen *et al.*, 1973; Pant *et al.*, 1977). The concentrations of oestradiol and progesterone in jugular venous plasma are those published by Hauger *et al.* (1977). The concentration of androstenedione and testosterone in ovarian venous plasma are from Baird *et al.* (1976b, 1981). The inset is to represent the rise in basal level and increased frequency of LH pulses in the follicular phase of the cycle.

adequate gonadotrophic support. Nevertheless, TRH-induced hyperprolactinaemia during the preovulatory period may result in a reduced secretion of oestradiol in some ewes without affecting LH pulsatility (McNeilly & Baird, 1977).

At present the evidence suggests that prolactin is probably required for normal follicular development and is certainly necessary for normal corpus luteum function.

### Control of gonadotrophin secretion

The synthesis and secretion of gonadotrophins from the anterior pituitary gland is stimulated by LH-RH released from the hypothalamus into the hypothalamic-hypophysial portal vessels (Fink, 1979).

There is thought to be only one releasing hormone (LH-RH) for gonadotrophins. The amount of gonadotrophin secreted is a function of the amount of LH-RH reaching the pituitary gland and the responsiveness of the anterior pituitary to LH-RH. Ovarian steroids influence the hypothalamus and pituitary to modulate the secretion of gonadotrophins. Because it is not possible to monitor the electrical activity of LH-RH-secreting neurones or to measure LH-RH in portal blood, changes in hypothalamic activity can only be inferred from changes in the frequency of LH pulses. LH is released in pulses at intervals ranging from one per 24 h in anoestrus (Yuthasastrakosol *et al.*, 1977) to more than one every hour during the late follicular phase of the cycle (Baird, 1978a). Each pulse of LH is presumed to represent the response of the

anterior pituitary to a pulse of LH-RH. Neutralization of LH-RH with specific antiserum immediately blocks LH pulses (Clarke, Fraser & McNeilly, 1978). Thus steroid hormones influence the secretion of gonadotrophins by changing the frequency and magnitude of release of LH-RH from the hypothalamus as well as altering the responsiveness of the pituitary gland to LH-RH.

Oestradiol is probably the most important hormone in regulating the release of LH and FSH. After ovariectomy or immunization against oestradiol there is a very marked rise in the concentration of both gonadotrophins. Because of the inability of immunized ewes to respond to oestrogen and produce a surge of LH, ovulation does not occur although luteinized cysts are common (Pant, Dobson & Ward, 1978). However, during the breeding season the post-castration rise of LH cannot be prevented fully by the administration of oestradiol alone in physiological amounts (Legan, Karsch & Foster, 1977). The addition of progesterone in amounts normally secreted by the corpus luteum are necessary if luteal phase levels of LH are to be achieved (Legan & Karsch, 1979; Karsch, Legan, Ryan & Foster, 1980). Progesterone causes a marked reduction in the frequency of LH pulses although their amplitude increases. Thus the secretory products of the follicles (oestradiol) and the corpus luteum (progesterone) are necessary for the control of tonic LH secretion during the luteal phase of the oestrous cycle.

The effects of steroids on FSH secretion have been less well studied. In the ovariectomized ewe FSH is inhibited by oestradiol but not progesterone although a 2–3-week treatment period is required for physiological amounts of oestradiol to reduce FSH values to levels similar to those of intact animals (Cumming *et al.*, 1974). Oestradiol and large amounts of LH-RH will also induce the release of FSH under the same conditions as LH is released (Jonas *et al.*, 1973). However, smaller pulses of LH are not always accompanied by release of FSH, suggesting that, as in the ram, the relative proportion of LH and FSH may be determined by both the frequency and amplitude of LH-RH pulses (Text-fig. 2).

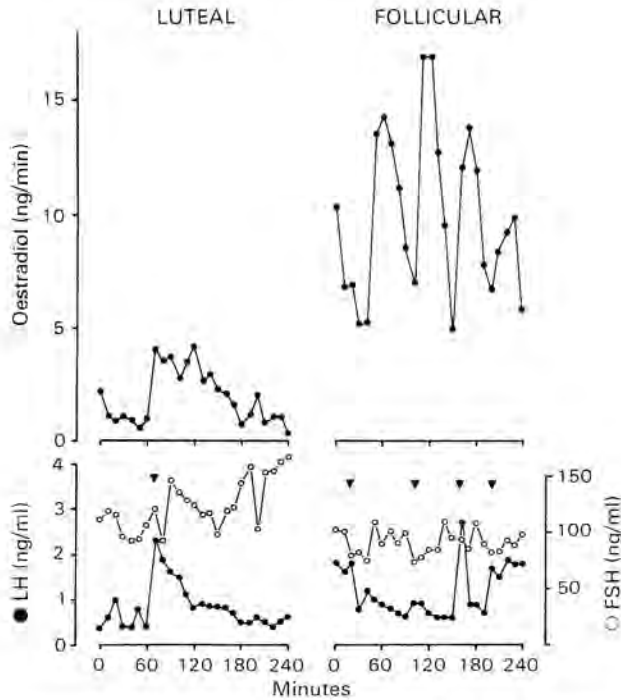
Experiments with ewes suggest that androgens may play some role in regulating the secretion of gonadotrophins. Immunization against androstenedione results in multiple follicular development and an increase in ovulation rate (Scaramuzzi, Davidson & Van Look, 1977). Although the secretion of LH is elevated the concentration of FSH is suppressed (Martensz & Scaramuzzi, 1979). The reason for the suppression of FSH is probably the increased ovarian secretion of oestradiol due to multiple development of non-atretic large follicles (Scaramuzzi, Baird, Clarke, Martensz & Van Look, 1980). These immunized ewes exhibit many of the endocrine characteristics of the high fecundity breeds, e.g. decreased sensitivity to the feedback effects of oestrogen, implying that androstenedione may normally modulate the sensitivity of the hypothalamic–pituitary unit to the feedback effect of ovarian steroids.

### **Hormones and follicular function throughout the oestrous cycle**

It is convenient to divide the 17-day cycle (Day 0 = oestrus) into the luteal phase lasting from Day 2 to 13 and the peri-ovulatory period from Day 14 (oestrus –3) to Day 1 (Text-fig. 1).

#### *Luteal phase*

During the luteal phase the corpus luteum secretes increasing quantities of progesterone so that its concentration in peripheral plasma reaches a plateau between Days 6 and 12. Consistently one or two large follicles develop on Day 2 or 3 as indicated by a rise in the secretion of oestradiol (Holst, Braden & Mattner, 1972). Following this peak the secretion of oestradiol and androgens continues to fluctuate as follicles develop up to 4 mm diameter before becoming atretic. Although the concentration of FSH shows no consistent trend at this time, the secretion of LH gradually declines. Closer inspection of the pattern of LH concentration reveals



**Text-fig. 2.** The concentration of LH and FSH in jugular venous plasma and the ovarian secretion rate of oestradiol in a ewe with an ovarian autotransplant to the neck. Samples of blood were collected every 10 min for 4 h from 10:00 to 14:00 on Day 10 of the oestrous cycle (luteal) and 24–28 h after luteal regression had been induced by the injection of 100  $\mu$ g of a prostaglandin analogue (cloprostenol; ICI) (follicular). Note the greater secretion of oestradiol in the follicular phase associated with an increased frequency of LH pulses ( $\blacktriangledown$ ). (Data from Baird *et al.*, 1981.)

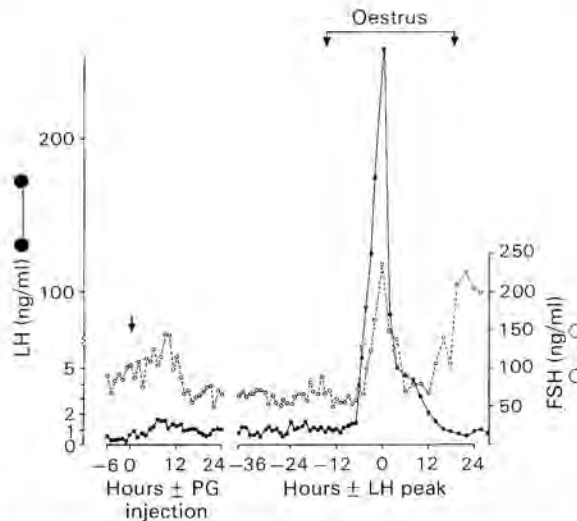
that LH is secreted in pulses which occur at intervals of approximately every 3 h during the luteal phase. These pulses of LH stimulate secretion of oestradiol and androgens from the follicle (Baird *et al.*, 1976a). The suppression of the basal level and pulse frequency of LH by progesterone during the luteal phase reduces the effective stimulation of oestrogen secretion from the follicle. Progesterone has an additional important effect on the uterus which requires a period of priming of 7–10 days before it will synthesize adequate quantities of prostaglandin (PG) F-2 $\alpha$  (Baird, 1978b). It is this feedback loop between the ovary and the uterus which regulates the length of the luteal phase. In the presence of the low basal levels of LH which exist by Day 12–13, the corpus luteum becomes increasingly sensitive to the luteolytic effect of PGF-2 $\alpha$  which is released in small amounts into the uterine vein. By Day 13 sufficient PGF-2 $\alpha$  reaches the ovary via a counter-current transfer between the utero-ovarian vein and the ovarian artery to cause a decline in secretion of progesterone. Further release of PGF-2 $\alpha$  from the uterus is enhanced by this decline in progesterone levels so that the whole cascade of events proceeds until functional and eventually structural luteal regression is complete.

#### *Peri-ovulatory period*

Because antral follicles (up to 5 mm diameter) are usually present in the ovary at all times during the luteal phase and even during anoestrus, the factors regulating the final 2 or 3 days of follicular maturation of the ovulatory follicle are of particular interest. During the interval

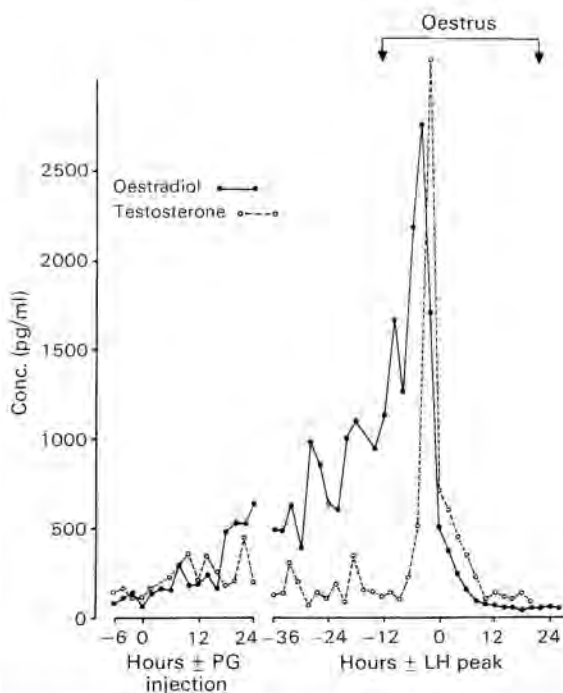
between luteal regression and the end of oestrus, the dominant follicle(s) undergo a series of structural and functional changes which culminate in ovulation (Hay & Moor, 1975). The sequence of events during this peri-ovulatory period is similar whether luteal regression occurs spontaneously or whether it is induced by surgical enucleation of the corpus luteum or administration of exogenous PGF-2 $\alpha$  (Baird & Scaramuzzi, 1976; Legan & Karsch, 1979; Karsch, Foster Legan, Ryan & Peter, 1979). For convenience we have studied the hormone changes following luteal regression induced by the injection of the potent synthetic analogue of PGF-2 $\alpha$ , cloprostenol, on Day 10 of the cycle, as well as during the spontaneous cycle (Baird & Scaramuzzi, 1975; Baird, Land, Scaramuzzi & Wheeler, 1976b). The endocrine events during the peri-ovulatory period can be divided into three phases.

*Luteal regression to LH surge.* The period between luteal regression and the onset of the LH surge is characterized by a striking increase in the secretion of oestradiol from the follicle(s) which is going to ovulate (Text-figs 1 and 2). The rise in secretion of androstenedione and testosterone is much less so that there is a progressive increase in the oestrogen to androgen ratio (Text-fig. 4). This change in ratio is also found in follicular fluid (Moor *et al.*, 1978) and probably reflects the increasing utilization of androgen precursor as it is aromatized to oestrogen by the preovulatory follicle. The increasing secretion of oestradiol from the preovulatory follicle has three important effects—it stimulates further PGF-2 $\alpha$  release from the uterus and hastens the onset of irreversible structural regression of the corpus luteum; it induces oestrous behaviour; and it suppresses the secretion of FSH so that during the final 48 h before the LH surge the level of FSH falls significantly (Text-fig. 3). The follicle(s) destined to ovulate are probably protected from the deleterious effect of declining levels of FSH by the high intrafollicular concentration of FSH and oestradiol. It is possible, however, that this decline in FSH before ovulation hastens atresia in those large antral follicles which will not ovulate but which are very dependent on gonadotrophic support.



**Text-fig. 3.** Mean concentration of LH and FSH in jugular venous plasma in 4 ewes with ovarian autotransplants injected with 100  $\mu$ g cloprostenol (arrow) on Day 10 of the oestrous cycle. The samples have been grouped around the injection of cloprostenol or the LH peak. The mean interval from the injection to the LH peak was 60 h (range 54–69 h). Oestrous behaviour started at 48 h and extended to 79 h. Note the 5-fold rise in basal LH following luteal regression induced by injection of cloprostenol. LH expressed in ng NIH-LH-S14 and FSH in ng NIH-FSH-S10. (Data from Baird *et al.*, 1981.)





**Text-fig. 4.** Mean concentration of oestradiol and testosterone in ovarian venous plasma of 4 ewes with ovarian autotransplants. Note the marked rise in concentration of testosterone which occurs as the LH surge commences. See legend Text-fig. 3 for explanation of grouping of samples. (Data from Baird *et al.*, 1981.)

The question now arises—what is the stimulus for this final rise in secretion of oestradiol? There is strong evidence that the factor responsible is the rise in basal secretion of LH which occurs in response to the decline in the concentration of progesterone associated with luteal regression (Baird & Scaramuzzi, 1976; Hauger, Karsch & Foster, 1977; Karsch *et al.*, 1979). If the fall in progesterone concentration is prevented by the insertion of a progesterone-releasing implant, the rises in basal LH and preovulatory oestradiol secretion are suppressed. LH stimulates a rapid increase in the secretion of androgens and oestrogens when infused into the ovary *in vivo* (McCracken *et al.*, 1969). Each spontaneous LH pulse is followed within 10 min by an increased secretion of oestradiol from the ovary. Taken together with the known steroidogenic effect of LH these results support the concept that LH stimulates preovulatory oestrogen secretion. Associated with the rise in basal LH concentration is a marked increase in the frequency of LH pulses (Baird, 1978a). By 24 h after the decline of progesterone secretion the pulse frequency has doubled to one pulse every 75 min. This increase in pulse frequency stimulates the largest non-atretic antral follicle so that the secretion of oestradiol rises steeply. Although the pulse frequency increases, the amplitude decreases, probably due to a direct effect of the increasing levels of oestradiol on the anterior pituitary (R. L. Goodman & F. J. Karsch, unpublished). In spite of the decrease in pulse amplitude, the ovary responds to each pulse of LH with a bigger increase in oestradiol secretion. This increase in ovarian sensitivity to LH is probably related to the increased number of LH receptors present on the theca cells of the large antral follicle (Carson *et al.*, 1979).

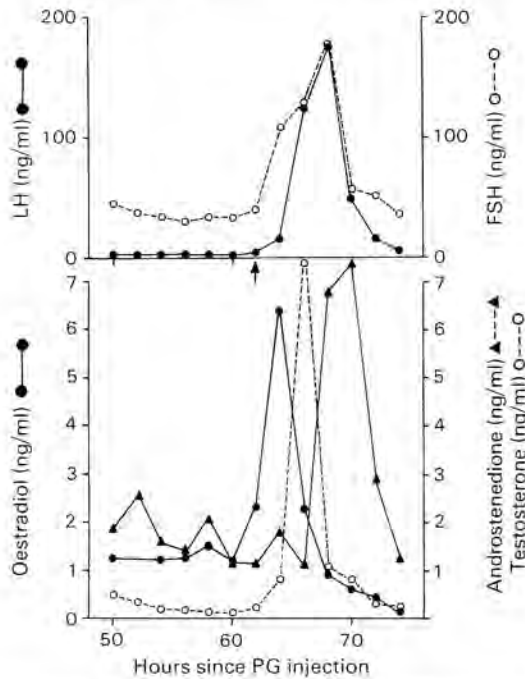
Further evidence of the importance of LH in stimulating the final maturation of the Graafian follicles comes from experiments in which LH or hCG was given to ewes during anoestrus (Legan & Karsch, 1979; Goodman & Karsch, 1980). In 4/7 ewes a rise in the secretion of

oestradiol, an LH surge and ovulation occurred following the infusion of hCG alone. When LH was injected into anoestrous ewes repeatedly for 72 h in amounts to mimic the changes in LH pulses which occur during the follicular phase of the cycle in the breeding season, hormone changes characteristic of the peri-ovulatory period occurred, i.e. rise in oestradiol secretion and fall in FSH concentration followed by a surge of LH and FSH (A. S. McNeilly, M. A. O'Connell & D. T. Baird, unpublished). These results indicate that during seasonal anoestrus ovulation does not occur in spite of the presence of large antral follicles because LH pulses occur too infrequently to stimulate oestrogen secretion to a level at which it will induce a preovulatory surge of LH.

*Preovulatory LH surge.* Approximately 60 h after the initiation of luteal regression, at about the time of the onset of behavioural oestrus, there is a marked rise in the concentration in plasma of both FSH and LH. We have defined the onset of this preovulatory LH surge as commencing when the concentration of LH reaches at least 5 ng/ml. There is strong experimental evidence for most species studied, including the sheep, that the preovulatory surge of LH and FSH is stimulated by the rise in oestrogen secretion from the preovulatory follicle (Goding *et al.*, 1973). It is likely that the positive feedback effect of oestradiol is exerted at the levels of both the hypothalamus and pituitary. By oestrus (Day 0) the pulsatile discharges of LH are occurring so frequently (every 45 min or less) that it becomes difficult to distinguish individual pulses from the rising basal level of LH. At the same time the sensitivity of the anterior pituitary gland to LH-RH is increased by oestrogen so that by Day 0 small amounts of exogenous LH-RH will release large quantities of LH (Reeves *et al.*, 1971). In the rhesus monkey positive feedback can be obtained by oestrogen acting on the pituitary gland alone (Knobil, Plant, Wildt, Belchetz & Marshall, 1980). These monkeys in which endogenous LH-RH had been abolished by radiofrequency lesions in the arcuate region of the hypothalamus were maintained on a pulse frequency of LH-RH (one per h) which closely approximates that observed during the final stages of development of the preovulatory follicle in the sheep and women (Santen & Bardin, 1973; Baird, 1978a). The preliminary evidence for sheep suggests that the increase in pulse frequency of LH is essential if adequate follicular development and oestrogen secretion is to occur (A. S. McNeilly, M. A. O'Connell & D. T. Baird, unpublished). In sheep and women progesterone decreases and oestradiol increases the frequency of LH pulses, indicating that under physiological situations these steroids act in part at the level of the hypothalamus (Santen & Bardin, 1973; Legan & Karsch, 1979). Unfortunately, it is not yet possible to address these questions directly in the rhesus monkey because pulsatile release of gonadotrophins cannot be demonstrated in the intact animal (Knobil, 1980). Whether this is a true species difference or (more likely) a limitation of the existing radioimmunoassays available for measuring gonadotrophins in the monkey is not yet known. However the fact that, in contrast to intact animals, progesterone is unable to inhibit the positive feedback effect of oestradiol in hypothalamic-lesioned monkeys maintained on hourly injections of LH-RH indicates that at least this steroid has an effect on hypothalamic activity as in other species (Wildt, Hutchison, Marshall & Knobil, 1980). It would be of interest to know whether normal cyclic activity of the ovary and positive feedback effect of oestradiol could be observed in lesioned animals maintained at an exogenous pulse frequency of  $<1/h$ .

The preovulatory surge of LH initiates a sequence of events in the ovary which lead to ovulation about 24 h later. There is an initial stimulation of the secretion of oestradiol and androgens by the dominant follicle(s) followed by a marked inhibition of steroid secretion (Text-fig. 5). The secretion of testosterone and androstenedione is increased to a greater extent than that of oestradiol so that the oestrogen/androgen ratio, which rises progressively throughout the follicular phase, very rapidly declines.

These paradoxical effects of LH, i.e. stimulation and inhibition of steroid secretion, appear to be dependent on dose and duration. Infusion of LH into ewes on Day 10 of the oestrous cycle first stimulates and then inhibits oestrogen secretion from the dominant follicle (Baird, McNeilly,



**Text-fig. 5.** Concentration of oestradiol, testosterone and androstenedione in ovarian venous plasma and LH and FSH in jugular venous plasma of a ewe with ovarian autotransplant. The samples were collected between 50 and 76 h after the induction of luteal regression by the injection of 100 µg cloprostenol on Day 10 of the cycle. The start of the LH surge, defined as a concentration >5 ng/ml, is indicated by the arrow. (Data from Baird *et al.*, 1981.)

O'Connell & Swanston, 1980). The fact that the secretion of oestradiol is inhibited before that of androgen suggests that aromatase may be inhibited directly (Moor, 1974). However, the eventual total suppression of steroid secretion would be compatible with desensitization and loss of LH receptors such as occurs in the rat (Webb & England, 1979). Probably both mechanisms are involved.

The LH surge induces important changes in the morphology and structure of the granulosa cells. In contrast to that for women and rats, there is very little cytological evidence of luteinization of granulosa cells before ovulation in the sheep (Bjersing *et al.*, 1972) although there is a slight increase in the concentration of progesterone in ovarian venous blood (Wheeler, Baird, Land & Scaramuzzi, 1975) and follicular fluid in the 12 h prior to ovulation. The cytoplasm of the theca cells shrinks shortly after the onset of oestrus so that by ovulation the theca interna is insignificant (Bjersing *et al.*, 1972).

While the LH surge prepares the dominant follicle for ovulation, it probably has disastrous consequences for the remaining large follicles (>2 mm) which are already suffering from the relative decline in FSH. At ovulation all but one or two large follicles show prominent signs of atresia. Administration of hCG together with PMSG increases the proportion of atretic follicles (Turnbull *et al.*, 1977). From experiments with rats it has been concluded that the increase in follicular atresia produced by hCG is mediated by androgens (Louvet *et al.*, 1975). Probably the theca cells of those follicles which have not developed to the stage at which the granulosa cells contain adequate amounts of LH receptors are unable to luteinize in response to LH (hCG) and are stimulated to produce androgens in large quantities.

*LH surge to ovulation.* Ovulation occurs approximately 24 h after the preovulatory LH

surge (Cumming *et al.*, 1971). During this time the secretion of oestradiol, androstenedione and testosterone decline rapidly so that at ovulation steroid secretion by the ovary is lower than at any other time in the oestrous cycle. Although the concentrations of LH and prolactin also decline during this period, the concentration of FSH rises again to reach a peak comparable in size to that occurring 24 h earlier.

The cause and function of this second peak of FSH remains unknown although it occurs in many other species, e.g. rat and hamster. Primates including man are an exception and it may be relevant that the decline in ovarian steroid secretion at ovulation in primates is not nearly so complete as in other species. The primate corpus luteum secretes oestradiol as well as progesterone early in its formation and helps to maintain a high concentration of ovarian steroids (Baird, Baker, McNatty & Neal, 1975).

Whatever the mechanism it seems likely that this second peak of FSH is in some way related to the events which follow the preovulatory LH surge. Infusion of exogenous LH in the ewe on Day 10 of the oestrous cycle is followed within 24 h by a second peak of FSH (Baird *et al.*, 1980). As oestrogen secretion is inhibited following the infusion of LH, it is tempting to relate the rise in FSH secretion to the reduction in negative feedback effect of this steroid. However, in similar experiments with rats, Chappel & Barraclough (1977) were unable to prevent the second peak of FSH when steroid levels were maintained with oestradiol implants and concluded that the second peak of FSH was due to lack of ovarian 'inhibin' acting at the pituitary. In the ewe the first but not the second FSH peak can be inhibited by the administration of pentobarbitone or antiserum to LH-RH (Dobson & Ward, 1977; Narayana & Dobson, 1979). During the second FSH peak there is no coincidental rise in LH secretion and the frequency of LH pulses remains unaltered. All these experiments would point to a change in the sensitivity of the pituitary gonadotroph to LH-RH as being responsible for the selective release of FSH at this time. Direct experiments with exogenous LH-RH would help to confirm this hypothesis.

It is hard to believe that the second FSH peak has no function in the ewe. In rats if its effect is neutralized by the administration of antiserum to FSH, the number of follicles available for ovulation at the next oestrus is reduced (Sheela Rani & Moudgal, 1977). In sheep, because the second FSH peak is larger in breeds with high rates of ovulation, it has been suggested that it determines the number of small antral follicles which are recruited for development and which will eventually ovulate 17 days later (Cahill, 1979). However, it is difficult to reconcile this hypothesis with the fact that the rate of ovulation is apparently unaffected when the corpus luteum is regressed prematurely at any stage after Day 4 (Bindon, Blanc, Pelletier, Terqui & Thimonier, 1979). It is perhaps more likely that the two or three large antral follicles present on Days 3 or 4 of the cycle are a result of the second FSH peak.

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