Reproduction Supplement **61**, 463–476

The differential secretion of FSH and LH: regulation through genes, feedback and packaging

A. S. McNeilly[1], J. L. Crawford[1]*, C. Taragnat[2], L. Nicol[1] and J. R. McNeilly[1]

[1]*MRC Human Reproductive Sciences Unit, University of Edinburgh Centre for Reproductive Biology, The Chancellor's Building, New Royal Infirmary, 49 Little France Crescent, Old Dalkeith Road, Edinburgh EH16 4SB, UK; and* [2]*INRA Station Physiologie de la Reproduction et des Comportements, 37380 Nouzilly, France*

While the role of oestradiol and progesterone in the control of GnRH pulsatile secretion and generation of the preovulatory GnRH surge to induce release of the LH surge has been fully investigated, less attention has been given to changes in the pituitary gland that may sensitize gonadotrophs to switch from pulsatile release to surge release of LH, in particular. Furthermore, in the follicular phase while pulsatile secretion of LH is maximal, FSH secretion is reduced, yet both hormones are produced by the same gonadotrophs. The mechanisms whereby this differential release can occur are still unclear. The main regulator of FSH secretion is through the negative feedback effects of oestradiol and inhibin, which directly affect FSHβ mRNA content and subsequent synthesis of FSH. FSH is then released predominantly via a constitutive pathway and the amount released is closely related to the rate of synthesis. In contrast, while basal LH secretion occurs via a constitutive pathway, the principal release of LH through pulsatile secretion is through the regulated pathway with GnRH stimulating the release of pre-synthesized LH contained in storage granules without significant changes in LHβ mRNA. Secretogranin II (SgII) is associated with LH in these electron-dense storage granules and LH–SgII granules appear to be the principal form of granule released in response to GnRH through the regulated pathway. At the time of the preovulatory LH surge, granule movement to the gonadotrope cell membrane abutting a capillary, polarization, appears to play an important part in the priming mechanism for release of LH during the preovulatory LH surge in response to the GnRH surge. As there appears to be limited or no gonadotroph cell division in the adult pituitary gland, each gonadotroph passes through this synthesis and secretion pathway repeatedly through successive oestrous

*Present address: Reproductive Biology Group, AgResearch, Wallaceville Animal Research Centre, PO Box 40063, Upper Hutt, New Zealand
Email: a.mcneilly@hrsu.mrc.ac.uk

© 2003 Society for Reproduction and Fertility

cycles. Packaging of LH and FSH into different secretory granules within the same cell is thus pivotal for the differential secretion of these gonadotrophins.

Introduction

Control of the number of babies born and, in some species, the time of year of birth are critical in promoting the well-being of the offspring and, hence, the species as a whole. Thus, the co-ordination of the events that control follicle selection, and subsequent ovulation, at the correct time is crucial, and relies on a tight control of the secretion of both FSH to control the number of follicles and LH to provide the signal from the follicles to time ovulation when the eggs are fully matured. Ovulation is not a common event when the natural course of reproduction is followed, that is, pregnancy followed by lactational infertility, sometimes coinciding with seasonal infertility, for example in sheep and deer. Thus, the preovulatory surge may be generated only 20 times in the lifespan of a sheep, 50 times in women and even in the prolific mouse about 15 times. Therefore, the timing of the surge relative to follicle growth is critical. This paper will review some of the factors that are involved, concentrating principally on changes within the pituitary gonadotrophs which may be necessary to allow generation of the preovulatory surge.

Of equal importance are the factors that regulate the differential secretion patterns of FSH and LH during the follicular phases of the oestrous cycle. In all species so far examined, including cows, both LH and FSH are present in the same gonadotrophs, and cells expressing only FSH or only LH are rare. LH is stored in the gonadotrophs and, therefore, is easily detected by immunocytochemistry, whereas FSH is released mainly through a constitutive pathway, and there may be little storage. Therefore, it is likely that in most instances in which LH only cells are observed, these cells are bi-hormonal but the production of FSH is greatly diminished and FSH cannot be detected in these cells. Of critical importance to the regulation of reproduction is that LH is released in discrete pulses due to the pulsatile release of GnRH. Therefore, LH release is governed by how frequently GnRH pulses are released, and regulation of the GnRH clock is the simple regulator of the oestrous and menstrual cycles. In humans, the only natural regulator of fertility, apart from severe nutritional imbalances, is breastfeeding, and it is now clear that, as in other species, lactational infertility is caused by the suckling stimulus disrupting the natural pattern of GnRH pulsatile release and, hence, pulsatile LH release from the pituitary gland to drive steroidogenesis from developing follicles (McNeilly, 2001). At the time of the LH surge, the mode of GnRH release switches from pulsatile to a maintained surge, which then generates the preovulatory LH surge leading to ovulation. In species in which this has been examined in any detail it appears that the gonadotrophs become sensitized to GnRH, so-called priming, such that subsequent stimulation causes an increased release of LH. This process is deemed essential to allow gonadotrophs to release most of their content of LH to generate the LH surge. However, the precise factors that may prime gonadotrophs have not been established, and although in some studies it is thought that priming involves increased LH production, there is little support for this concept. At the same time that priming may be occurring, pulsatile release of LH is maximal before the onset of the LH surge, but FSH release is inhibited and plasma concentrations of FSH are declining. Although it is known that the majority of gonadotrophs contain both LH and FSH, there is very limited information as to which cells actually release LH, FSH or both gonadotrophins. For differential secretion of LH and FSH to occur there must either be subsets of cells releasing only FSH, or only LH, or if they are released from the same cell, there must be different

pathways for release of FSH and LH. The present review describes some of the studies that we have undertaken to address this issue.

Cells

There appears to be little or no change in the gonadotroph cell population within the adult pituitary gland since cell division of gonadotrophs is almost never observed, even after castration (J. R. McNeilly, unpublished). Furthermore, ablation of gonadotrophs in fetal life using alpha gonadotrophin subunit (alpha GSU; Kendall *et al.*, 1991; Seuntjens *et al.*, 1999), FSHβ promoter (Markkula *et al.*, 1996) or the LHβ promoter (McNeilly *et al.*, 2001) to drive ablation agents results in a permanent, partial, but not complete, depletion of gonadotrophs in adults confirming that there are no progenitor cells within the pituitary gland that can repopulate the gonadotroph pool of cells. Thus, in considering the changes that occur to allow gonadotrophin secretion, these must be dynamic changes within the existing population of gonadotrophs.

Although it is recognized that in all species for which adequate data are available LH and FSH are present in the same cells (for example, sheep: Taragnat *et al.* 1998), no studies have addressed the question of whether all gonadotrophs release both FSH and LH. The few studies that have assessed LH release in rats indicate that only 20–40% of gonadotrophs release LH in dioestrus, which increases to 60–70% at pro-oestrus before the onset of the LH surge and that oestradiol treatment increases the number of cells releasing LH (Neill *et al.*, 1987). Similar studies using the reverse haemolytic assay in sheep failed to show a difference in the numbers of gonadotrophs releasing LH between the luteal and the follicular phases of the oestrous cycle (C. Taragnat, unpublished). These results are supported by studies on the changes in the ultrastructure of gonadotrophs during the oestrous cycle in sheep and concluded that, at the time when LH pulses are being released before the LH surge, only about 20–30% of gonadotrophs are actually releasing LH (Currie and McNeilly, 1995). There is no reliable information about FSH release, so it is unknown whether the same or different gonadotrophs release LH and FSH.

It is clear that GnRH is the factor that controls the release of LH pulses. However, LH release can occur in a basal mode in most species in the absence of GnRH, and although concentrations may be very low, there is, nevertheless, some release via a non-GnRH pathway. LHβ gene expression appears to function in a non-GnRH- and GnRH-dependent manner (Brown and McNeilly, 1999), although GnRH is absolutely essential for normal LHβ mRNA expression associated with normal production of LH. During the follicular phase of the oestrous cycle, there is a modest increase in GnRH receptor mRNA and GnRH binding capacity (Brooks *et al.*, 1992, 1993), an increase that in sheep appears to occur in response to an increase in oestradiol rather than the increase in pulsatile GnRH secretion (Brooks and McNeilly, 1994). In rats, this increase in GnRH receptor expression appears to relate to an increase in the number of gonadotrophs expressing the receptor so that by the time of the preovulatory LH surge about 90% of LH cells bound biotinylated GnRH (Childs *et al.*, 1994). However, it is important to note that expression of the GnRH receptor does not necessarily equate to release of LH or the amount of LH released if release occurs (Neill *et al.*, 1987). Nothing is known about the expression of GnRH receptors on gonadotrophs at different stages of the reproductive cycles in other species, as there are no antisera that will recognize the GnRH receptor, and detailed *in situ* analyses have not been performed. Furthermore, if oestradiol is the principal mediator of the increase in GnRH receptor expression then it will be important to determine the relationship between the expression of GnRH receptors and oestrogen receptors (ER) in gonadotrophs.

Changes in gonadotrophin gene expression

Throughout the oestrous cycle there is little evidence for a major change in LHβ mRNA in the pituitary gland (Brooks *et al.*, 1992, 1993), but there is a large decrease in the rate of transcription between the luteal and the follicular phase of the oestrous cycle in sheep (Brown and McNeilly, 1997). At the time of reduced transcription, there is an increase in polyadenylation of the LHβ mRNA leading to a potential increase in LHβ stability, which in turn would lead to maintenance of steady state values of LHβ mRNA (Crawford and McNeilly, 2002). This increase in poly-A tail length could be due to either a withdrawal of an effect of progesterone, or the increased input of GnRH and oestradiol either together or separately in the follicular phase. Whichever mechanisms are operating, there appears to be little change in the synthesis of LHβ and, hence LH, before the onset of the preovulatory LH surge. Furthermore, LHβ mRNA is very stable with a half-life in rats of about 40 h (Bouamoud *et al.*, 1992), a duration similar to that estimated from the time to decline of LHβ mRNA content in sheep pituitary gland after interruption of GnRH input (McNeilly *et al.*, 1991). However, in view of the changes in polyadenylation of LHβ mRNA, the rates of LH synthesis and the number of gonadotrophs in which this may be occurring throughout the ovarian cycle needs to be examined.

In contrast to LHβ mRNA, FSHβ mRNA concentrations change under the influence of the negative feedback action of gonadal steroids, principally oestradiol and inhibin. These factors act directly at the gonadotroph, and indicate that FSH release is much more closely tied to production of FSH after a mainly constitutive pathway rather than regulated pathway of release (McNeilly, 1988; Farnworth, 1995). Although the initiation of FSHβ gene expression is dependent on GnRH, the frequency of GnRH pulsatile secretion can alter FSHβ mRNA concentrations, with slow pulses favouring FSH transcription (sheep: Molter-Gerard *et al.*, 1999; Padmanabhan and McNeilly, 2001). There has been much debate about the presence of a specific FSH releasing factor, but the bulk of the evidence indicates that such a factor may not exist and no putative factor has been isolated (Padmanabhan and McNeilly, 2001). The FSHβ mRNA is intrinsically unstable with a short half-life of about 1 h, related to the long AU-rich poly-A tail (Bouamoud *et al.*, 1992). In transgenic mice, substitution of this 3′UTR with the LHβ 3′UTR results in stabilization of FSHβ mRNA in transgenic mice (Brown *et al.*, 2001), and leads to greater producton of FSH *in vitro* (Mountford *et al.*, 1992). Furthermore, although inhibin shortens the half-life of FSHβ mRNA (Attardi and Winters, 1993), inhibin also reduces the transcription rate of the FSHβ gene (Clarke *et al.*, 1993) possibly by interfering with local activin signalling at the pituitary gland. However, activin increases the half-life of FSHβ mRNA through a protein-mediated step requiring *de novo* protein synthesis (Attardi and Winters, 1993).

In sheep, an increase in plasma FSH equivalent to that seen after ovariectomy can be induced by immunoneutralization of both oestradiol and inhibin, although each individually causes only a partial response (Mann *et al.*, 1990). These negative regulatory effects of oestradiol and inhibin on FSH secretion acting directly at the pituitary gland in sheep overcome any natural GnRH input, and appear to explain the difference in duration of oestrus in sheep and menstrual cycles in humans which in many other endocrine senses are equivalent. As first proposed by Baird *et al.* (1975), the main difference between the ovarian and menstrual cycles is that the human corpus luteum secretes oestradiol and progesterone which suppresses FSH during the luteal phase. Antral follicle growth is arrested and is stimulated only at the onset of luteolysis when the feedback effects of oestradiol on FSH are removed, plasma concentrations of FSH increase, and follicle growth can resume over the next 14 days leading to preovulatory follicle development. The human corpus luteum produces large amounts of inhibin A

(Groome *et al.*, 1994) which would act with oestradiol to enhance this negative feedback effect on FSH. In contrast, sheep corpus luteum, although also present for 14 days of the oestrous cycle, produces only progesterone, which after priming of the hypothalamus by oestradiol released from follicles on days 3–4 of the luteal phase (Campbell *et al.*, 1990) suppresses pulsatile LH secretion in the luteal phase of the sheep oestrous cycle (Wallace and McNeilly, 1986). In the absence of oestradiol in the early part of the luteal phase, progesterone no longer acts to suppress GnRH–LH pulse frequency (Wallace and McNeilly, 1986). The absence of major negative feedback from the ovary in the sheep luteal phase allows plasma concentrations of FSH to remain unsuppressed; waves of follicle growth are maintained and the time from luteal regression to development of preovulatory follicles and ovulation is considerably shortened compared with the human menstrual cycle. Nevertheless, during the follicular phase in both humans and sheep, plasma concentrations of FSH decline at the time of follicle selection due to the combined negative feedback effects of oestradiol and inhibin A in sheep (Souza *et al.*, 1997; Knight *et al.*, 1998), as sheep appear to secrete only inhibin A into plasma (McNeilly *et al.*, 2002), whereas women secrete oestradiol and inhibin B (Groome *et al.*, 1996).

At the cellular level, it is unclear whether the negative feedback effects of oestradiol and inhibin act on all or a subset of gonadotrophs, since, as reported above, there is no information on the numbers of gonadotrophs that actually release FSH. As inhibin (Brooks *et al.*, 1992; Mercer *et al.*, 1987) and oestradiol (Mercer *et al.*, 1989, 1993) both reduce FSHβ concentrations *in vivo* in line with the decline in FSH secretion, it is probable that most gonadotrophs respond to these negative feedback signals. Interestingly, ERα is expressed only in about 60% of gonadotrophs in the luteal phase increasing to about 80% of gonadotrophs in the follicular phase of the reproductive cycle in sheep (Sheng *et al.*, 1998; Tobin *et al.*, 2001; A. S. McNeilly and C. Sheng, unpublished). ERβ expression appears to be low in many cells in the sheep pituitary gland, located mainly in the cytoplasm and does not change during the oestrous cycle (A. S. McNeilly, M. Millar and G. Scobie, unpublished). The probable component parts of the inhibin receptor system, activin receptor IIB and betaglycan, are both expressed on gonadotrophs in the sheep pituitary gland (A. S. McNeilly and C. Taragnat, unpublished) but the interactions of oestradiol and inhibin on FSH in individual gonadotrophs require proper investigation.

Of further importance is the intracellular regulation of FSHβ gene expression through the presence of the activin βB subunit expression within gonadotrophs themselves, which may result in the production of activin B. This intra-pituitary system for regulating FSH secretion has received much attention in rats, but very limited work has appeared in other species (Padmanabhan and McNeilly, 2001). In culture, sheep pituitary cells produce large amounts of FSH independently of GnRH (Tsonis *et al.*, 1986) and also produce follistatin (Farnworth *et al.*, 1995), an activin binding protein that abolishes the action of activin. As follistatin concentrations increase, FSH secretion declines (Farnworth *et al.*, 1995). The origin of follistatin in sheep pituitary cells is unknown, but it may be derived from folliculostelate cells as in the primate pituitary gland (Kawakami *et al.*, 2002). These interactions between follistatin and activin in the sheep pituitary gland appear to be important, but the relevant importance of this system, GnRH positive input, and the negative feedback effects of ovarian-derived oestradiol and inhibin remain to be elucidated, although the ovarian component appears to dominate.

It is clear that the control of release of FSH in the follicular phase is vital to ensure that only the correct numbers of follicles develop. In terms of secretion, FSH must be released through a pathway independent of LH since, at the time that FSH concentrations are declining in the follicular phase, pulsatile secretion of LH is increasing markedly. These LH pulses drive the increase in oestradiol secretion by the preovulatory follicle with oestradiol acting as

the co-ordinating signal to the hypothalamus and pituitary gland indicating the readiness of the follicle for ovulation (Baird *et al.*, 1981).

The main question to be answered is how FSH and LH molecules are partitioned to the different pathways within the same gonadotroph to allow differential secretion. This is particularly intriguing as they are made in the same cell and have the same common alpha subunit.

Packaging, granules and regulated and constitutive release of LH and FSH

There are three aspects of LH and FSH packaging that are important to recognize. The first aspect is the requirement to allow release of FSH and LH in different amounts at the same time potentially from within the same gonadotroph. If LH and FSH were always present in the same granule then differential secretion of FSH and LH would not be possible. However, it is still not known whether LH and FSH are released from the same gonadotroph at the same time, or whether some gonadotrophs release only LH while others release only FSH.

Secondly, LH is released in discrete pulses that are not usually accompanied by a release of FSH. Some FSH pulses may be associated with GnRH and, hence, LH pulses, but the increase in FSH concentrations often precedes that of either GnRH or LH (Padmanabhan *et al.*, 1997). However, FSH is also released independently of direct GnRH input in intact sheep, and in ovariectomized ewes the majority of FSH secretory episodes are released in this way (Padmanabhan *et al.*, 1997; Clarke *et al.*, 2002). For an LH pulse in sheep this amounts to a bolus release of approximately 2 μg LH over a period of a few seconds, as the profile of LH in plasma after such a treatment is nearly identical to a natural LH pulse in the follicular phase of the reproductive cycle (McNeilly *et al.*, 1982). Thus, packaging of LH in granules allowing regulated release of LH in response to GnRH pulses is essential. Thirdly, sufficient LH, in particular, must be stored in the gonadotroph to allow enough LH to be released to maintain the preovulatory LH surge. With a half-life in plasma of only 20 min (McNeilly *et al.*, 1982) the maintenance of the plasma concentrations of LH during the preovulatory LH surge in a sheep requires the release of up to 80% of the total pituitary content (Brooks *et al.*, 1993) resulting in the release of most, if not all, intracellular LH granules at the time of the preovulatory LH surge (Currie and McNeilly, 1995; Crawford *et al.*, 2000).

In sheep, although LH and FSH are co-localized in the same gonadotroph, at a gross morphological level they are packaged separately (Thomas and Clarke, 1997). At the ultrastructural level, LH is packaged in electron-dense core granules (Currie and McNeilly, 1995; Crawford and McNeilly, 2002), whereas FSH appears to be present in less dense granules. In addition, granules containing an FSH-positive electron-light matrix surrounding an LH-positive electron-dense core have also been observed. LH and FSH are mainly packaged in separate granules and this is clearly illustrated in studies of the changes in morphology of gonadotrophs after the preovulatory LH surge in sheep. Within 24 h of an induced preovulatory LH surge, all gonadotrophs contained few, if any, LH positive granules, although at this time the second FSH surge was occurring with increased FSH secretion from these gonadotrophs (Fig. 1; Crawford *et al.*, 2000). Furthermore, the absence of LH granules was associated with a lack of pusatile secretion but maintained basal LH secretion, associated with the presence of LHβ in the rough endoplasmic reticulum indicating continued synthesis of LH in gonadotrophs (Crawford, 2000). Subsequently, the resumption of pulsatile LH secretion with time was associated with the reappearance of electron-dense LH-containing granules (Crawford *et al.*, 2000) (Fig. 1). Thus, granules appear to be essential for the regulated release of LH in response to GnRH pulsatile secretion as well as providing the store of LH required to generate the preovulatory LH surge. In contrast, FSH and basal LH secretion are not associated with

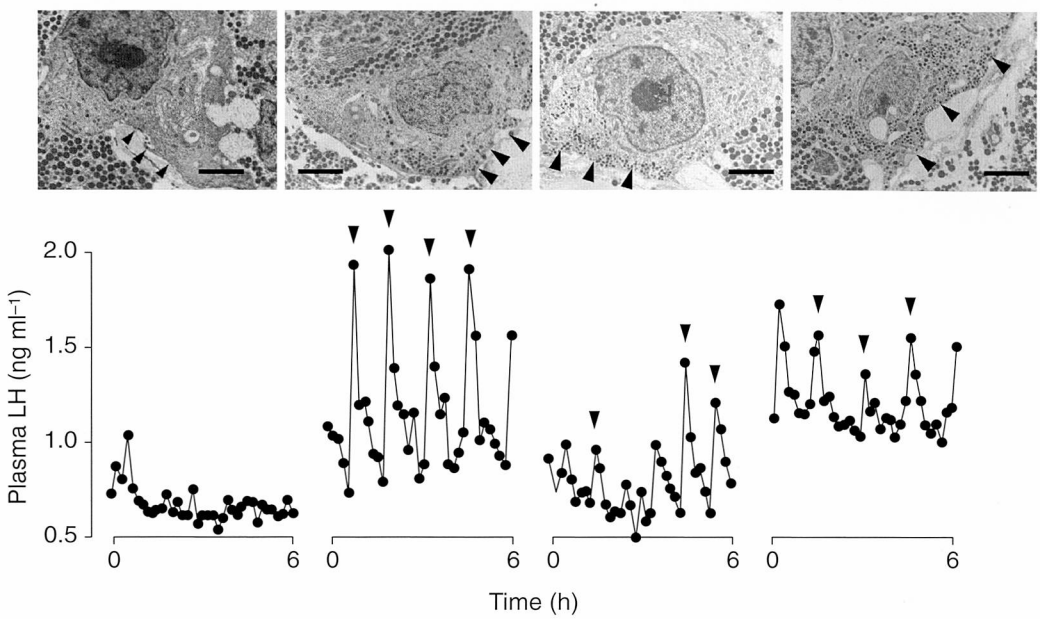

Fig. 1. Changes in LH-immunopositive granules in gonadotrophs in the sheep pituitary gland at different times after the induction of a preovulatory LH surge with the GnRH analogue Buserelin, in relation to the pattern of LH pulsatile secretion in the 6 h period immediately before collection of the pituitary glands. At 24 h after Buserelin (B + 24 h) there is a lack of LH pulsatile secretion and few or no LH granules present, although LH is readily localized in the rough endoplasmic reticulum confirming active LH synthesis. Pulses return by B + 48 h and thereafter, and this is associated with the appearance of LH granules in gonadotrophs (arrowheads indicate LH granules near the cell membrane abutting a capillary border). (Redrawn from Crawford *et al.* 2000.)

storage in electron-dense granules, and most if not all parameters of FSH secretion are typical of a constitutive mode of secretion in which most of the FSH release occurs as a result of alterations in the rate of synthesis, ultimately controlled by gene expression and available mRNA for translation.

Studies in rats (Watanabe *et al.*, 1993, 1998), mice (Crawford *et al.*, 2002b) and sheep (Currie and McNeilly, 1995; Crawford *et al.*, 2000, 2002a; Crawford and McNeilly, 2002) support a role for the granin class of protein in the organization of granule formation. These granins are glycoproteins that aggregate at low pH and high salt concentrations equivalent to that in the intra-Golgi environment, bind calcium and, at the ultrastructural level, are closely associated with LH and FSH. Secretogranin II (SgII) is present within the electron-dense core granules associated with LH, and in mice it has been shown that it is these granules that are released in response to GnRH (Crawford *et al.*, 2002b). In contrast, less electron-dense granules containing only LH and no SgII are associated with basal, constitutive, non-GnRH-dependent release of LH in mice (Crawford *et al.*, 2002b). Furthermore, the release of LH in response to GnRH is associated with the release of SgII, whereas basal LH release occurs independently of SgII (Nicol *et al.*, 2002a,b). Chromogranin A (CgA) is also associated with granules but appears to be present on the periphery of, rather than within, the electron dense LH-containing granules in the sheep pituitary gland (Crawford and McNeilly, 2002), whereas the rat CgA is associated with FSH in electron light–dense bodies (Watanabe *et al.*, 1993, 1998). In the mouse LβT2 gonadotroph cell line in culture, CgA is released

only in small amounts, and it is not usually associated with the GnRH-induced release of LH and SgII (Nicol *et al.*, 2002b). Furthermore, although FSH is associated with CgA in rats (Watanabe *et al.*, 1993), there was no association between the release of FSH and CgA in the LβT2 gonadotrophs *in vitro* (Nicol *et al.*, 2002a). Thus, although there are no associations between granins and gonadotrophins within granules, their precise interactions, requirements for packaging, and the potential ability of granins to determine the intracellular pathways that facilitate differential release of LH and FSH still requires clarification. However, at present there is sufficient evidence to indicate that the association between LH and SgII to form dense-core granules probably dictates that these granules are destined for release via the GnRH-dependent regulated pathway of secretion and that CgA probably plays only an intracellular role in packaging and the secretory pathway, rather than being part of the granule that is released (Crawford *et al.*, 2002b).

Priming gonadotrophs for LH release

After the gonadotrophins have been packaged within the cell, it is also necessary to regulate how exocytosis is controlled, and how many gonadotrophs will release LH through the regulated pathway in response to GnRH stimulation. It would appear that movement of granules within the gonadotroph to the cell membrane abutting a capillary, a feature termed polarization (Currie and McNeilly, 1995), may play an important role in this. Treatment of mouse pituitary glands with GnRH resulted in the marginalization of LH granules to the periphery of the gonadotroph through alterations in actin filament alignment, and exocytosis of LH granules of about 120 nm in diameter was observed only at the cell membrane abutting a capillary (Lewis *et al.*, 1985). In sheep, Currie and McNeilly (1995) showed that in about 20–30% of gonadotrophs, LH granules were polarized with the number increasing to more than 80% during the preovulatory LH surge. On analysis of granule sizes in these polarized gonadotrophs, it was noted that there was a depletion of LH granules within the 120 nm range, equivalent to that released in the mouse. Thus, it was surmised that polarized gonadotrophs were in active secretory mode and, except during the LH surge, only about 20–30% of gonadotrophs are releasing LH at any one time, a figure similar to that observed by plaque assay for LH release in sheep (C. Taragnat, unpublished) and rats (Neill *et al.*, 1987). Since all gonadotrophs appear to discharge their LH granule contents during the LH surge (Crawford *et al.*, 2000), priming of gonadotrophs would have to take place to increase the number of GnRH-responsive gonadotrophs, and polarization of granules to a readily releasable position in the cell may be the priming mechanism involved.

Priming of gonadotrophs is used to describe the phenomenon of increased LH release in response to a second GnRH challenge either *in vitro* or *in vivo* after the first GnRH challenge, the so-called self-priming effect of GnRH (Fink, 1995). During the normal oestrous or menstrual cycle this is rarely observed as feedback effects of steroids *in vivo* appear to modify the response directly at the pituitary gland. The key time when priming is important is during the LH surge, when all gonadotrophs appear to be required to respond maximally to GnRH. Oestradiol increases priming in rats, but priming requires the continued input of GnRH (see Fink, 1995). In sheep, polarization of LH granules can be induced with oestradiol (R. J. Currie and A. S. McNeilly, unpublished; Thomas and Clarke, 1997), an effect that would prime gonadotrophs to respond to GnRH. However, although oestradiol is clearly involved, progesterone appears to play a key role in many species.

In rats (Turgeon *et al.*, 1999), mice (Turgeon and Waring, 2001), rhesus monkeys (Remohi *et al.*, 1988) and women (Baird *et al.*, 1995; Baird, 2001), progesterone appears to play a key role in the generation of the preovulatory LH surge, as blockade of progesterone

receptors with anti-gestogens prevents the surge, even when the cycle is being driven with exogenous GnRH pulses (Batista *et al.*, 1994). In these species, the start of the preovulatory LH surge induces an increase in progesterone secretion from the preovulatory follicles which would then amplify the effect of the GnRH surge driving LH release. Progesterone receptors (PR) are located in gonadotrophs in rhesus monkey (Spangers *et al.*, 1990) and rat (Turgeon and Waring, 1994) pituitary glands, and in gonadotrophs and lactotrophs in mice (Turgeon and Waring, 2001). In rats, the priming effect of GnRH in increasing the LH response to a second dose of GnRH is reduced or abolished after treatment with antigestagen RU486 even in the absence of progesterone, and enhanced with added progesterone (see Turgeon and Waring, 1992). Although the priming effect of GnRH and the preovulatory LH surge is also reduced or abolished in PR knockout mice, the priming effect of progesterone is much more limited than in rats (Turgeon and Waring, 2001).

The situation in sheep appears to be very different. Although the presence of PR in the pituitary gland is unknown, it is clear that there is no increase in progesterone secretion by the preovulatory follicle(s) at the start of the LH surge (Thorburn *et al.*, 1973; Baird *et al.*, 1981), and that blocking progesterone action either by immunoneutralization of progesterone (Thomas *et al.*, 1987) or by treatment with the anti-gestagen RU486 (Campbell *et al.*, 2000) does not affect the preovulatory LH surge. Indeed, during the surge the dominant steroids are androgens, since oestradiol secretion is inhibited at the start of the LH surge (Baird *et al.*, 1981), and progesterone is not secreted until follicle luteinization after the LH surge. Oestradiol is not required in the immediate period before the onset of, or during, the LH surge (Evans *et al.*, 1997), but oestradiol priming before the surge is important (Clarke, 2002). However, progesterone priming does appear to play an important role in the generation of a full GnRH surge, but this effect is through the hypothalamus, and not at the pituitary gland (Caraty and Skinner, 1999; Harris *et al.*, 1999). Thus, it is unlikely that progesterone plays any significant role in the generation of the preovulatory surge in sheep. Indeed, it is not clear that there is any priming of gonadotrophs in the sheep pituitary gland either before the LH surge in ewes (McLeod and McNeilly, 1991) or in rams (Evans *et al.*, 1995). In anoestrous ewes, the LH response to a treatment with GnRH pulses at a constant frequency from 1.0 to 1.5 h intervals and constant dose is not increased before the onset of the LH surge (McLeod and McNeilly, 1991). However, blocking the natural increase in plasma concentrations of oestradiol associated with the stimulation of follicle growth by this treatment in anoestrous ewes (McLeod and McNeilly, 1991) or in the follicular phase of the normal oestrous cycle (Wallace and McNeilly, 1986) does result in an increase in the amplitude of LH pulses indicating a negative effect of oestradiol on LH release directly at the pituitary gland. Furthermore, a study by Crawford *et al.* (2002a) has failed to demonstrate any significant increase in the LH response to GnRH between the luteal phase and the late follicular phase, just before the onset of the LH surge in the oestrous cycle of sheep. This finding indicates that the priming phenomenon may occur only during the LH surge in sheep at a time when gonadotrophs are exposed to constant high concentrations of GnRH.

Although the factors regulating priming of gonadotrophs may be different between species, there would appear to be mechanisms to increase either the responsiveness of individual gonadotrophs to respond to GnRH, or an increase in recruitment of gonadotrophs that can respond to GnRH to generate the preovulatory LH surge.

Conclusions

After synthesis, LH predominantly enters storage granules associated with secretogranin II and these electron-dense granules are released in response to GnRH stimulation through

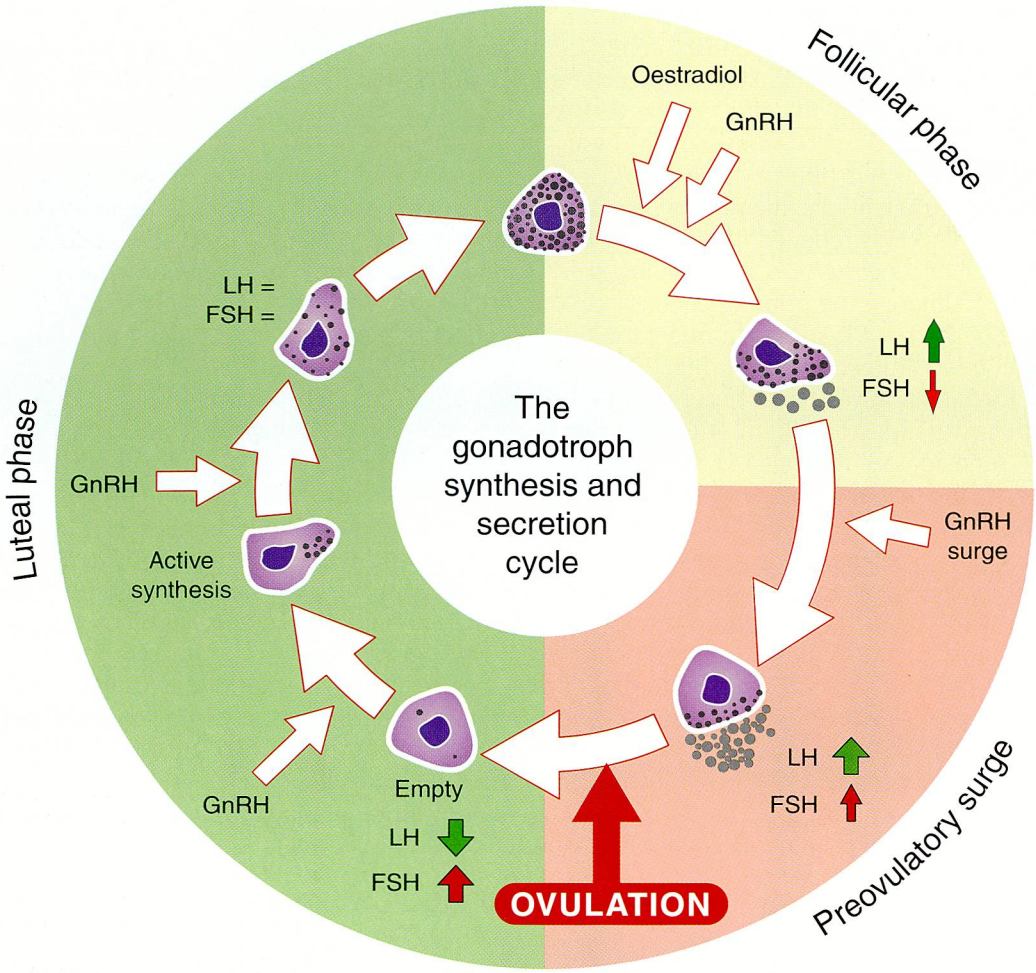

Fig. 2. The gonadotroph synthesis–secretion cycle illustrating the changes within an individual gonadotroph during the oestrous cycle in sheep. After the preovulatory LH surge the gonadotrophs are devoid of LH granules and do not release LH pulses in response to GnRH. FSH secretion is increased due to the loss of negative feedback effects of inhibin A and oestradiol at the time of ovulation. Subsequently the gonadotroph refills with LH-containing granules and pulsatile secretion of LH resumes. At the end of the luteal phase the increase in GnRH pulse frequency increases the pulsatile release of LH granules, while at the same time the increased secretion of oestradiol and inhibin A from the selected follicle(s) inhibits FSH secretion by a direct action at the gonadotroph. During this time in response to increasing plasma oestradiol there is recruitment of gonadotrophs into LH release mode probably through movement of LH granules to the part of the gonadotroph abutting a capillary. Thus at the time of the GnRH surge, all gonadotrophs respond by releasing their stores of LH-containing granules to generate the preovulatory LH surge. The cycle within each gonadotroph then resumes. Throughout these changes FSH is released mainly through a constitutive pathway, while LH is principally released through a regulated pathway with secretion stimulated by GnRH.

a classical regulated pathway. Pulsatile secretion occurs only when granules are present in gonadotrophs. In contrast, FSH is principally released through a constitutive pathway in which the amount of FSH released is closely coupled to the synthesis and hence the gene expression and FSHβ mRNA content. FSH is loosely associated with CgA, but not SgII, in electron

light–dense bodies, but the precise packaging mechanisms for FSH are still unclear. Although these intracellular packaging and secretory pathways can explain the ability of individual gonadotrophs to release LH and FSH differentially at different stages in the oestrous cycle, it is still unclear whether the same gonadotrophs release both FSH and LH at the same time. Only about 20% of gonadotrophs release LH at any time other than around and at the preovulatory LH surge, when all gonadotrophs appear to respond to GnRH by releasing all stored LH-containing granules. Although progesterone is important for the generation of the preovulatory LH surge in a number of species, this is not the case in sheep, as progesterone concentrations are unaltered during the surge which is dominated by androgens. In sheep, priming of the pituitary gland before the LH surge does not appear to occur, but pre-exposure of gonadotrophs to oestradiol appears necessary for the full release of LH during the surge. Finally, because gonadotrophs do not appear to change in number throughout normal adult reproductive life, each gonadotroph appears to pass through a synthesis and secretion cycle during each oestrous or menstrual cycle (Fig. 2). The challenge remains to determine how individual gonadotrophs respond to the myriad of positive and negative input signals to control synthesis, storage and secretion of FSH and LH to allow the controlled differential pattern of gonadotrophin secretion essential for regulation of the reproductive cycle.

The authors would like to thank all their colleagues who have contributed to their research, particularly M. Stridsberg and D. Baird. A. S. McNeilly and J. R. McNeilly, and C. Taragnat are grateful to the ALLIANCE scheme for financial support. J. L. Crawford was supported by a Post-doctoral fellowship from the Journal of Reproduction and Fertility, and Society for Reproduction and Fertility.

References

Attardi B and Winters SJ (1993) Decay of follicle stimulating hormone-β messenger RNA in the presence of transcriptional inhibitors and/or inhibin, activin or follistatin *Molecular Endocrinology* **7** 668–680

Baird DT (2001) Antigestogens: the holy grail of contraception *Reproduction, Fertility and Development* **13** 723–728

Baird DT, Baker TG, McNatty KP and Neal P (1975) Relationship between the secretion of the corpus luteum and the length of the follicular phase of the ovarian cycle *Journal of Reproduction and Fertility* **45** 611–619

Baird DT, Swanston IA and McNeilly AS (1981) Relationship between LH, FSH and prolactin concentration and the secretion of androgens and estrogens by the preovulatory follicle in the ewe *Biology of Reproduction* **24** 1013–1025

Baird DT, Thong KJ, Hall C and Cameron ST (1995) Failure of oestrogen induced luteinizing hormone surge in women treated with mifepristone (RU486) every day for 30 days *Human Reproduction* **10** 2270–2276

Batista MC, Cartledge TP, Zellmer AW, Nieman LK, Loriaux DL and Merriam G (1994) The antiprogestin RU486 delays the midcycle gonadotropin surge and ovulation in gonadotropin-releasing hormone-induced cycles *Fertility and Sterility* **63** 28–34

Bouamoud N, Lerrant Y, Ribot G and Counis R (1992) Differential stability of mRNAs coding for the alpha and gonadotropin β subunits in cultured rat pituitary cells *Molecular and Cellular Endocrinology* **88** 143–151

Brooks J and McNeilly AS (1994) Regulation of gonadotrophin-releasing hormone receptor mRNA expression in the sheep *Journal of Endocrinology* **143** 175–182

Brooks J, Crow WJ, McNeilly JR and McNeilly AS (1992) Relationship between gonadotrophin gene expression, GnRH receptor content and pituitary and plasma gonadotrophin concentrations during the rebound release of FSH after the treatment of ewes with inhibin during the luteal phase of the cycle *Journal of Molecular Endocrinology* **8** 109–118

Brooks J, Taylor PJ, Saunders PTK, Eidne KA, Struthers WJ and McNeilly AS (1993) Cloning and sequencing of the sheep pituitary gonadotropin-releasing hormome receptor and changes in expression of its mRNA during the estrous cycle *Molecular and Cellular Endocrinology* **94** R23–R27

Brown P and McNeilly AS (1997) Steroidogenic factor-1 (SF-1) and the regulation of expression of luteinising hormone and follicle stimulating hormone β-subunits in the sheep anterior pituitary *in vivo*. *International Journal of Cell and Molecular Biology* **29** 1513–1524

Brown P and McNeilly AS (1999) Transcriptional regulation of gonadotrophin genes *Reviews of Reproduction* **4** 117–124

Brown P, McNeilly JR, Evans JG, Christian HC, Crawford GM, Walker M and McNeilly AS (2001) Manipulating the *in vivo* mRNA expression profile of FSHβ to resemble that of LHβ does not promote a concomitant increase in intracellular storage of FSH *Journal of Neuroendocrinology* **13** 48–60

Campbell BK, Mann GE, McNeilly AS and Baird DT (1990) The pattern of ovarian inhibin, estradiol and androstenedione secretion during the estrous cycle of the ewe *Endocrinology* **127** 227–235

Campbell BK, de Souza C and Baird DT (2000) Progesterone as cycle organiser: mifepristone causes ovulation of first wave follicles in sheep *Journal of Reproduction and Fertility Abstract Series* **26** Abstract 2

Caraty A and Skinner DC (1999) Progesterone priming is essential for the full expression of the positive feedback effect of estradiol in inducing the preovulatory gonadotropin-releasing hormone surge in the ewe *Endocrinology* **140** 165–170

Childs GW, Unabia G and Miller BT (1994) Cytochemical detection of gonadotropin-releasing hormone-binding sites on rat pituitary cells with luteinizing hormone, follicle stimulating hormone and growth hormone antigens during diestrous up-regulation *Endocrinology* **134** 1943–1951

Clarke IJ (2002) Multifarious effects of estrogen on the pituitary gonadotrope with special emphasis on studies in the ovine species *Archives of Physiology and Biochemistry* **110** 62–73

Clarke IJ, Rao A, Fallest PC and Shupnik MA (1993) Transcription rate of the FSH beta subunit gene is reduced by inhibin in sheep but this does not fully explain the decrease in mRNA *Molecular and Cellular Endocrinology* **91** 211–216

Clarke IJ, Moore L and Veldhuis J (2002) Intensive direct cavernous sinus sampling identifies high-frequency, nearly random patterns of FSH secretion in ovariectomized ewes: combined appraisal by RIA and bioassay *Endocrinology* **143** 117–129

Crawford J and McNeilly AS (2002) Co-localisation of gonadotropins and granins in gonadotropes at different stages of the oestrous cycle in sheep *Journal of Endocrinology* **174** 179–194

Crawford J, Currie R and McNeilly AS (2000) Replenishment of LH stores of gonadotrophs in relation to gene expression, synthesis and secretion of LH after the preovulatory phase of the sheep oestrous cycle *Journal of Endocrinology* **167** 453–463

Crawford J, McNeilly JR and McNeilly AS (2002a) Gonadotropin secretory patterns suggest lack of priming of gonadotropes prior to preovulatory LH surge, in spite of changes in granule morphology in sheep *84th Annual Meeting of the US Endocrine Society* P1–114

Crawford J, McNeilly JR, Nicol L and McNeilly AS (2002b) Enhancement of secretogranin II (sgII) storage in GnRH-deprived male mice promotes co-aggregation with LHβ and intra-cellular granule storage in gonadotropes *Reproduction* **124** 267–277

Currie RJW and McNeilly AS (1995) Mobilization of LH secretory granules in gonadotrophs in relation to gene expression, synthesis and secretion of LH during the preovulatory phase of the sheep oestrous cycle *Journal of Endocrinology* **147** 259–269

Evans NP, McNeilly JR and Webb R (1995) Effects of indirect selection for pituitary responsiveness to gonadotropin-releasing hormone on the storage and release of luteinizing hormone and follicle stimulating hormone in prepubertal male lambs *Biology of Reproduction* **53** 237–243

Evans NP, Dahl GE, Padmanabhan V, Thrun LA and Karsch FJ (1997) Estradiol requirements for induction and maintenance of the gonadotropin-releasing hormone surge: implications for neuroendocrine processing of the estradiol signal *Endocrinology* **138** 5408–5414

Farnworth PG (1995) Gonadotrophin secretion revisited. How many ways can FSH leave a gonadotroph? *Journal of Endocrinology* **145** 387–395

Farnworth PG, Thean E, Robertson DM and Schwartz J (1995) Ovine anterior pituitary production of follistatin *in vitro*. *Endocrinology* **136** 4397–4406

Fink G (1995) The self-priming effect of LHRH: a unique servomechanism and possible cellular model for memory *Frontiers in Neuroendocrinology* **16** 183–190

Groome NP, Illingworth PJ, O'Brien M, Cooke I, Ganesan TS, Baird DT and McNeilly AS (1994) Detection of dimeric inhibin throughout the human menstrual cycle by two-site enzyme immunoassay *Clinical Endocrinology* **40** 717–723

Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather J and McNeilly AS (1996) Measurement of dimeric inhibin-B throughout the human menstrual cycle *Journal of Clinical Endocrinology and Metabolism* **81** 1401–1405

Harris TG, Dye S, Robinson JE, Skinner DC and Evans NP (1999) Progesterone can block transmission of the estradiol-induced signal for luteinizing hormone surge generation during the specific period of time immediately after activation of the gonadotropin-releasing hormone surge-generating system *Endocrinology* **140** 827–834

Kawakami S, Fujii Y and Winters SJ (2002) Paracrine regulation of FSH by follistatin in folliculostellate cell-enriched primate pituitary cell cultures *Endocrinology* **143** 2250–2258

Kendall SK, Saunders TL, Jin L, Lloyd RV, Glode LM, Nett TM, Keri RA, Nilson JH and Camper SA (1991) Targeted ablation of pituitary gonadotropes in transgenic mice *Molecular Endocrinology* **5** 2025–2036

Knight PG, Feist SA, Tannetta DS, Bleach ECL, Fowler PA, O'Brien M and Groome NP (1998) Measurement of inhibin-A (aβA dimer) during the oestrous cycle, after manipulation of ovarian activity and

during pregnancy in ewes *Journal of Reproduction and Fertility* **113** 159–166

Lewis CE, Morris JF and Fink G (1985) The role of microfilaments in the priming effect of LH-releasing hormone: an ultrastructural study using cytochalasin B *Journal of Endocrinology* **106** 211–218

McLeod BJ and McNeilly AS (1991) Manipulation of plasma FSH concentrations by administration of bFF changes patterns of LH secretion in seasonally anoestrous ewes *Animal Reproduction Science* **25** 115–124

McNeilly AS (1988) The control of FSH secretion *Acta Endocrinologica (Copenhagen)* **288** 31–40

McNeilly AS (2001) Lactational control of reproduction *Reproduction, Fertility and Development* **13** 583–590

McNeilly AS, O'Connell M and Baird DT (1982) Induction of ovulation and normal luteal function by pulsed injections of luteinizing hormone in anestrous ewes *Endocrinology* **110** 1292–1299

McNeilly AS, Souza CJH, Baird DT, Swanston IA, McVerry J, Crawford J, Cranfield M and Lincoln GA (2002) Production of inhibin A not B in rams: changes in plasma inhibin A during testis growth and regression and expression of inhibin/activin subunit mRNA and protein in the adult testis reproduction gene expression *Reproduction* **123** 827–835

McNeilly JR, Brown P, Clarke J and McNeilly AS (1991) GnRH modulation of gonadotrophins: evidence for differential effects on gene expression and hormone secretion *Journal of Molecular Endocrinology* **7** 35–43

McNeilly JR, Brown P, Crawford G, Huhtaniemi I and McNeilly AS (2001) Gonadotrophe ablation in fetal mice leads to a permanent reduction in adult plasma and pituitary LH *Reproduction Abstract Series* **27** Abstract 58

Mann GE, Campbell BK, McNeilly AS and Baird DT (1990) Effects of passively immunizing ewes against inhibin and oestradiol during the follicular phase of the oestrous cycle *Journal of Endocrinology* **125** 417–424

Markkula M, Kananen K, Klemi P and Huhtaniemi I (1996) Pituitary and ovarian expression of the endogenous follicle-stimulating hormone (FSH) subunit genes and the FSH beta-subunit promoter-driven herpes simplex virus thymidine kinase gene in transgenic mice; specific partial ablation of FSH-producing cells by antiherpes treatment *Journal of Endocrinology* **150** 265–273

Mercer JE, Clemens JA, Funder JW and Clarke IJ (1987) Rapid and specific lowering of pituitary FSHβ mRNA levels by inhibin *Molecular and Cellular Endocrinology* **53** 251–254

Mercer JE, Clemens JA, Funder JW and Clarke IJ (1989) Regulation of follicle-stimulating hormone-β and common α-subunit messenger ribonucleic acid by gonadotropin-releasing hormone and estrogen in the sheep pituitary *Neuroendocrinology* **50** 321–326

Mercer JE, Phillips DJ and Clarke IJ (1993) Short-term regulation of gonadotropin subunit mRNA levels by estrogen: studies in the hypothalamo–pituitary intact and hypothalamo–pituitary disconnected ewe *Journal of Neuroendocrinology* **5** 591–596

Molter-Gerard C, Fontaine J, Guerin S and Taragnat C (1999) Differential regulation of the gonadotropin storage pattern by gonadotropin-releasing hormone pulse frequency in the ewe *Biology of Reproduction* **60** 1224–1230

Mountford PS, Brandon MR and Adams TE (1992) Removal of the 3′ untranslated sequences dramatically enhances the transient expression of ovine follicle stimulating hormone beta gene messenger ribonucleic acid *Journal of Neuroendocrinology* **4** 655–658

Neill JD, Smith PF, Luque EH, Munzo de Toro M, Nagy G and Mulchahey JJ (1987) Detection and measurement of hormone secretion from individual pituitary cells *Recent Progress in Hormone Research* **43** 175–229

Nicol L, McNeilly JR Stridsberg M and McNeilly AS (2002a) Differential release of gonadotropins: the role of secretoganin II in the regulated and constitutive release of LH and FSH *84th Annual Meeting of the US Endocrine Society* P1–153

Nicol L, McNeilly JR Stridsberg M, Crawford JL and McNeilly AS (2002b) The influence of steroids and gonadotrophin-releasing hormone on the biosynthesis and secretion of secretogranin II and chromogranin A in relation to luteinising hormone secretion in LβT2 cells *Journal of Endocrinology* **174** 473–483

Padmanabhan V and McNeilly AS (2001) Is there an FSH-releasing factor? *Reproduction* **121** 21–30

Padmanabhan V, McFadden K, Mauger DT, Karsch FJ and Midgley AR (1997) Neuroendocrine control of FSH secretion 1. Direct evidence for separate episodic and basal components of FSH secretion *Endocrinology* **138** 424–432

Remohi J, Balmaceda JP, Rojas FJ and Asch RH (1988) The role of preovulatory progesterone in the midcycle gonadotropin surge, ovulation and subsequent luteal phase: studies with RU 486 in rhesus monkeys *Human Reproduction* **3** 431–435

Seuntjens E, Vankelcom H, Quaegebeur A, Van de Vijver V and Denef C (1999) Targeted ablation of gonadotrophs in transgenic mice affects embryonic development of lactotrophs *Molecular and Cellular Endocrinology* **150** 129–139

Sheng C, McNeilly AS and Brooks AN (1998) Distribution of estrogen receptor α and luteinizing hormone β subunit in the ovine pituitary gland during fetal development *Journal of Neuroendocrinology* **10** 713–718

Souza CJH, Campbell BK, Webb R and Baird DT (1997) Secretion of inhibin A and follicular dynamics throughout the estrous cycle in the sheep with and

without the Booroola gene (*Fec*^{B}) *Endocrinology* **138** 5333–5340

Spangers SA, West NB, Brenner RM and Bethea CL (1990) Regulation and localization of estrogen and progesterone receptors in the pituitary of steroid-treated monkeys *Endocrinology* **126** 1133–1142

Taragnat C, Bernier A and Fontaine J (1998) Gonadotropin storage patterns in the ewe during the estrous cycle or after long-term treatment with a GnRH agonist *Journal of Endocrinology* **156** 149–157

Thomas GB, Oldham CM, Hoskinson RM, Scaramuzzi RJ and Martin GB (1987) Effect of immunization against progesterone on oestrus, cycle length, ovulation rate, luteal regression and LH secretion in the ewe *Australian Journal of Biological Science* **40** 307–313

Thomas SG and Clarke IJ (1997) The positive feedback action of estrogen mobilizes LH-containing, but not FSH-containing secretory granules in ovine gonadotropes *Endocrinology* **138** 1347–1350

Thorburn GD, Cox RI, Currie WB, Restall BJ and Schneider W (1973) Prostaglandin F and progesterone concentrations in the utero–ovarian venous plasma of the ewe during the oestrous cycle and early pregnancy *Journal of Reproduction and Fertility* **18** 151–158

Tobin VA, Pompolo S and Clarke IJ (2001) The percentage of pituitary gonadotropes with immunoreactive oestradiol receptors increases in the follicular phase of the ovine oestrous cycle *Journal of Neuroendocrinology* **13** 846–854

Tsonis CG, McNeilly AS and Baird DT (1986) Measurement of exogenous and endogenous inhibin in sheep serum using a new and extremely sensitive bioassay for inhibin based on inhibition of ovine pituitary FSH secretion *in vitro*. *Journal of Endocrinology* **110** 341–352

Turgeon JL and Waring DW (1992) Functional cross-talk between receptors for peptide and steroid hormones *Trends in Endocrinology and Metabolism* **3** 360–365

Turgeon JL and Waring DW (1994) Activation of the progesterone receptor by the gonadotropin-releasing hormone self-priming signal pathway *Molecular Endocrinology* **8** 860–869

Turgeon JL and Waring DW (2001) Luteinizing hormone secretion from wild-type and progesterone receptor knockout mouse anterior pituitary cells *Endocrinology* **142** 3108–3115

Turgeon JL, Van Patten SM, Shyamala G and Waring DW (1999) Steroid regulation of progesterone receptor expression in cultured rat gonadotropes *Endocrinology* **140** 2318–2325

Wallace JM and McNeilly AS (1986) Changes in FSH and the pulsatile secretion of LH during treatment of ewes with bovine follicular fluid throughout the luteal phase of the oestrous cycle *Journal of Endocrinology* **111** 317–327

Watanabe T, Jeziorowski T, Wuttke W and Grube D (1993) Secretory granules and granins in hyperstimulated male rat gonadotropes *The Journal of Histochemistry and Cytochemistry* **41** 1801–1812

Watanabe T, Banno T, Jeziorowski T, Ohsawa Y, Waguri S, Grube D and Uchiyama Y (1998) Effects of sex steroids on secretory granule formation in gonadotropes of castrated male rats with respect to granin expression *Endocrinology* **139** 2765–2773