

Control and regulation of folliculogenesis – a symposium in perspective

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The Intervet Symposium 'Control and Regulation of Folliculogenesis' took place at the Annual Conference of the Society for the Study of Fertility in University College, Dublin in July, 1995. Five papers were presented, of which abstracts have been published*. Discussion centred on monotocous species and principally addressed the question 'How is the ovulatory follicle selected and nurtured?' Many follicles start to develop simultaneously, but most become atretic. During the luteal phase of the cycle, several waves of follicles are initiated, and although one in each wave gains dominance, all ultimately atrophy. The dominant follicle in the wave produced towards the end of the luteal phase ovulates. The biochemical characteristics of dominant and subordinate follicles were contrasted, and the endocrine environment in which they waxed and waned was analysed. Interaction of autocrine and paracrine effectors, principally with FSH, LH and their receptors, ultimately determines follicular destiny.

The initial stages of folliculogenesis occur independently of gonadotrophic hormones. Antral follicles initially become responsive to and then dependent on FSH. There are continual transient increases in FSH in cattle during the oestrous cycle and anoestrus which cause the recurrent emergence and development of cohorts of follicles. Jim Ireland (East Lansing) highlighted key endocrine and biochemical events involved in the emergence and development of these waves of follicles. Determination of antral follicle inventories, use of Indian ink marking of follicles, determination of the health of follicles after sequential ovariectomy, and measurement of utero-ovarian venous concentrations of oestradiol led to the hypothesis of turnover of dominant follicles during the oestrous cycle in heifers. Subsequently, daily ultrasound scanning of ovaries confirmed that generally two (but sometimes only one) non-ovulatory follicle waves occur in the luteal phase of the cycle, before the development of the ovulatory dominant follicle that occurs after luteal regression (Fig. 1). The key question is 'What is the critical step initiating this sequence of events?' Jim Ireland hypothesized that the turnover of dominant follicles during the oestrous cycle is regulated by the differential response of selected and unselected follicles in the cohort to alterations in patterns of secretion of gonadotrophins, which, in turn, result in a differential production of intrafollicular stimulatory or inhibitory factors that control selection, dominance and atresia.

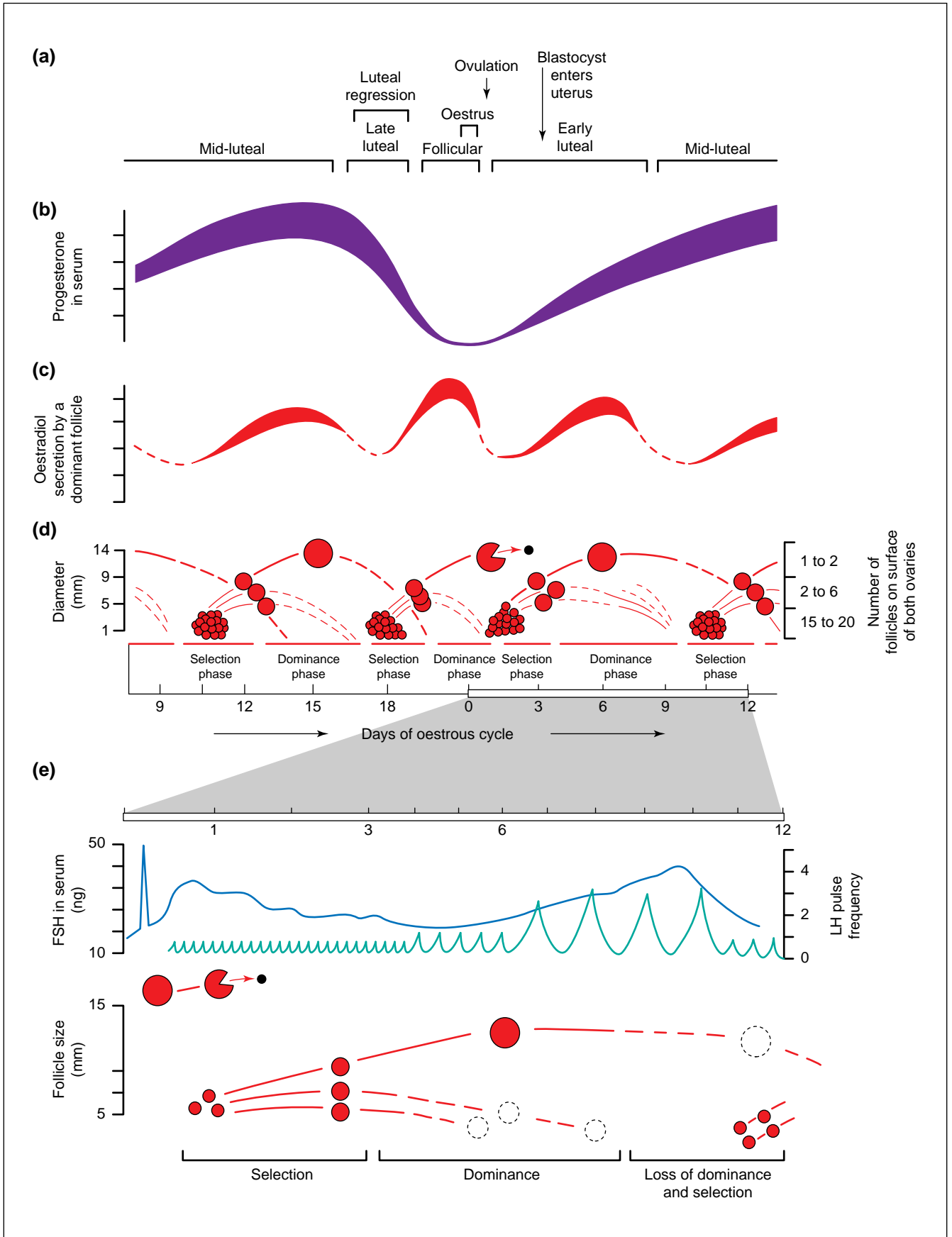
The role of growth factors

Inhibins, activin, insulin-like growth factor I (IGF-I) and their binding proteins have direct and indirect effects on granulosa and theca cells that can modulate follicular development and steroidogenesis. Inhibins have both autocrine and paracrine effects; they increase LH-induced androgen synthesis in thecal

cells, and their production is stimulated by steroids and FSH. Thus, there may be a local feedback loop within individual follicles involving a sequential change of inhibins, activins and their binding proteins, which ultimately determines the different fates of the selected and unselected follicles that develop in the same systemic environment of gonadotrophins and growth hormone. In the light of these findings, the working hypothesis was modified such that growth of the dominant follicle and enhanced oestradiol production are characterized by relatively low concentrations of inhibin, activin and IGF-binding proteins, and perhaps high concentrations of IGFs. In contrast, onset of atresia and loss of capacity of follicles to produce oestradiol are characterized by relatively high concentrations of inhibin, activin or IGF-binding proteins. This hypothesis was tested using quantitative immunoblot analysis to measure alterations in intrafollicular concentrations of inhibins, activin, IGF-I or IGF-binding protein 2 (IGFBP-2) during selection (days 2–4 of the oestrous cycle), dominance (day 5), and loss of dominance phases (days 8–12) for the first-wave dominant non-ovulatory follicle of cattle (Fig. 2).

Further to the changes illustrated (Fig. 2), subluteal phase concentrations of progesterone (provided through use of progesterone intravaginal devices or ear implants of progestagen in the absence of the cow's own corpus luteum) result in an increase in LH pulse frequency, suppression of turnover of follicle waves and the sequential FSH rises, and the formation of a persistent dominant follicle that maintains its oestrogenic activity. This persistent dominant follicle lacks the increase in inhibin (34 kDa) and IGFBP-2 that occurs in the atretic dominant follicle. These findings imply that the loss of LH receptors in the dominant follicle that is about to become 'oestrogen-inactive' is a key step in the onset of atresia; subtle changes in LH pulse frequency may be sufficient to trigger loss of LH receptors in this terminally differentiated follicle which can now undergo only atresia (low LH pulse frequency) or luteinization (high LH pulse frequency). This view is also in accord with known changes in steroidogenic enzymes: the decrease in

* Baird (1995), Driancourt (1995), Ireland *et al.* (1995), McNeilly *et al.* (1995), and Padmanabhan (1995)



oestrogen production by the first dominant follicle of cattle is not due to a loss of activity of the three major steroidogenic enzymes, P450-aromatase, 3 β -hydroxysteroid dehydrogenase and P450-17 α -hydroxylase (Carriere *et al.*, 1995). The question is 'What regulates these subtle changes in LH pulse frequency and loss of LH receptors resulting in the dramatic loss of oestrogen production?'

Since intrafollicular contents of inhibin (34 kDa), activin and IGFBP-2 are inversely related to oestradiol-producing capacity of dominant and subordinate follicles (Fig. 2), these findings, coupled with previous studies *in vitro* showing inhibitory roles for inhibin, activin and IGF-binding proteins on FSH-stimulated oestradiol production (reviewed by Ying, 1988; Ui *et al.*, 1989; Knight, 1991; Mather *et al.*, 1992; Findlay, 1993; and Erickson *et al.*, 1994), indicate that growth factors have key local negative roles on gonadotrophic action that may be involved in controlling the processes of follicle selection and dominance. Current knowledge suggests that, during the bovine oestrous cycle, differential changes in patterns of secretion of FSH and LH stimulate alterations in intrafollicular concentrations of inhibin (34 kDa), activin and IGFBP-2 which, in turn, result in selection, dominance and loss of dominance of dominant follicles.

Role of other follicular fluid proteins

In follicular fluid, many proteins other than inhibins, activin, IGF-I and their respective binding proteins may be important in selection, dominance and steroidogenesis. The impact of these factors is difficult to examine in intact animals because of interactions between the putative direct ovarian and systemic feedback effects of such proteins on gonadotrophin secretion. Hence, Marc Driancourt (Nouzilly) reported work *in vitro*, in which one parameter measured was aromatase activity in the walls of ovine and bovine follicles. Another marker studied was cell proliferation assessed from [³H]thymidine incorporation. Interactions between follicles were tested using media

conditioned by large follicles or ovarian venous serum. Neither conditioned medium (ovine) nor ovarian venous serum (ovine or bovine) contained compounds that affected aromatase activity. In sheep, co-culture of large healthy follicles with smaller follicles did not affect basal or FSH-stimulated granulosa cell proliferation. Hence, using these tests, between-follicle interactions were not demonstrated in sheep and cattle. In contrast, in these species, follicular fluid from dominant (but not small or atretic) follicles contained an aromatase inhibitor that showed dose dependency, was unaffected by the presence of FSH, was retained in charcoal-treated follicular fluid and was not present in follicle-conditioned medium. This inhibitor was produced by granulosa cells and stored in follicular fluid. Two possible candidate molecules were identified by image analysis of two-dimensional PAGE samples of newly synthesized proteins in follicular fluid (active material) versus incubation medium (inactive material). After HPLC, activity was assigned to a slightly acidic 90 kDa protein that had physicochemical features like those of heat shock protein 90 (hsp 90). Indeed, using western blotting, an hsp 90-like protein can be detected in follicular fluid but not in serum.

Because current knowledge of ovarian proteins is restricted to a few families (IGF-binding proteins, inhibin/activin, proteoglycans), the array of proteins newly synthesized by ovarian follicles was characterized by two-dimensional PAGE and image analysis. About 100 proteins could be detected following incubation for 24 h with [³⁵S]methionine. No qualitative difference related to follicle status (size, atresia, Booroola genotype) could be detected. In contrast, quantitative differences were identified related to size (three spots), atresia (five spots) and Booroola genotype (six spots). Western blotting showed that two spots were tissue inhibitor of metalloproteinase (TIMP). Two other spots probably represented IGFBP-3 and IGFBP-4. Unequivocal elucidation of the identity of all these proteins and the factors that regulate their function will undoubtedly lead to a better understanding of the mechanisms controlling ovarian function.

Fig. 1. Follicular events based on analysis of oestradiol:progesterone ratios and gonadotrophins in follicular fluid of all follicles >6 mm in diameter on different days of the bovine oestrous cycle (Ireland and Roche, 1982, 1983a,b). (a) The stage of the bovine oestrous cycle when selection, dominance and loss of dominance occur during each developmental wave of turnover of follicles (Ireland, 1987; Ireland and Roche, 1987). (b) Activity of the corpus luteum in terms of its progesterone output. (c) Correlation of oestradiol production with the development of the dominant follicle in each wave. (d) The waves of follicular development throughout the cycle. Cohorts of follicles arise, and while one is selected to become dominant, the others become atretic. In the early and mid-luteal phases, the dominant follicle also atrophies, but in the cohort that develops in the late luteal phase, the dominant follicle ovulates. (e) Events that take place in the different phases (selection, dominance, loss of dominance) in turnover of the first-wave dominant follicle (days 0–12 of the cycle, corresponding to the bar on the axis in (d)). In each wave of follicles coincident with a transient increase of FSH (blue line), two to five medium size follicles develop, from which one is selected to continue growing between day 2 and day 3 of the cycle to become the dominant follicle by day 4 or 5. Selection is preceded by a two- to fourfold transient increase in basal concentrations of FSH in serum (blue line), whereas selection of the dominant follicle begins while concentrations of FSH are declining (blue line) (Ireland and Roche, 1983a,b; Adams *et al.*, 1992; Sunderland *et al.*, 1994) and the pulse amplitude of LH is increasing (green line) (Rahe *et al.*, 1980; Savio *et al.*, 1993; Cupp *et al.*, 1995). The subordinate follicles (shown as dashed circles) become 'oestrogen-inactive' (greater concentrations of progesterone than oestradiol in follicular fluid), lose FSH receptors (see Fig. 2) and undergo atresia during the dominance phase while serum FSH is low (blue line). Simultaneously, the dominant follicle continues to grow, gains LH receptors (see Fig. 2) on granulosa cells, and secretes greater amounts of oestradiol (c), despite a reduction in the number of FSH receptors and a low concentration of FSH in serum (blue line). The dominant follicle then becomes dependent on LH, and its final fate, atresia or ovulation, is determined by LH secretion. The end of each dominance phase is preceded by a loss of FSH and LH receptors and oestrogen production by the dominant follicle (see Fig. 2), and a decrease in LH pulse frequency may also occur (green line), (Rahe *et al.*, 1980; Savio *et al.*, 1993; Cupp *et al.*, 1995). In addition, loss of dominance coincides with the emergence of a new wave of follicles, which is again preceded by a transitory increase in FSH (blue line). Red circles indicate that follicles are 'oestrogen-active' (oestradiol concentration in follicular fluid greater than that of progesterone); dashed circles indicate that follicles are 'oestrogen-inactive'. Changes in patterns of LH frequency (green line) do not represent actual values, but indicate only a frequency shift.

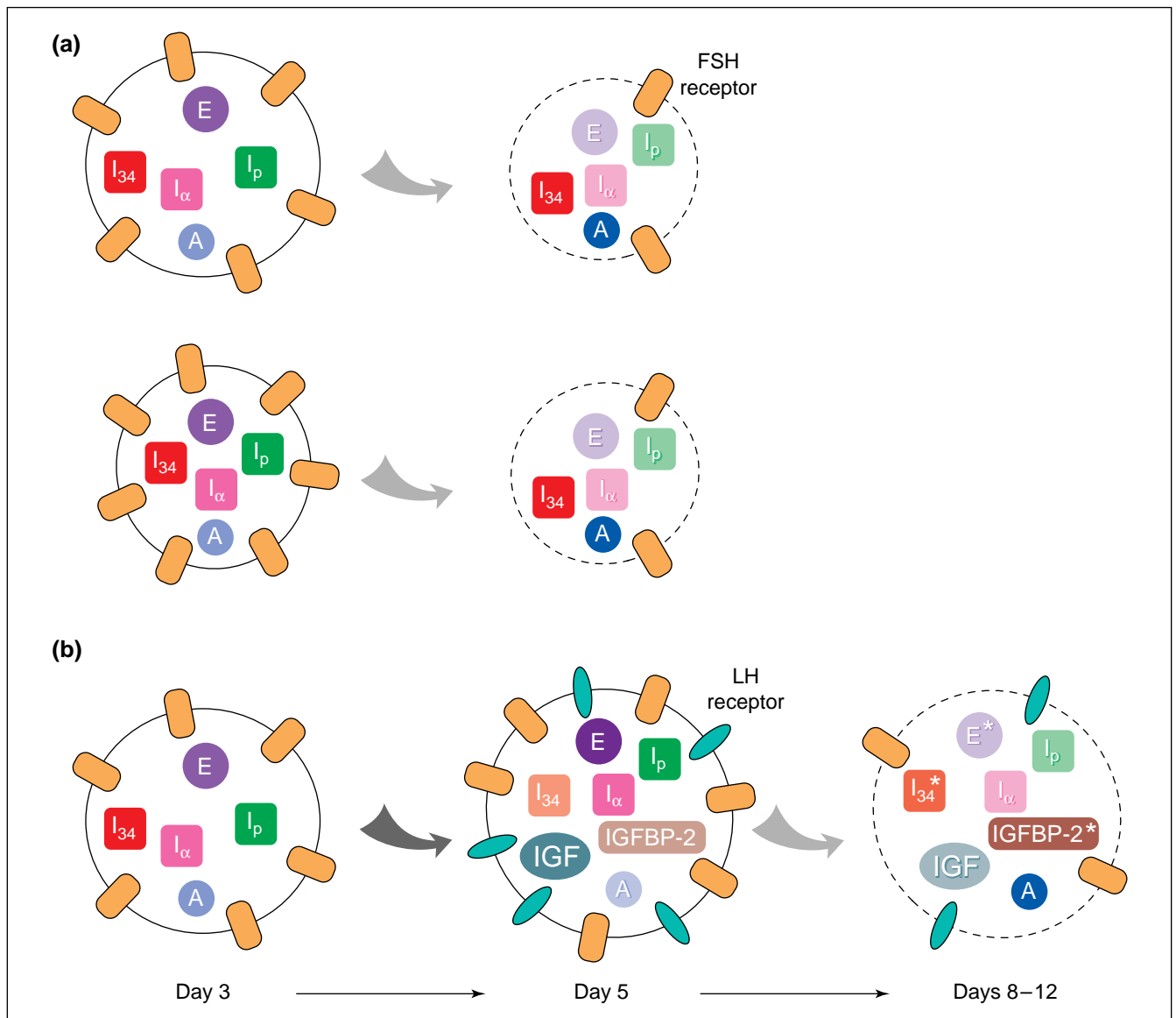


Fig. 2. (a) Differences between follicles that become (a) subordinate or (b) dominant during the first wave of recruitment in the bovine oestrous cycle. (a) Ultrasound analysis was used to monitor follicular development, radioimmunoassay to measure concentrations of oestradiol (E), a two-site immunoradiometric assay to measure concentrations of activin (A) and quantitative immunoblot analysis to measure concentrations of the different forms of inhibin (I) in follicular fluid (M. Mihm, J. Ireland, P. Knight and J. Roche, unpublished). (b) Ultrasound analysis was used to monitor follicular development, radioimmunoassay to measure oestradiol and insulin-like growth factor I (IGF), and quantitative immunoblot analysis to measure insulin-like growth factor-binding protein 2 (IGFBP-2) and the different forms of inhibin in follicular fluid (R. de la Sota, T. Good, J. Ireland, J. Ireland and W. Thatcher, unpublished). Quantitative immunoblot analysis was used to determine activin in a separate study, using luteal phase 'oestrogen-active' (greater concentrations of oestradiol than progesterone in follicular fluid) and 'oestrogen-inactive' (greater concentrations of progesterone than oestradiol in follicular fluid) follicles collected from a local abattoir (T. Good, J. Ireland and J. Ireland, unpublished). Ireland and Roche (1983a) determined FSH and LH receptors in a previous study.

On day 3, all follicles were 'oestrogen-active', whereas by day 5 this was true only of the dominant follicle (b). Subordinate follicles (those not becoming dominant, dotted circles) (a) on day 5 were 'oestrogen-inactive'. In the follicle that became dominant by day 5 (b), production of oestradiol was enhanced simultaneously with a marked decline in inhibin (34 kDa) (I₃₄) compared with 'oestrogen-active' follicles on day 3 (left column) or 'oestrogen-inactive' subordinants on day 5 (a), dotted circles). In the dominant follicle on day 5 (b), concentrations of inhibin precursors (I_p) remained unchanged compared with 'oestrogen-active' follicles on day 3. LH receptors also develop on granulosa cells. From day 3 to day 5 in subordinate follicles ((a), dotted circles), activin increased but oestradiol, inhibin precursors and inhibin α -subunits (I _{α}) showed a precipitous decline compared with the dominant follicle on day 5(b), or with 'oestrogen-active' follicles on day 3. In subordinate compared with 'oestrogen-active' follicles on day 3 (a), inhibin (34 kDa) was unaltered, but activin had increased in subordinate follicles. Numbers of FSH receptors are markedly lower in subordinate follicles compared with the dominant follicle on day 5 (b) or 'oestrogen-active' follicles on day 3 (Ireland and Roche 1983a,b). Treatment with relatively low doses of FSH during days 2–3 unexpectedly prolonged the

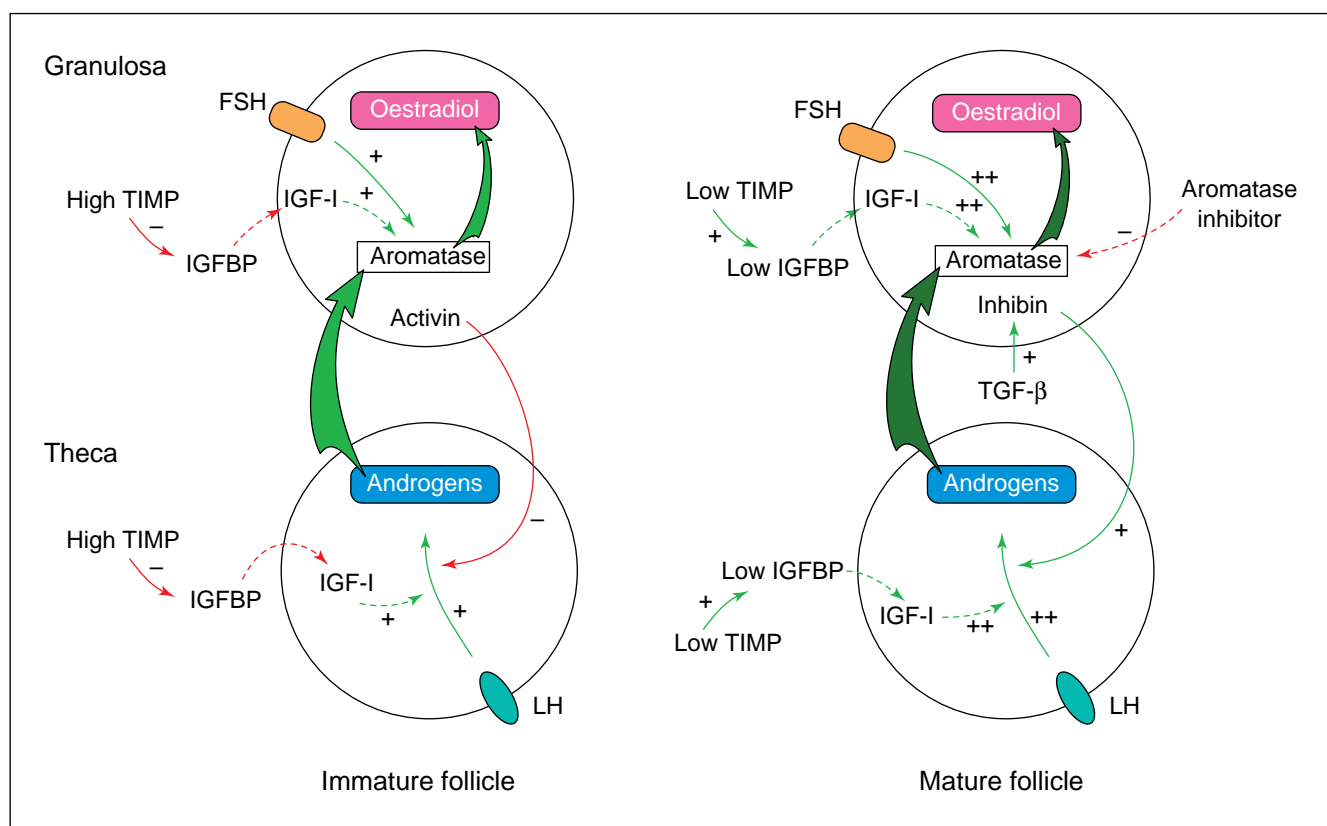


Fig. 3. A model for the interaction between various growth factors, steroidogenesis and the putative aromatase inhibiting peptide in follicular fluid in theca and granulosa cells from immature and mature follicles. Immature follicles are characterized by (i) high concentrations of tissue inhibitor of metalloproteinase (TIMP), (ii) high concentrations of insulin-like growth factor binding proteins (IGFBP) and (iii) a high activin:inhibin ratio in follicular fluid. As TIMP is likely to inhibit the proteolytic cleavage of IGF-I from IGFBP, the high TIMP production observed in immature follicles should limit the bioavailability of IGF-I and its synergy with LH to promote thecal androgen production, and with FSH to stimulate aromatase activity in granulosa cells. In addition, the high activin concentration will also reduce androgen production by thecal cells (Hillier *et al.*, 1991). In contrast, mature follicles feature (i) decreased production of TIMP, (ii) decreased amounts of IGFBP through a decrease in IGFBP-2 and IGFBP-4 (Monger *et al.*, 1993) and (iii) high inhibin:activin ratios in follicular fluid. Inhibin acts on thecal cells to promote LH-stimulated androgen production (Findlay, 1993). The high IGF-I availability resulting from the decreased production of TIMP, and the decreased amounts of IGFBP will also stimulate thecal androgen production. These high amounts of androgens are continuously transferred to granulosa cells to FSH (McNatty and Henderson, 1987) and to the amplifying effects of IGF-I on FSH action. High thecal androgen production and high aromatase activity of granulosa cells explain the high oestradiol output of the dominant follicle. At this stage, an aromatase inhibitor (hsp 90?) is produced. Its role would be to maintain oestradiol production within limits compatible with fertilization and early embryonic development.

Fig. 2 continued

selection phase, inhibited dominance and blocked all the described changes in amounts of oestradiol and the different inhibin forms that spontaneously occur during selection on days 3–5 (M. Mihm, J. Ireland, P. Knight and J. Roche, unpublished). However, the increase in activin that occurred in subordinate follicles was not blocked. This study showed that FSH has a role in regulation of inhibins within the follicle in the intact animal. It also implied that the decline in serum concentrations of FSH after day 1 (see Fig. 1), rather than the later increase in FSH in serum, triggers the end of selection and the beginning of dominance during turnover of the first-wave dominant follicle.

The dominant follicle from the first wave changes as it loses dominance after day 5 (b). This follicle on day 5 was 'oestrogen-active', whereas most dominant follicles on day 8 and all on day 12 were 'oestrogen-inactive'. During days 5–8 (b), the first-wave dominant follicle loses FSH and LH receptors and its capacity to produce oestradiol and inhibin precursors (I_p). Despite the loss of oestradiol production and decline in production of inhibin precursors, amounts of inhibin (34 kDa), activin, and insulin-like growth factor binding protein 2 increased markedly. When a combination of low progesterone and prostaglandin was used to increase LH pulsatility, which prevents loss of dominance of the first-wave dominant follicle, amounts of oestradiol, inhibin (34 kDa) and insulin-like growth factor binding protein 2 did not decrease (*).

Density of colour reflects the amount of each intrafollicular factor.

Table 1. Different parameters of LH and FSH secretion and regulation in the anterior pituitary gland

Parameter	FSH	LH
Pituitary content ratio	1	40
Plasma concentrations (ng ml ⁻¹)	0.5–6.0	0.5–100.0
Half-life in plasma (min)	150	20
GnRH regulation of secretion	Partial	Absolute
mRNA β -subunit stability	Unstable	Stable
Main regulatory control	Negative feedback by oestradiol and inhibin	GnRH secretion
Secretory mode	Constitutive	Regulated

Modified from McNeilly (in press)

A hypothesis about how these factors might interact (Fig. 3) was presented by Marc Driancourt.

Control of FSH secretion

FSH is the key hormone stimulating the emergence of waves of follicles, and its decline is associated with selection of a dominant follicle, which then becomes dependent on LH for its final fate when concentrations of FSH are minimal. There is great divergence in the patterns of secretion of the two gonadotrophic hormones, yet they are produced in the same cell under the control of a single releasing hormone. Alan McNeilly (Edinburgh) discussed this paradox in which the differential release of LH and FSH from the same gonadotroph requires different intracellular control mechanisms. The synthesized gonadotrophins are stored in secretory granules within the cytoplasm, and are secreted differentially by exocytosis. There appears to be prolonged storage of LH during the cycle, but only short-term storage of FSH, and the content of FSH in the sheep anterior pituitary is only 2–3% of that of LH. During the oestrous cycle of ewes, up to 50% of FSH, but only 1–5% of LH, is released each day.

GnRH binds to specific receptors on gonadotrophs, and triggers the release of intracellular Ca²⁺, causing a transient release of both FSH and LH. The gonadotrophs become insensitive to GnRH, and the pulsatile nature of its release is necessary to prevent downregulation of its receptor. GnRH also results in episodic release of LH, the frequency of which is determined by progesterone or oestradiol feedback. FSH release is not pulsatile in character, and generally not co-incident with LH release.

In addition, treatment of ewes with a GnRH antagonist results in an immediate decrease in LH pulse frequency, but the effect on FSH is delayed for 48–72 h. During the oestrous cycle of ewes, the patterns of secretion of LH and FSH are different, with an increase in LH pulse frequency of 1 per hour during pro-oestrus, while there is a coincident decrease in FSH due to the increase in oestradiol and perhaps inhibins. There is a major depletion of LH after the preovulatory surge. Thus, LH release is a regulated pathway mediated by GnRH action, whereas FSH release is a more constitutive pathway, in which synthesis is followed by release rather than by storage.

Inhibins and oestradiol act directly at the anterior pituitary to decrease expression of the gene encoding the FSH subunit.

They act to reduce both transcription and stability of mRNA, effects which override GnRH action on FSH release. Oestradiol causes a major decrease in FSH, while LH is initially decreased and then increased. Inhibins suppress FSH, without affecting LH. Immunoneutralization of either inhibin or oestradiol during the oestrous cycle increases FSH, and combined neutralization of both is equivalent to ovariectomy (see Table 1).

FSH isoforms

The differential regulation and secretion of LH and FSH by both GnRH and an activin-mediated autocrine–paracrine system raises questions regarding the types and functions of secretory granules within gonadotrophs, the types and functions of gonadotroph subpopulations, and the regulation and control of activin and inhibin and other peptides involved in autocrine or paracrine control. The GnRH-independent release of FSH is probably due to selective stimulation of FSH synthesis by activin in association with sequential changes in the activin-binding protein, follistatin. Thus, paracrine mechanisms within the pituitary that selectively affect FSH are partially responsible for the differential release of FSH and LH from the gonadotroph. However, not only are there differences in the secretory patterns of LH and FSH from the pituitary, but there are also variations in the structure of the individual hormones, and these were considered by Vasantha Padmanabhan (Ann Arbor). Evidence for the existence and importance of gonadotrophin heterogeneity is now compelling. Like many other protein hormones, FSH consists of a family of related isoforms. The isoforms can be separated on the basis of size, charge, biological to immunological potency relationships, and circulatory clearance rates (Chappel *et al.*, 1983; Blum and Gupta, 1989; Robertson, 1989; Beitins and Padmanabhan, 1991). A mixture of circulating gonadotrophin isoforms reaches the target tissues to influence a variety of biological endpoints: cellular growth, development, steroidogenesis and synthesis of proteins. Relative proportions of the various gonadotrophin isoforms within the circulation have the potential to cause qualitatively different endocrine effects on target tissues.

Although heterogeneity has been studied extensively in the pituitary, regulation of FSH heterogeneity in the circulation is poorly understood, due in part to limitations imposed by the relatively low concentrations of circulating FSH. Results from recently developed, sensitive, *in vitro* bioassays for FSH have provided indirect evidence for the regulation of circulating bioactive FSH (B-FSH) heterogeneity: the ratio B-FSH:I-FSH (immunoreactive FSH) varies with endocrine status. If the potential interferences in the measurement system by substances in the serum are considered, the existence of heterogeneity in serum requires that the changes in B-FSH:I-FSH ratios are accompanied by changes in the distribution pattern of the isoforms.

Although the factors affecting final isoform distribution within the circulation are multifaceted and complex, endocrine changes clearly regulate the proportions of FSH isoforms both within the pituitary and in the peripheral circulation (Fig. 4). Various techniques have been used to correlate qualitative differences in the content of FSH in the pituitary in several species with age, sex and stage of the oestrous cycle (Chappel *et al.*, 1983; Blum and Gupta, 1989; Robertson, 1989; Beitins

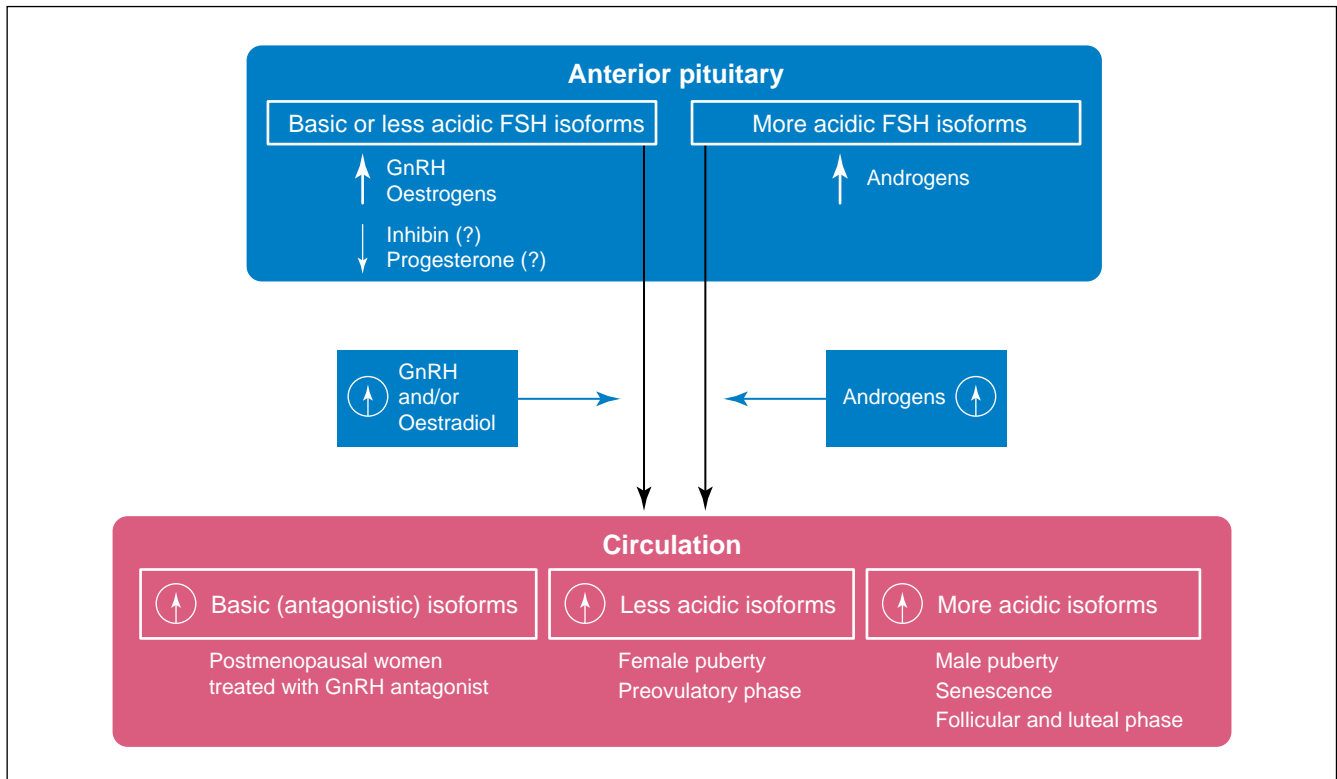


Fig. 4. The neuroendocrine regulation of FSH heterogeneity. Hormonal factors influencing synthesis and release of the different isoforms from the pituitary gland are summarized as well as the physiological conditions associated with their relative abundance in the systemic circulation.

and Padmanabhan, 1991). A majority of the studies show that oestradiol is associated with increased release of less acidic isoforms of FSH, and androgens are associated with increased release of more acidic isoforms of FSH. Studies in ovariectomized, nutritionally growth-retarded lambs have shown that GnRH, in the absence of ovarian feedback, does not alter the heterogeneity of pituitary or circulating FSH (Hassing *et al.*, 1993).

Although mounting evidence documents the existence of FSH heterogeneity and its regulation by the endocrine milieu, before attributing physiological significance to heterogeneity, it is essential to establish that: (i) changes in FSH heterogeneity are of sufficient magnitude to alter the net potency of the hormone, and/or (ii) the various isoforms differ in their functional attributes. Major shifts in the distribution pattern of circulating FSH isoforms, and an associated increase in FSH bioactivity, were shown during the onset of puberty (experimentally induced with GnRH) in sheep (Padmanabhan *et al.*, 1992) (Fig. 4) and during the preovulatory phase in normal cyclic women (periods characterized by high frequency GnRH input and increased oestradiol concentrations) (Padmanabhan *et al.*, 1988). The magnitude of these changes appears sufficient to influence the net potency of the hormone, and elicit important biological consequences.

FSH isoforms differ in their immunopotency, biopotency, receptor binding ability and circulatory clearance (Chappel *et al.*, 1983; Blum and Gupta, 1989; Robertson, 1989; Beitins and Padmanabhan, 1991). In general, less acidic FSH isoforms are

more bioactive in bioassays *in vitro*, but appear to have a shorter half-life. This opens up very interesting alternatives: do these seemingly opposite effects *in vitro* and *in vivo* simply cancel one another or can target cells respond differentially to the pattern of imposed FSH signals, much as T cells respond differentially to small changes in ligand (Marx, 1995)? Studies characterizing patterns of FSH secretion from the pituitary have shown that FSH is secreted in discrete pulses (Padmanabhan *et al.*, 1994). As FSH secreted in pulses is biologically more potent, and rapid changes in functional interactions between FSH and its receptor are possible, the pulsatile input of biopotent FSH isoforms, such as that occurring during the onset of puberty and the preovulatory period, could provide a potent and necessary stimulus for development of the preovulatory follicle in spite of faster clearance.

In addition to changes in FSH heterogeneity having an impact on the final potency, the functional attributes of the various FSH isoforms may differ. The molecular modifications underlying the changes in the distribution of FSH isoforms have the potential to lead to changes in affinity for classic FSH receptors and/or crossreactivity with non-FSH receptors. Studies using chemically deglycosylated (DG-oFSH) and intact ovine FSH (oFSH) in a bioassay using cAMP, oestradiol and inhibin production as endpoints (Padmanabhan *et al.*, 1993) have shown differential effects on these parameters, supporting the hypothesis that the various isoforms can exert differential effects. When cAMP is used as an endpoint, oFSH increases production, whereas

DG-oFSH does not. In terms of oestrogen production, DG-oFSH is less potent than oFSH. These studies, although preliminary, suggest that changes in FSH heterogeneity have the potential to provide an exquisitely fine-tuned mechanism to control gonadal function. If heterogeneity proves to be biologically important, it should be possible to design FSH isoforms for desired functions: for example, less biopotent, long-lived isomers for growth and maintenance of reproductive organs, more biopotent and short-lived isomers for acute dynamic events, such as pubertal onset and ovulation, and antagonistic FSH isoforms for potential use in contraception.

Clinical applications

The final application of research is to use emerging knowledge to develop more effective practical applications to regulate fertility. Fertility regulation consists generally of induction of ovulation in anovulatory conditions, regulation of time of ovulation to allow avoidance or optimization of time of insemination, or increased ovarian stimulation for oocyte or embryo recovery. This topic was reviewed by David Baird (Edinburgh).

In monotocous species, like humans, a single large dominant follicle emerges for further development a few days before ovulation. The process by which this single preovulatory follicle acquires dominance over other follicles is complex, involving both endocrine regulation of FSH and LH as well as paracrine control of the responsiveness of the follicle to gonadotrophins. In clinical medicine, gonadotrophins are used to induce ovulation and, hence, restore fertility in hypogonadotrophic states, as well as to stimulate the formation of multiple follicles in techniques of assisted conception, including IVF. The goals of the two types of treatment, therefore, are quite different. In anovulatory infertility, the aim is to induce a single ovulation by simulating the endocrine changes that occur in the normal ovarian cycle. Until recently, gonadotrophins used clinically, which were extracted from the urine of postmenopausal women (hMG), were impure and contained a mixture of LH and FSH. The synthesis of FSH and LH by recombinant DNA techniques has provided a means of testing therapeutic strategies that may restrict the number of ovulatory follicles and, hence, reduce the risk of multiple births. Similar techniques can be used to produce synthetic gonadotrophins with characteristics designed for other purposes. For example, by fusing the carboxy-terminal extension of the β -subunit of hCG on to the β -subunit of FSH, a very long-acting gonadotrophin with FSH activity is obtained which could be used for stimulation of multiple follicular development before IVF and in the stimulation of spermatogenesis in hypogonadotrophic men. Use of these recombinant techniques to produce chimaeric molecules should provide the means to prepare gonadotrophins with characteristics designed for specific therapeutic uses.

References

Key references are identified by asterisks.

- Adams GP, Matteri RL, Kastelic JP, Ko JCH and Ginther OJ (1992) Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers *Journal of Reproduction and Fertility* **94** 177–188
- Baird DT (1995) Clinical applications of gonadotrophins in stimulating follicle activity *Journal of Reproduction and Fertility Abstract Series* **15** Abstract 55
- Beitins IZ and Padmanabhan V (1991) Bioactivity of gonadotropins *Endocrinology and Metabolism Clinics of North America* **20** 85–120
- Blum WF and Gupta D (1989) Follicle stimulating hormone structure–function relationships. In *Microheterogeneity of Glycoprotein Hormones* pp 127–148 Ed. BA Keel and HE Grotjan, Jr CRC Press, Boca Raton, FL
- Carriere PD, Harvey D and Cooke GM (1995) Decreased oestradiol production in the bovine first-wave dominant follicle is not due to a decrease in P₄₅₀ aromatase, P₄₅₀ 17 α -hydroxylase or 3 β -hydroxysteroid dehydrogenase activity *Journal of Reproduction and Fertility Abstract Series* **15** Abstract 77
- Chappel SC, Ulloa-Aguirre A and Coutifaris C (1983) Biosynthesis and secretion of follicle-stimulating hormone *Endocrine Reviews* **4** 179–211
- Cupp AS, Stumpf TT, Kojima FN, Werth LA, Wolfe MW, Roberson MS, Kitto RJ and Kinder JE (1995) Secretion of gonadotrophins change during the luteal phase of the bovine oestrous cycle in the absence of corresponding changes in progesterone or 17 β -oestradiol *Animal Reproduction Science* **37** 109–119
- Driancourt MA (1995) Local control of follicular growth by ovarian follicles *Journal of Reproduction and Fertility Abstract Series* **15** Abstract 54
- Erickson GF, Nakatani A, Liu XJ, Shimasaki S and Ling N (1994) Role of insulin-like growth factor (IGF) and IGF-binding proteins in folliculogenesis. In *Molecular Biology of the Female Reproductive System* Ed. JK Findlay. Academic Press, San Diego, CA
- *Findlay JK (1993) An update on the role of inhibin, activin, and follistatin as local regulators of folliculogenesis *Biology of Reproduction* **48** 15–23
- Hassing JM, Klette GB, l'Anson H, Wood RL, Beitins IZ, Foster DL and Padmanabhan V (1993) Pulsatile administration of GnRH does not alter the FSH isoform distribution pattern of pituitary or circulating FSH in nutritionally growth-restricted ovariectomized lambs *Endocrinology* **132** 1527–1536
- Hillier SG, Yong EL, Illingworth PJ, Baird DT, Schwall RH and Mason AJ (1991) Effect of recombinant activin on androgen synthesis in cultured human thecal cells *Journal of Clinical Endocrinology and Metabolism* **72** 1206–1211
- Ireland JJ (1987) Control of follicular growth and development *Journal of Reproduction and Fertility* **34** 39–54
- Ireland JJ and Roche JF (1982) Development of antral follicles in cattle after prostaglandin-induced luteolysis: changes in serum hormones, steroids in follicular fluid and gonadotropin receptors *Endocrinology* **111** 2077–2086
- Ireland JJ and Roche JF (1983a) Development of nonovulatory antral follicles in heifers: changes in steroids in follicular fluid and receptors for gonadotropins *Endocrinology* **112** 150–156
- Ireland JJ and Roche JF (1983b) Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: changes in concentration of hormones in follicular fluid and specific binding of gonadotropins to follicles *Journal of Animal Science* **57** 157–167
- *Ireland JJ and Roche JF (1987) Hypotheses regarding development of dominant follicles during a bovine estrous cycle. In *Follicular Growth and Ovulation Rate in Farm Animals* pp 1–18 Ed. JF Roche and D O'Callaghan. Martinus Nijhoff Publishers, Dordrecht/Boston/Lancaster for the Commission of the European Communities
- Ireland JJ, Ireland JLH, Good TEM, Knight PG, Sunderland SJ, Mihm M, Boland MP, Roche JF, de la Sota RL, Thatcher WW, Thompson D and Martin F (1995) Regulation of dominant follicle turnover during the bovine oestrous cycle *Journal of Reproduction and Fertility Abstract Series* **15** Abstract S1
- Knight PG (1991) Identification and purification of inhibin and inhibin-related proteins *Journal of Reproduction and Fertility* **43** 111–123
- McNatty KP and Henderson KM (1987) Gonadotrophins, fecundity genes, and ovarian follicular function *Journal of Steroid Biochemistry* **27** 365–373
- McNeilly AS The control of FSH secretion *Journal of Reproduction and Development* (in press)
- McNeilly AS, Brooks J, McNeilly JR and Brown P (1995) Synthesis and release of FSH *Journal of Reproduction and Fertility Abstract Series* **15** S2
- Marx J (1995) The T cell receptor begins to reveal its many facets *Science* **267** 459–460
- Mather JP, Woodruff TK and Krummen LA (1992) Paracrine regulation of reproductive function by inhibin and activin *Proceedings of the Society for Experimental Biology and Medicine* **201** 1–15
- *Monger P, Monniaux D, Pisselet C and Durand P (1993) Changes in insulin-like growth factor-I (IGF-I), IGF-II and their binding proteins during growth and atresia of ovine ovarian follicles *Endocrinology* **132** 1438–1446
- Padmanabhan V (1995) Neuroendocrine control and physiologic relevance of FSH heterogeneity *Journal of Reproduction and Fertility Abstract Series* **15** Abstract S3

- *Padmanabhan V, Lang LL, Sonstein J, Kelch RP and Beitins IZ (1988) Modulation of serum follicle-stimulating hormone bioactivity and isoform distribution by estrogenic steroids in normal women and in gonadal dysgenesis *Journal of Clinical Endocrinology and Metabolism* **67** 465–473
- Padmanabhan V, Miehler CD, Borondy M, I'Anson H, Wood RL, Landefeld TD, Foster DL and Beitins IZ (1992) Circulating bioactive follicle-stimulating hormone and less acidic follicle-stimulating hormone isoforms increase during experimental induction of puberty in the female lamb *Endocrinology* **131** 213–220
- Padmanabhan V, Kersey KA, Sairam MR, Cole LA, Ireland JJ and Beitins IZ (1993) A multiple endpoint bioassay to assess physiological relevance of FSH heterogeneity *75th Annual Meeting of the Endocrine Society* Abstract 1820
- Padmanabhan V, McFadden KL, Evans NP, Dahl GE, Mauger DT and Karsch FJ (1994) Pulsatile FSH secretion in the ovariectomized ewe is controlled by both GnRH-dependent and GnRH-independent mechanisms *Society of Neuroscience Abstracts* **20** 1058
- Rahe CH, Owens RE, Fleegeer LH, Newton HJ and Harms PJ (1980) Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon period of the estrous cycle *Endocrinology* **107** 498–503
- Robertson D (1989) Follicle-stimulating hormone microheterogeneity. In *Microheterogeneity of Glycoprotein Hormones* pp 107–126 Ed. BA Keel and HE Grotjan. CRC Press, Boca Raton, FL
- Savio JD, Keenan L, Boland MP and Roche JF (1988) Pattern of growth of dominant follicles during the oestrous cycle of heifers *Journal of Reproduction and Fertility* **83** 663–671
- Savio JD, Thatcher WW, Badinga L, de la Sota RL and Wolfenson D (1993) Regulation of dominant follicle turnover during the oestrous cycle in cows *Journal of Reproduction and Fertility* **97** 197–203
- *Sunderland SJ, Crowe MA, Boland MP, Roche JF and Ireland JJ (1994) Selection, dominance and atresia of follicles during the oestrous cycle of heifers *Journal of Reproduction and Fertility* **101** 547–555
- Ui M, Shimonaka M, Shimasaki S and Ling N (1989) An insulin-like growth factor binding protein in ovarian follicular fluid blocks follicle stimulating hormone steroid production by ovarian granulosa cells *Endocrinology* **125** 912–916
- Ying S-Y (1988) Inhibins, activins, and follistatins: gonadal proteins modulating the secretion of follicle-stimulating hormone *Endocrine Reviews* **9** 267–293