Roles of follicle stimulating hormone and luteinizing hormone in controlled ovarian hyperstimulation

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Introduction

With the advent of recombinant follicle stimulating hormone (FSH) and luteinizing hormone (LH), free from contamination with each other (Loumaye et al., 1995), it is possible to dissect the individual contributions of FSH and LH to the regulation of ovarian function and to define optimal gonadotrophin usage in controlled ovarian hyperstimulation (COH) (Hillier, 1994). Basic and clinical research evidence is accumulating to propose that the future use of these new pharmaceuticals will allow systematic improvements in the treatment regimes used to achieve COH (Hillier et al., 1995). Here, I briefly re-examine some of the physiological principles that need to be borne in mind as these developments take place.

Physiological control of follicular maturation

FSH and LH secreted by the anterior pituitary gland act on target cells in the ovaries to stimulate preovulatory follicular development and oestrogen secretion. During follicular growth (also known as ‘recruitment’; Goodman and Hodgen, 1983), FSH acts via granulosa cell FSH receptors coupled to cAMP-mediated post-receptor signalling to stimulate the formation of factors that locally modulate cell proliferation and differentiation (Richards, 1994). Locally-produced factors that affect FSH-regulated granulosa cell proliferation include growth/differentiation factors such as activin and transforming growth factor (TGF)-β that activate serine/threonine kinase-mediated post-receptor signalling (Miró and Hillier, 1995). Paracrine factors of thecal cell origin capable of influencing FSH action include androgens, i.e. aromatase substrates. There is experimental evidence that the granulosa cell androgen receptor (AR) that mediates paracrine androgen action is developmentally regulated by FSH. Immunocytochemical studies of the AR content of non-human primate and rat ovaries reveal the presence of AR almost entirely in granulosa cells of preantral, and early-intermediate antral follicles,
disappearing in the preovulatory follicle. In-vivo experiments on immature female rats confirm that granulosa cell AR mRNA levels are negatively regulated by FSH (Tetsuka et al., 1995). A current working hypothesis is that negative regulation of the granulosa cell AR by FSH is part of the intraovarian mechanism that determines which follicle(s) becomes dominant and hence secretes oestrogen in the normal menstrual cycle.

During follicular dominance (also known as ‘selection’), LH-stimulated thecal androgen is required as an oestrogen precursor in FSH-stimulated granulosa cells. In-vitro studies reviewed below on isolated granulosa and thecal cells (human, non-human primate and rat) and whole follicles (rat) reveal that FSH stimulates granulosa cells to produce a factor(s) that up-regulates LH-stimulated thecal P450c17α-mRNA expression and androgen synthesis during preovulatory follicular maturation (Smyth et al., 1993, 1994, 1995). Growth/differentiation factors implicated in this positive feedback loop are inhibin and insulin-like growth factors (IGF-I and IGF-II) (Giudice, 1992). Although physiological (intrafollicular) concentrations of IGF-I and IGF-II augment LH-stimulated human thecal androgen production in vitro, their effects are greatly enhanced by the additional presence of inhibin (Nahum et al., 1995). Thus paracrine inhibin may have special relevance to the maintenance of follicular dominance in the human menstrual cycle, as discussed below.

Pharmacological control of follicular maturation

Follicular dominance is irrelevant to most COH treatment regimens, since the aim is to induce multiple preovulatory follicles. The dose and duration of FSH treatment necessary to induce/activate a critical level of aromatase activity in the most responsive follicle sets the baseline FSH dose. In COH, other follicles with higher ‘threshold’ FSH requirements must also be activated. Thus a supraphysiological FSH dose becomes necessary. Amongst other factors, follicular FSH thresholds are set by granulosa cell sensitivity to FSH. Sensitivity to FSH is modulated by regulatory substances of thecal cell origin. Since LH regulates thecal cell function, tonic stimulation by LH might indirectly sensitize granulosa cells to FSH (i.e. modulate FSH thresholds). This has yet to be proven clinically but, as discussed below, paracrine signalling (granulosa on theca) initiated by FSH has been shown to operate experimentally in vivo and in vitro.

The ability of FSH to induce granulosa-on-theca paracrine signalling was demonstrated by Smyth et al. (1995), treating hypophysectomized, immature female rats with recombinant human (rh) gonadotrophins to manipulate ovarian follicular development in vivo. After injection for 2 days with rhFSH either with or without rhLH, ovaries were removed and (i) used to isolate granulosa and thecal/interstitial cells for assessment of basal and gonadotrophin-responsive steroidogenesis in vitro; or (ii) homogenized to extract total RNA for Northern analysis of cytochrome P450c17α mRNA. Serum oestradiol and uterine weight were measured as indices of ovarian oestrogen production; androstenedione was
Roles of FSH and LH in COH

measured to reflect ovarian androgen production. Consistent with the 2-cell, two-gonadotrophin model of oestrogen synthesis (Hillier et al., 1994), increased ovarian oestrogen secretion occurred only if both rhFSH and rhLH were given simultaneously. Treatment with rhFSH alone stimulated ovarian weight gain and granulosa cell aromatase activity without oestrogen secretion, whereas rhLH alone stimulated thecal androgen synthesis and androgen secretion. When the total rhLH dose was fixed at 1 IU, giving rise to an unmeasurably low serum concentration of rhLH, additional treatment with rhFSH (10–100 IU) dose-dependently stimulated serum androgen concentrations as well as oestrogen concentrations. The ~2.0 Kb-sized P450c17α mRNA transcript was undetectable in the ovaries of untreated control animals but was abundantly expressed in the ovaries of animals treated with 15 IU pregnant mare’s serum gonadotrophin as a positive control. Treatment with 1 IU rhLH alone barely induced a P450c17α mRNA signal and treatment with 30 IU rhFSH alone was completely ineffective. However, combined treatment with 1 IU rhLH and 30 IU rhFSH markedly enhanced the P450c17α mRNA signal to a level approaching the positive control. Since it was previously shown that P450c17α mRNA is expressed exclusively in thecal cells (Smyth et al., 1993), which do not possess FSH receptors, it was concluded that: (i) rhFSH up regulates thecal P450c17α mRNA and hence follicular androgen synthesis via granulosa-on-theca paracrine signalling; and (ii) tonic stimulation by LH is required to facilitate thecal responsiveness to this FSH-activated paracrine signal(s).

Relevance of follicular oestrogen synthesis

Recruitment of multiple oestrogen-secretory follicles is crucial to successful COH. Oestrogen synthesis per se may not be essential for fertilizable oocytes to develop during COH (Rabinovici et al., 1989). However, locally-produced oestrogen is thought likely to influence oocyte ‘quality’. FSH induces the potential for oestrogen synthesis through induction of granulosa cell aromatase activity, however, LH is necessary for oestrogen synthesis to occur because androgen, of thecal origin, is an obligatory oestrogen precursor. The plasma concentration of LH necessary to facilitate oestrogen synthesis is low (<1 IU/l), generally being adequate in COH regimens incorporating suppression of endogenous LH by gonadotrophin-releasing hormone (GnRH) agonist therapy (Schoot et al., 1994). Presumably ‘maintenance’ LH concentrations exist under these circumstances that are adequate to sustain thecal responsiveness to FSH-induced granulosa cell-derived paracrines (e.g. inhibin), which facilitate oestrogen synthesis by exerting positive feedback regulation of thecal androgen synthesis (Figure 1).

Previous in-vitro studies on human ovarian tissues showed that LH coordinately stimulates aromatase activity and inhibin synthesis in granulosa cells from the dominant follicle, and that inhibin potently enhances LH-stimulated thecal androgen synthesis (Hillier et al., 1991). Thus inhibin was suggested to participate in the paracrine system that leads to enhanced secretion of oestrogen
Figure 1. How 'pure' follicle stimulating hormone (FSH) given in conjunction with gonadotrophin-releasing hormone (GnRH) agonist therapy can achieve controlled ovarian stimulation (COH). (A) Chronic treatment with GnRH agonist incompletely suppresses endogenous luteinizing hormone (LH) such that thecal cells in immature follicles still synthesize androgen and presumptive paracrine modulators (curved arrow) of granulosa cell function. However, preovulatory follicular growth and oestrogen synthesis does not occur in the absence of exogenous FSH. (B) When appropriate amounts of exogenous FSH are administered, the granulosa cells of responding follicles increasingly express aromatase and synthesize paracrine modulators, e.g. inhibin and insulin-like growth factor (IGF)-II, of thecal responsiveness to LH. Thus androgen production in maturing follicles increases sufficiently to sustain oestrogen synthesis and COH. (C) Thecal and granulosa cells in the immature follicles of some polycystic ovary syndrome (PCOS) patients seem to be inherently more responsive than normal to LH and FSH respectively. The paracrine feedback-loop established by FSH treatment may therefore be accentuated in such patients, explaining their tendency to become excessively hyperstimulated.

by the dominant follicle (Hillier, 1991). However, insulin-like growth factors (IGFs) and insulin itself are also implicated in the regulation of thecal androgen synthesis (Adashi et al., 1991; Giudice, 1992). The hypothesis that IGF-I might be a paracrine regulator of thecal androgen synthesis was built largely on
experiments in rats, in which species IGF-I appears to be the major IGF produced by gonadotrophin-stimulated granulosa cells. It was subsequently shown that human granulosa cells produce mainly IGF-II instead of IGF-I (Hernandez et al., 1992). Recently, Nahum et al. (1995) compared the abilities of IGF-I, IGF-II and insulin to stimulate androgen production by human thecal cells and assessed interactions with LH and inhibin. Serum-free monolayer cell cultures were established from the ovaries of euniodrogenic women undergoing hysterectomy with oophorectomy for non-ovarian indications. Androstenedione production was determined after 4 days of culture in the presence of insulin or either of the IGFs (10–100 ng/ml), with/without LH (10 ng/ml) and/or inhibin (30 ng/ml). The three metabolic hormones exerted similar dose-related effects on androgen production (ED$_{50}$ ≤10 ng/ml), which were augmented 2–3-fold in the presence of LH and further increased several-fold by the additional presence of inhibin. Treatment with insulin, IGF-I or IGF-II did not measurably stimulate thecal cell growth, but all treatments caused striking morphological changes consistent with enhanced steroidogenesis. These data reveal potent regulatory effects of metabolic hormones on human thecal androgen synthesis in vitro, implying endocrine roles for circulating insulin and IGFs and a possible paracrine role for granulosa-derived IGF-II in regulating ovarian androgen production. The finding that the actions of these metabolic hormones can be substantially overridden by inhibin, further implicates inhibin as a major paracrine regulator of thecal androgen biosynthesis in the human ovary (Hillier, 1991; Findlay, 1993).

Summary and implications

FSH and LH are of primary importance in the regulation of follicular growth and dominance. However, there is experimental evidence that gonadotrophin action depends vitally on locally produced steroidal and non-steroidal factors that: (i) facilitate the mitogenic action of FSH during follicular recruitment; and (ii) amplify LH-induced androgen and oestrogen synthesis within the dominant follicle.

COH outcome is mainly determined by the dose and duration of treatment with FSH. LH is of secondary importance but might be used to modulate follicular sensitivity and response to FSH. It is uncertain whether oestrogen affects oocyte quality; either way, its synthesis occurs during COH (Devroey et al., 1994; Recombinant Human FSH Study Group, 1995).

In the absence of pharmaceutical LH formulations, human chorionic gonadotrophin (HCG) is usually given as surrogate LH to induce ovulation or time oocyte collection during COH cycles. Treatment with HCG, instead of exogenous progesterone, is then optional to maintain the luteal phase during which FSH is irrelevant. Both rhCG and rhLH will soon be available for clinical use and it will be interesting to see if rhLH offers advantages over rhCG for either purpose. Meanwhile, extrapolation of the existing experimental evidence (Smyth et al., 1993, 1994, 1995; Hillier et al., 1994) to the clinical situation suggests that the
following benefits might arise out of the use of pure FSH and LH in ovarian stimulation regimens.

**COH for assisted reproduction: gonadotrophin stimulation in association with GnRH agonist therapy**

COH regimens used in conjunction with assisted reproduction procedures typically combine suppression of endogenous LH by chronic treatment with GnRH agonist with the administration of exogenous gonadotrophins to stimulate multiple follicular development. Repeated exposure of pituitary gonadotropes to GnRH agonists causes ‘down-regulation’ involving microaggregation of GnRH receptors and internalization of agonist–receptor complexes, such that LH and FSH concentrations in blood fall to near undetectable concentrations. Despite the suppression of endogenous LH caused by GnRH agonist, administration of ‘pure’ FSH alone to such patients usually stimulates multiple follicular development and oestrogen secretion to degrees comparable with those achieved when FSH and LH (i.e. human menopausal gonadotrophin) are given simultaneously (Edelstein et al., 1990; Shoham et al., 1991a). If FSH activates a paracrine mechanism that up-regulates LH-responsive androgen synthesis, and hence oestradiol synthesis, as suggested by Smyth et al. (1993, 1995; Figure 1), it becomes evident why different women might be more-or-less responsive to FSH, depending upon the extent to which their endogenous LH activity is being suppressed by the GnRH-agonist therapy. A direction for future clinical research is to quantify and understand the relationship between circulating LH concentrations and follicular responsiveness to FSH.

**Patients with WHO group II type infertility**

Women with anovulatory infertility who are not devoid of endogenous LH often receive ovulation induction therapy, with or without pituitary down-regulation. Such patients frequently over-respond to FSH therapy and if care is not taken they can develop ovarian hyperstimulation (Shoham et al., 1991b). Many of these women have polycystic ovaries (PCO) associated with high basal serum LH concentrations. Thecal cells from PCO follicles undertake higher rates of androgen synthesis than those of ‘normal’ follicles of a similar size (Gilling-Smith et al., 1994). Since androgens enhance the induction by FSH of granulosa cell function (including inhibin production) in vitro (Hillier et al., 1991) and inhibin and/or other granulosa cell factors have potentials to promote LH-responsive thecal androgen synthesis (Hillier, 1991) it is evident how reciprocal paracrine signalling between LH-stimulated thecal cells and FSH-stimulated granulosa cells might bring about follicular hypersensitivity to FSH. A clinical challenge is to determine how the follicular paracrine system can be manipulated pharmaceutically to minimize this effect.
Figure 2. Why patients with hypogonadotropic hypogonadism need 'low-dose' luteinizing hormone (LH) to respond appropriately to exogenous follicle stimulating hormone (FSH). (A) Such patients have varying degrees of gonadotrophin deficiency such that antral follicular development is unable to occur in the absence of exogenous gonadotrophin therapy. (B) Treatment with FSH alone can stimulate follicular growth, associated with induction of granulosa cell aromatase activity and onset of granulosa-on-theca paracrine signalling. However, paracrine stimulation of androgen synthesis to levels adequate to sustain preovulatory follicular oestrogen synthesis cannot occur in the complete absence of LH. (C) Patients with complete LH deficiency must therefore be supported with low-dose LH therapy in order to allow exogenous FSH to drive the paracrine feedback loop that sustains androgen (and hence oestrogen) synthesis in the preovulatory follicle.

**Patients suffering from complete LH deficiency (WHO group I type infertility)**

When these patients are given ovarian stimulation therapy, the usual aim is to induce monovulation so that conception can occur in vitro (Couzin et al., 1988; Schoot et al., 1994). Thus a normal pattern of oestrogen production is integral
to a successful therapeutic outcome (Shoham et al., 1991a). An adequate ovarian response to ‘pure’ FSH will therefore depend on simultaneous administration of LH: either at doses that stimulate thecal androgen synthesis directly or in reduced amounts sufficient to promote the indirect responsiveness of thecal cells to FSH (Shoham et al., 1994; Figure 2). Clinical studies are required to establish the duration and dose of treatment with LH that optimally facilitate the action of FSH in such women.

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References


Roles of FSH and LH in COH


