Introduction

The design of effective ovarian stimulation therapy requires knowledge of basic concepts of follicular dynamics and of the respective roles of FSH and LH in regulating the development of a single ovulatory follicle in the spontaneous ovarian cycle. Although FSH and LH are of paramount importance to the regulation of follicular development and ovulation, gonadotrophin action depends vitally on locally produced steroidal and nonsteroidal factors that mediate and modify the actions of FSH and LH within the ovaries. In recent years, understanding of the molecular and cellular mechanisms underpinning gonadotrophin action on the ovaries has grown, aided by experimental work in rodent (Richards et al., 1998) and domestic animal species (Webb et al., 1999). Armed with this knowledge, and the availability of genetically engineered, pharmaceutical grade, pure FSH and LH (Howles 1996; Olivje et al., 1996), the way is open systematically to manipulate the ovarian paracrine system to achieve single or multiple ovulation according to clinical requirements (Hillier et al., 1995). This brief review summarizes the physiological principles that underpin the design of modern ovarian stimulation protocols and points the way to safer and more effective forms of treatment for the future.

Spontaneous follicular development

About 400 follicles mature sequentially and ovulate during the reproductive lifetime of an average woman. From birth to the menopause, the other approximately 99.98% of her follicles initiate development but never complete it (Baker and Sum, 1976). Instead, these follicles default to atresia due to inadequate or inappropriate stimulation by gonadotrophins (Hillier, 1994). The follicle life-cycle is a continuum, with four phases of different requirements for stimulation by FSH and LH: (1) initiation, which occurs from birth to senescence independent of gondotrophic support; (2) progression, which requires tonic stimulation by FSH; (3) preovulatory maturation, which occurs during menstrual cycles and requires appropriate stimulation by adequate amounts of both FSH and LH; and (4) ovulation, which is induced by the mid-cycle gonadotrophin surge.

Initiation

The size of the initial oocyte stock, the proportion that degenerates and the subsequent rate of growth initiation are genetically determined variables (Cahill, 1981; Faddy et al., 1983; Faddy and Gosden, 1996). The stock exists as a pool of non-growing primordial follicles consisting of the oocyte in the prophase of the first meiotic division surrounded by an incomplete or whole layer of flattened spindle-shaped cells and separated from the surrounding ovarian stroma by a...
basement membrane. When a primordial follicle begins to grow, the oocyte increases in size and the flattened cells surrounding it become cuboidal and proliferate to form the granulosa cell layer. It is then a primary follicle.

The mechanism of initiating follicular growth remains unknown. Gonadotrophins are not necessary (Dufour et al., 1979; Halpin et al., 1986; Tapanainen et al., 1998; Abel et al., 2000) but follicular responsiveness to FSH is acquired once granulosa cells are formed (Hirshfield 1985; Mayerhof et al., 1997). The initial stimulus for growth may originate in the oocyte or surrounding somatic cells (Eppig et al., 1997). Paracrine signalling between oocytes and adjacent somatic cells occurs throughout folliculogenesis and involves various growth and differentiation factors, including members of the activin–transforming growth factor β (TGF-β) superfamily (Elvin et al., 2000). Targeted deletion of the growth and differentiation factor 9 (GDF-9) gene in mice results in sterility, with no normal follicular growth occurring beyond the two-layer granulosa cell stage (Dong et al., 1996; Carabatos et al., 1998). Since expression of the GDF-9 gene is oocyte-specific, this experiment proves that oocyte-derived GDF-9 is vital to mammalian folliculogenesis and oogenesis. Oocytes also express dopamine beta-hydroxylase, rendering them capable of synthesizing adrenaline from granulosa cell-derived dopamine (Mayerhof et al., 1998). Since granulosa cells express beta-adrenergic receptors, activation of which stimulates cyclic AMP formation, oocyte-derived noradrenaline may be a primary stimulus for the induction of FSH receptors and subsequent follicular responsiveness to FSH (Mayerhof et al., 1997).

All primary follicles that develop during infancy are destined to undergo atresia, since the level of gonadotrophic support needed to promote full preovulatory growth is absent before puberty. Granulosa cells in healthy primary follicles continue to divide to produce two or more layers while the zona pellucida forms around the enlarging oocyte. Outside the basement membrane, a layer of cells differentiates from the stroma and arranges itself as the theca. At this secondary or preantral stage of development the follicle becomes increasingly dependent on stimulation by FSH.

**Progression**

FSH acts via its receptor in the granulosa cell surface membrane to stimulate cell division (Richards et al., 1998) and formation of antral fluid. When about three layers of granulosa cells have formed in preantral follicles, fluid-filled spaces appear between the granulosa cells due to the increased secretion of glycosaminoglycans into the extracellular spaces. As they increase in size, these spaces gradually become confluent to form a single large antrum. Antrum formation is dependent on FSH beginning in all species in follicles of about 200 μm in diameter containing approximately 5000 granulosa cells. The duration of the subsequent antral phase of growth is a species-specific variable related to follicular size at the time of ovulation. Thus, in mice and rats, in which preovulatory follicles eventually attain diameters of around 1 mm, antral growth lasts 4–5 days (Pederson, 1970). In sheep and humans, in which maximal follicular diameters of about 5 mm and 20 mm are achieved, it lasts approximately 50 (Cahill, 1981) and 60 (Gougeon, 1996) days, respectively. This means that, in women, the follicle(s) that ovulate in a particular menstrual cycle will have begun its antral phase of development approximately two cycles earlier.

Antral follicular growth in humans continues to depend on tonic FSH support until diameters of approximately 5 mm are reached (McNatty et al., 1983). Healthy follicles at this ‘precursor’ stage are on the brink of entering terminal maturation, subject to appropriate stimulation by FSH. Before adulthood, any follicles that survive to this stage are atretic (Peters et al., 1981). However, the circulating serum concentration of FSH at the beginning of each adult menstrual cycle is typically sufficient to prevent atresia and thereby initiate preovulatory maturation.

**Preovulatory maturation**

**Role of FSH.** As the blood FSH concentration increases, multiple precursor follicles in both ovaries are ‘recruited’ (Goodman and Hodgen, 1983) to begin preovulatory development. However, usually only one of these is ‘selected’ to attain ‘dominance’ during the late follicular phase (Fig. 1). This seems to be the follicle in which the granulosa cells are most responsive to FSH, and therefore it becomes the first follicle to secrete oestrogen. FSH also induces the synthesis of inhibin (Groome et al., 1996; Eldar-Geva et al., 2000), which synergizes with oestrogen in the negative feedback regulation of FSH secretion by the anterior pituitary gland. Thus, the oestrogen and inhibin secreted by this follicle drive down the circulating FSH concentration until it is insufficient to sustain the development of those follicles that are less responsive to FSH. These less responsive follicles become nonovulatory and undergo atresia, while the dominant follicle continues to grow and secrete oestrogen, reaching a diameter of 20–25 mm by the time it ovulates.

**Role of LH.** Tonic exposure to LH promotes follicular responsiveness to FSH during follicular recruitment and selection, initially through sustaining thecal androgen and polypeptide growth factor production, and later (after the induction of LH receptors by FSH) through stimulating granulosa cell function directly (Hillier, 1994). In the granulosa cells of the preovulatory follicle, aromatase and inhibin production are functionally coupled to the LH receptor, such that LH stimulates both androgen synthesis and aromatization, thereby directly regulating follicular oestrogen secretion (Zeleznik and Hillier, 1984). The increased sensitivity of the preovulatory follicle to FSH and LH, resulting in a sustained increase in granulosa cell aromatase activity and increased formation of the androgen required as an aromatase substrate, is crucial to follicular dominance. Paracrine signalling (granulosa on theca) explains these unique features of the preovulatory follicle, and holds the key to efficient and effective clinical strategies for ovarian stimulation, as discussed below.

**Paracrine signalling.** FSH activates paracrine mechanisms in mammalian granulosa cells through the occupation of cell-
Growth–differentiation factors such as members of the activin–TGF-β superfamily, which activate serine–threonine kinase-mediated post-receptor signalling (Dorrington et al., 1988; Gaddy-Kurten et al., 1995; Miró and Hillier, 1995) in adjacent thecal cells, as well as within the granulosa cells themselves. Conversely, LH acts on thecal cells to induce the formation of paracrine factors such as androgens, which are also granulosa cell aromatase substrates, and which exert receptor mediated action on adjacent granulosa cells. There is experimental evidence that the granulosa cell androgen receptor that mediates paracrine androgen action is developmentally regulated by FSH. Immunocytochemical studies of the androgen receptor content of non-human primate and rat ovaries revealed its presence almost entirely in granulosa cells of preantral, and early–intermediate antral follicles, but did not detect it in the preovulatory follicle. In vivo experiments on immature female rats confirm that granulosa cell androgen receptor mRNA concentrations are negatively regulated by FSH. A working hypothesis is that negative regulation of the granulosa cell androgen receptor by FSH is part of the intraovarian mechanism that determines which follicle(s) becomes dominant and hence secretes oestrogen during the normal menstrual cycle (Hillier and Tetsuka, 1997).

Studies in vitro 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 upregulates 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 include inhibin (Roberts et al., 1993) and insulin-like growth factors (IGF-I and IGF-II) (Giudice, 1992; Hernandez et al., 1992; el-Roeiy et al., 1994). 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 signalling in preovulatory follicles is of special relevance to clinical ovulation induction protocols.

Ovulation

Oestrogen secreted by the preovulatory follicle eventually triggers the mid-cycle LH surge through positive feedback action within the hypothalamo–pituitary axis. The bolus of LH intensely activates cyclic AMP-mediated post-receptor signalling, which suppresses granulosa cell division and activates the inflammatory gene cascade that leads to proteolytic breakdown of the follicle wall, release of the oocyte and formation of the corpus luteum (Adashi, 1998). The response to LH includes increased local production of pro- and anti-inflammatory mediators (Terranova and Rice, 1997) that tightly control this natural sequence of damage and repair to the ovarian surface (Hillier and Tetsuka, 1998). The extent of this damage and the speed of its resolution are remarkable, indicating that the cellular changes induced by LH in the follicle wall and at the ovarian surface may be of general relevance to the biology of wound healing. The high concentration of LH also serves to terminate the
development of less mature follicles, causing their premature luteinization and atresia (see above).

**Induction of multiple follicular development**

Most assisted reproduction protocols depend on controlled ovarian stimulation (COS) to allow the recovery of multiple mature oocytes from the ovaries. The basic assisted reproduction technique is in vitro fertilization and embryo transfer (IVF–ET) (Steptoe et al., 1980), which is used mainly to treat causes of female infertility other than ovulatory dysfunction, such as tubal disease, unexplained infertility or endometriosis. IVF–ET has been extended to the treatment of male infertility, wherein eggs from a fertile woman are inseminated by intracytoplasmic sperm injection (ICSI) using spermatozoa from the infertile male partner (Van Steirteghem et al., 1996). In this situation, women with perfectly normal ovarian function receive COS therapy. Whichever partner is infertile, the clinical challenge remains simply and safely to obtain the maximum number of developmentally competent oocytes consistent with the treatment objectives.

**Principle**

Three stages of antral follicular development are potential targets of COS therapy (Hillier et al., 1985) (Fig. 2): (1) precursor follicle development during the luteal phase; (2) development beyond the precursor stage during the first half of the follicular phase; and (3) the preovulatory (oestrogen secretory) stage. Stage 1 remains of theoretical interest only, since it is not known whether the number of precursor follicles can be regulated by extrinsic factors. Stages 2 and 3 are of practical importance because medicines are available to manipulate them directly, the strategy being to overcome the gonadotrophin ‘threshold’ (see below) requirements of multiple precursor follicles (treatment in the early–mid follicular phase) and then to sustain multiple preovulatory
follicular development through overriding the follicular selection process (treatment during the mid–late follicular phase).

**Box 1. The FSH ‘threshold’ hypothesis (Brown, 1978)**

- Ovarian follicles have development-related requirements for stimulation by FSH
- FSH, beyond a certain ‘threshold’, stimulates granulosa proliferation and functional maturation (for example, expression of aromatase, LH receptors and inhibin synthesis)
- Follicles become increasingly sensitive (lower threshold) to FSH as they mature
- During ovulation induction, FSH dose should exceed the threshold of the most mature follicle

**The FSH threshold.** The FSH threshold concept advanced by Brown (1978) is crucial to successful COS. The concept states that all follicles potentially capable of undergoing preovulatory development have individual threshold requirements for stimulation by FSH (Box 1). Further healthy development of an individual follicle occurs only when its FSH threshold is exceeded. In a spontaneous cycle, the follicle with the lowest threshold requirement for FSH will become the first to synthesize oestrogen, and is thereby selected to ovulate. At the cellular level, the granulosa cells of this follicle are more sensitive to FSH, leading to earlier expression of high LH receptor-coupled aromatase activity (see above). Hence, this follicle becomes uniquely equipped to undertake LH-responsive oestradiol production during the late follicular phase when plasma FSH concentrations decrease. Other follicles, with greater dependence on FSH and more limited responsiveness to LH, become nonovulatory and degenerate (Zeleznik and Hillier, 1984). All successful COS regimens have in common the aim to override the selection process by overcoming the FSH threshold requirements of multiple precursor follicles, and then to sustain their development to the point of oocyte retrieval.

**Practice**

In the past, urinary (u) gonadotrophins and anti-oestrogens were most commonly administered to achieve COS. Nowadays, recombinant (r) DNA technology is being applied to produce pharmaceutical grades of r-FSH and r-LH, opening the way to the development of improved new generation pharmaceuticals in COS are outlined below.

**Use of human menopausal gonadotrophin.** Human menopausal gonadotrophin (hMG) is an extract of menopausal urine containing u-FSH and u-LH, in similar proportions to each other or enriched with respect to FSH. Modern treatment regimens conventionally combine hMG or u-FSH with GnRH-agonist, which is administered simultaneously to suppress inappropriate secretion of endogenous LH (Fleming et al., 1982; Rutherford et al., 1988). With appropriate monitoring (ovarian ultrasonography and oestrogen assays), oocyte collection is then timed to an ovulation-inducing injection of human chorionic gonadotrophin (hCG) (Shoham et al., 1995). GnRH-antagonists have also been used to block the endogenous LH surge (Felberbaum and Diedrich, 1999; Ganirelix Dose-finding Study Group, 1998).

Use of hMG containing fixed proportions of FSH and LH to stimulate ovarian function ignores the fact that follicular responsiveness FSH and LH varies characteristically with preovulatory development (Fig. 1). While many women respond adequately to standard superovulation regimens using hMG or u-FSH (Agrawal et al., 2000), their follicles usually develop asynchronously and yield oocytes of variable quality, some of which do not fertilize or undergo normal post-fertilization development. This probably occurs because sustained high-level stimulation with FSH during the mid–late follicular phase causes immature and intermediately mature follicles to continue to develop at a time when they would otherwise undergo spontaneous regression due to withdrawal of stimulation by endogenous FSH. This time is when granulosa cells in more mature follicles (those expected to provide the ‘best’ oocytes) become directly responsive to LH.

**Use of recombinant gonadotrophin.** With the advent of r-FSH and r-LH, it is possible to dissect individual contributions of FSH and LH to the regulation of ovarian function (Hillier, 1990; Loumaye et al., 1995) (Fig. 3), potentially allowing systematic improvements to be made to clinical COS treatment regimens (Hillier et al., 1995; Recombinant Human FSH Study Group, 1995).

The outcome of COS is determined mainly by the dose and duration of exposure to FSH, with LH being of secondary importance (Homburg and Howles, 1999). Although both FSH and LH are required for normal follicular oestrogen biosynthesis, paradoxically, exogenous FSH alone is sufficient to stimulate ovarian oestrogen secretion in superovulatory cycles, even when given in combination with a GnRH agonist to suppress endogenous LH. Usually, the response is indistinguishable from that achieved when FSH and LH (that is, hMG) are given simultaneously (Edelstein et al., 1990), although there is some evidence that additional LH is beneficial (Filicori et al., 1999a), possibly due to activation by FSH of the paracrine mechanism that upregulates LH-responsive androgen synthesis in the preovulatory follicle. An equally logical explanation is that there is enough LH in the circulation – even after GnRH agonist suppression – to maintain normal thecal cell function (Sills et al., 1999).

Recombinant FSH is likely to replace u-FSH in COS regimens. Advantages of r-FSH over u-FSH are that it is easier to produce to a constant pharmaceutical specification and, as it is molecularly pure, it is safer. r-FSH is also reported to be more effective than u-FSH at inducing multiple follicular development (Bergh et al., 1997) and pregnancy outcome after IVF (Out et al., 1997). Both currently available pharmaceutical grade r-FSH preparations – follitropin alpha (‘Gonal-F’) and follitropin beta (Puregon) – appear to be equally suitable for use in ovarian stimulation for IVF (Brinsden et al., 2000; Harlin et al., 2000).
The theory behind the practice of ovulation induction using FSH and LH. The abscissae represent 'time' (that is, an arbitrary 14-day follicular phase); ordinates represent exogenous gonadotrophin dose (\( FSH \) starting at 75 iu per day with a 100% increment every three days; \( LH \) given throughout at a constant 'subceiling' daily dose) and follicular diameter (arrows, approximately 5 mm increasing to a maximum of approximately 20 mm, with direction of arrowhead implying continued growth (up) or degeneration (down). (a) When FSH alone is given chronically at a 'suprathreshold' dose, multiple preovulatory follicular development is initiated and persists as long as that dose of FSH is given. (b) If the suprathreshold FSH dose is withdrawn, responding follicles cease to develop and become atretic. (c) However, chronic administration of a 'subceiling' dose of exogenous LH encourages sustained development of the most mature (that is, most LH-responsive) follicle, despite the withdrawal of FSH. (Redrawn from Hillier, 1993.)

Fig. 3. The theory behind the practice of ovulation induction using 'pure' FSH and LH. The abscissae represent 'time' (that is, an arbitrary 14-day follicular phase); ordinates represent exogenous gonadotrophin dose (\( FSH \) starting at 75 iu per day with a 100% increment every three days; \( LH \) given throughout at a constant 'subceiling' daily dose) and follicular diameter (arrows, approximately 5 mm increasing to a maximum of approximately 20 mm, with direction of arrowhead implying continued growth (up) or degeneration (down). (a) When FSH alone is given chronically at a 'suprathreshold' dose, multiple preovulatory follicular development is initiated and persists as long as that dose of FSH is given. (b) If the suprathreshold FSH dose is withdrawn, responding follicles cease to develop and become atretic. (c) However, chronic administration of a 'subceiling' dose of exogenous LH encourages sustained development of the most mature (that is, most LH-responsive) follicle, despite the withdrawal of FSH. (Redrawn from Hillier, 1993.)

The potential benefits of introducing r-LH to COS regimens are less clear. As discussed above, the present trend is to use u-FSH or r-FSH preparations devoid of LH activity. However, there is physiological and clinical evidence that supplementation of FSH with LH can promote better follicle and oocyte development. The advantage of adjuvant therapy with LH may depend on the level of pituitary suppression achieved by concomitant GnRH analogue therapy, and whether an antagonist or an agonist of GnRH is being used for this purpose.

Use of anti-oestrogens. The effective use of anti-oestrogens such as clomiphene citrate in COS regimens depends upon the pharmaceutical formulation, dosage and timing of administration during the treatment cycle. Tamoxifen, a triphenylethylene derivative like clomiphene citrate (Adashi, 1984), is also effective (Edwards et al., 1984). The mechanism of action of these anti-oestrogens on the ovary is not fully understood but depends upon their biological properties as mixed agonistic (zu)–antagonistic (en) isomeric oestrogens that enhance pituitary FSH and LH secretion through actions in the hypothalamo–pituitary axis (Adashi, 1984). It has been established clinically that the active agent in clomiphene citrate is the anti-oestrogenic en isomer (Glasier et al., 1989a). Therefore, the use of clomiphene citrate formulations enriched with respect to the en isomer may have particular advantages in COS protocols but this remains to be tested clinically.

Conventional treatment regimens involve daily administration of between one and three 50 mg tablets of clomiphene citrate given for a maximum of 5 days beginning during the early follicular phase; hCG can then be injected at mid-cycle to induce ovulation and time egg collection (Kerin et al., 1983). Simultaneous or sequential clomiphene–hMG therapy can give rise to more large follicles suitable for egg collection than is usually the case with clomiphene alone. Thus, more viable eggs can be collected and more embryos suitable for intrauterine replacement frequently result. The disadvantage remains that, if the spontaneous LH surge occurs before hCG is injected, egg collection cannot be timed accurately unless LH is being monitored. Moreover, since LH secretion as well as FSH is increased by clomiphene (Adashi, 1984), inappropriate LH action can interfere with follicular maturation by causing early luteinization, atresia or the development of large cystic follicles in some treatment cycles.

In general, the use of anti-oestrogens in COS protocols has given way to combined GnRH-agonist–gonadotrophin regimens (see below) that allow cycle programming with the ability to recover more eggs for assisted reproduction procedures. However, given the recent interest in more 'gentle' forms of ovarian stimulation (Fauser et al., 1999), it seems likely that COS regimens involving clomiphene citrate may yet regain favour (Dickey et al., 1998; Branigan and Estes, 2000). An attractive regimen might be sequential clomiphene–r-FSH, with GnRH-agonist given during the mid–late follicular phase to prevent an unwanted endogenous LH surge (Diedrich and Felberbaum, 1998).

**Induction of monovulation**

The aim of monovulation therapy is to stimulate the development of a single ovarian follicle in an otherwise anovulatory woman, so that conception can occur in vivo with the production of a singleton healthy baby (Couzin et al., 1988; Schoot et al., 1994). A normal pattern of ovarian oestrogen secretion, requiring appropriate stimulation of the ovaries with adequate amounts of both FSH and LH, is therefore necessary for a favourable outcome (Glasier et al., 1989b; Fauser, 1997). Infertile women requiring such treatment generally fall into two categories of anovulatory infertility classified by the WHO Scientific Group on 'Agents Stimulating Gonadal Function in the Human' (WHO, 1973). Patients with WHO-type I infertility have failure of hypothalamo–pituitary function leading to primary or secondary amenorrhoea. Owing to low serum concentrations of FSH and LH, follicular development and endogenous oestrogen production are suppressed. Women with
WHO-type II infertility have hypothalamic dysfunction in association with a variety of menstrual cycle disturbances, including luteal phase insufficiency, anovulatory cycles or amenorrhoea. Such patients often have gonadotrophin concentrations within normal ranges with evidence for endogenous oestrogen production sufficient to stimulate endometrial proliferation (Breckwoldt et al., 1993). Women with polycystic ovary syndrome (PCOS) characterized by hyperandrogenism and anovulation, frequently associated with hyperinsulinism, are included in this group (Franks et al., 1998).

**Principle**

The conventional strategy using exogenous gonadotrophins is to increase the dose of FSH progressively (that is, ‘step-up’) sufficient to mimic the inter-cyclic FSH increase and overcome the threshold requirement of a single dominant follicle, or to recruit multiple preovulatory follicles using high dose FSH, which is then withdrawn progressively (that is, ‘step-down’) until the level of stimulation remains adequate to sustain a single dominant follicle. The flaw in either case is that the physiological role of LH is ignored.

**The LH ‘ceiling’**. Although LH is essential for oestrogen synthesis and maintenance of follicular dominance, there is clinical evidence that excessive stimulation of the ovaries by LH affects human preovulatory follicular development adversely. Depending on the stage of development, follicles exposed to inappropriately high concentrations of LH enter atresia or become prematurely luteinized, and oocyte development may be compromised (Chappel and Howles, 1991; Jacobs, 1991). The dose dependence of this LH effect was illustrated by studies of LH action on human granulosa cell proliferation and steroidogenesis in vitro: ‘low-dose’ treatment with LH generally enhancing steroidogenesis without inhibiting DNA synthesis but ‘high-dose’ LH causing enhanced synthesis of progesterone, suppression of aromatase activity and inhibition of cell growth (Yong et al., 1992, 1994). Thus, developing human follicles have to have finite requirements for stimulation by LH, beyond which normal development ceases. Whereas each follicle has a threshold beyond which it must be stimulated by FSH to initiate preovulatory development (Brown, 1978) (see above), it may also have a ‘ceiling’ below which it needs to be stimulated by LH, unless further normal development is terminated (Box 2) (Hillier, 1993). Therefore, during the second half of the follicular phase as plasma FSH concentrations decrease, the LH-dependent phase of preovulatory follicular development proceeds normally only if LH is present at concentrations beneath the ‘ceiling’ value. The implication of the LH ‘ceiling’ hypothesis for the use of exogenous LH in the induction of ovulation in anovulatory women is considered below.

**Practice**

Exploiting the transition from FSH-dependent to LH-responsive granulosa cell function during preovulatory follicular development is the key to inducing monofollicular development. The usual first-line therapy is clomiphene citrate, with the subsequent use of gonadotrophin therapy in clomiphene-resistant patients (Balen, 1998). Patients with central nervous system or hypothalamic amenorrhoea can be treated with pulsatile GnRH, to activate endogenous feedback signalling between the anterior pituitary gland and the ovaries (Schoemaker, 1996). Exogenous gonadotrophin therapy is reserved for pituitary-related amenorrhoea, in which cases the aim of treatment must be to simulate a natural ovulatory cycle. As discussed below, the availability of r-FSH and r-LH radically extends the clinical options.

**Pulsatile GnRH.** Pulsatile administration of GnRH is an established treatment for ovulation induction and pregnancy in patients with hypogonadotropic amenorrhoea (for example, see Hurley et al., 1983; Mason et al., 1984; Reid and Sauerbrei, 1984). A major advantage over other forms of ovulation induction claimed for GnRH treatment in these cases is that the ovarian–pituitary feedback loop is left intact and should therefore involve a lower risk of multiple preovulatory follicular development and hence of multiple pregnancy (for example, see Hurley et al., 1983). However, multiple pregnancy can still occur after intravenous pulsatile GnRH infusion (for example, see Heineman et al., 1984).

**Use of hMG.** Gonadotrophins, such as HMG or u-FSH, are used widely to treat women with WHO-type II and WHO-type I anovulation. In the conventional protocol, hMG or u-FSH is administered beginning at a low daily dose (15–150 iu per day) with increments every 5 days in steps of 75 iu until a responding follicle (recognized on the basis of oestrogen measurements and ultrasonography) emerges, at which point hCG is administered to produce ovulation. Such patients frequently over-respond to FSH therapy and, if care is not taken, can develop ovarian hyperstimulation syndrome (Shoham et al., 1991). The more conservative ‘chronic low dose protocol’ uses lower dose increments (37.5 iu per day) to seek the lowest (that is, threshold) FSH dose required to sustain monofollicular development. Hyperstimulation and multiple pregnancy remain problematic although less so than with the conventional protocol. Many women classified as WHO-type II anovulatory have polycystic ovaries associated with high basal serum LH concentrations. Thecal cells from polycystic follicles undertake higher rates of androgen synthesis in vitro than those from ‘normal’ follicles of a similar size (Gilling-Smith et al., 1994) and their

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**Box 2. The LH ‘ceiling’ hypothesis (Hillier, 1993)**

- Ovarian follicles have development-related requirements for stimulation by LH
- LH, beyond a certain ceiling value, suppresses granulosa proliferation, and initiates atresia (nondominant follicles) or premature luteinization (preovulatory follicle)
- Mature follicles are more resistant (higher ceiling) to LH than are immature ones
- During ovulation induction, LH dose should not exceed the ceiling of the most mature follicle
granulosa cells are hyper-responsive to FSH (Mason et al., 1994). Since androgens enhance the induction of granulosa cell function (including inhibin production) by FSH in vitro (Hillier, 1991), and inhibit and other granulosa cell factors have the potential to promote LH-responsive thecal androgen synthesis (Smyth et al., 1993, 1995), it appears that reciprocal paracrine signalling between LH stimulated thecal cells and FSH-stimulated granulosa cells may result in follicular hyper-responsiveness to FSH. The challenge remains to determine how to manipulate the follicular paracrine system most effectively to minimize this undesirable effect (Filicori et al., 1999b).

Use of recombinant gonadotrophins. Recombinant FSH is becoming favoured over hMG or u-FSH in monovulation-induction programmes, and is proving to be effective and safe in both the chronic low dose and conventional protocols (Shoham and Insler, 1996; Fauser, 1997; Hedon et al., 1998). Indeed, there is evidence that r-FSH performs favourably compared with u-FSH in women with clomiphene citrate-resistant, normogonadotrophic, chronic anovulation (Coelinge Bennink et al., 1998).

Pure r-LH may be of particular use in treatment regimens that aim to achieve monovulation for conception in vivo (Fig. 3). The efficacy of r-LH for supporting human r-FSH-induced follicular development has been established in hypogonadotrophic hypogonadal women (WHO group I anovulation) (European Recombinant Human LH Study Group, 1998). It has been proposed that once the appropriate (that is, LH-responsive) stage of follicular development has been achieved in response to treatment with an above threshold dose of FSH, there are theoretical grounds for reducing or completely withdrawing FSH and maintaining tonic (subceiling) stimulation of the dominant follicle with exogenous LH (Hillier, 1993, 1994). Such an LH ‘coast’, for no more than 1–2 days, could have the dual advantage of promoting the terminal maturation of a single preovulatory follicle and simultaneously arresting the development of multiple less mature follicles that would otherwise occur in response to treatment with FSH. This regimen would aim to approximate the situation in a natural ovarian cycle, encouraging the selective maturation of a single LH-responsive preovulatory follicle and minimizing the likelihood of multiple ovulation (Sullivan et al., 1999). However, it remains to be established which dose and frequency of LH administration are adequate to sustain the development of a single preovulatory follicle without exceeding the ’ceiling’ beyond which LH induces premature luteinization or disordered oocyte development. The upper dose is unknown but exceeds 375 iu per day (Sullivan et al., 1999). Multicentre clinical trials are currently underway to optimize this protocol and evaluate its efficacy.

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