The Role of LH in Controlled Ovarian Stimulation

16

Sandro C. Esteves and Carlo Alviggi

Abstract

Although exogenous FSH is the main regulator of follicular growth in stimulated cycles, LH plays a key role in promoting steroidogenesis and follicle development. Stimulation protocols with LH supplementation are mandatory in patients with hypogonadotropic hypogonadism who do not achieve adequate steroidogenesis by stimulation with FSH alone, but resume adequate estrogen production by LH supplementation. In normogonadotropic women undergoing controlled ovarian stimulation (COS), the hypogonadotropic state after GnRH analogues is short in duration, and the resting levels of LH are usually sufficient for promoting optimal follicular development. An increased body of evidence otherwise indicates that at least three subgroups of normogonadotropic patients indeed seem to benefit from the addition of LH activity to the stimulation protocol: (1) patients >35 years, (2) patients with a decreased ovarian reserve/poor response to COS (poor responders), and (3) patients with an initial poor response to rec-hFSH (hyporesponders). Possible reasons for a beneficial effect of LH activity supplementation include the biological aging of the ovary and pharmacogenetics involving the LH molecule and its receptor. The three gonadotropins containing LH activity are human menopausal gonadotropin (hMG), with 1:1 ratio of FSH/LH in which LH activity is driven by hCG; recombinant

S.C. Esteves, MD, PhD (✉)
ANDROFERT, Andrology and Human Reproduction Clinic, Av. Dr. Heitor Penteado, 1464 Campinas, São Paulo 13075-460, Brazil
e-mail: s.esteves@androfert.com.br

C. Alviggi, MD, PhD
Department of Neuroscience and Reproductive Medicine, University of Naples Federico II, Naples, Italy

S. Ghumman (ed.), *Principles and Practice of Controlled Ovarian Stimulation in ART*,
DOI 10.1007/978-81-322-1686-5_16

human LH (rec-LH), with only LH activity driven by pure LH; and a combination of recombinant FSH and recombinant LH, with 2:1 ratio of pure FSH/LH activity. In addition to the higher purity and specific activity of rec-hLH compared with hMG, LH activity is markedly different at the molecular and functional levels between these gonadotropins. The choice of the type of gonadotropin preparations containing LH activity should be considered when tailoring COS with LH supplementation because they may influence cycle outcome.

Keywords

Gonadotropins • Luteinizing hormone • Human chorionic gonadotropin • Controlled ovarian stimulation • Assisted reproductive technology

Abbreviations

3β-HSD	3β-Hydroxysteroid dehydrogenase
AA	Amino acids
ART	Assisted reproductive techniques
Asn	Asparagine
cAMP	Cyclic adenosine monophosphate
CHO	Chinese hamster ovary
CI	Confidence interval
COS	Controlled ovarian stimulation
DHEA	Dehydroepiandrosterone
FbM	Filled by mass
FSH	Follicle-stimulation hormone
GalNAc	N-acetylgalactosamine
GC	Granulosa cells
GlcNAc	N-acetyl glucosamine
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotropin
hMG	Human menopausal gonadotropin
HP-hMG	Highly purified human menopausal gonadotropin
ICSI	Intracytoplasmic sperm injection
IGF	Insulin-like growth factor
IVF	In vitro fertilization
LH	Luteinizing hormone
LMW	Low molecular weight
OR	Odds ratio
P4	Progesterone
P450arom	P450 aromatase
P450scc	Cholesterol side-chain cleavage enzyme
RCT	Randomized controlled trial
RD	Risk difference
rec-hCG	Recombinant human chorionic gonadotropin
rec-hFSH	Recombinant human follicle-stimulating hormone
rec-hLH	Recombinant human luteinizing hormone
RR	Relative risk
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SE-HPLC	Size-exclusion high-performance liquid chromatography
SO3-4GalNAc	Sulfonated β1–4-linked N-acetylgalactosamine
StAR	Steroidogenic acute regulatory protein
WMD	Weighted mean difference

16.1 Introduction

Gonadotropin therapy has a central role in ovarian stimulation. Its introduction in medical practice dates from almost one century ago and represents a major upgrade in infertility treatments. Treatment of anovulatory women with exogenous gonadotropin administration started in the 1960s and expanded to ovulatory women to promote multifollicular development in the 1980s [1–3]. Gonadotropins were first extracted from urine in the 1940s, and a decade later the first urinary forms of human chorionic gonadotropin (hCG) and human menopausal gonadotropin

(hMG) became commercially available [2, 3]. Improvements in the purification methods led to the production of follicle-stimulating hormone (FSH) – only products in the 1980s and advances in DNA technology enabled the development of recombinant human gonadotropins, which became commercially available approximately two decades later [2–4]. In 2000, recombinant human luteinizing hormone (rec-hLH) became commercially available, and recently, in 2007, a fixed combination of recombinant FSH (rec-hFSH) and rec-hLH was launched [3].

Although exogenous FSH is the main regulator of follicular growth in stimulated cycles, the question whether the LH hormonal environment achieved after administration of gonadotropin-releasing hormone (GnRH) analogues is really optimal for all categories of patients undergoing controlled ovarian stimulation (COS) or whether subgroups of patients exist that might actually benefit from exogenous LH supplementation has received increased attention. While FSH is the main antral follicular growth regulator, LH plays a key role in promoting steroidogenesis and development of the leading follicle and has different functions in different stages of both natural and stimulated cycles. During the early follicular phase, LH stimulates the production of androgens by theca cells. Androgens are then transferred to the GC and transformed into estrogens via aromatization [4]. From the mid-follicular phase onwards, LH upregulates FSH receptor expression and sustains FSH-dependent granulosa cell activities, including aromatase production and growth factors' release. In addition, LH sustains follicular growth and final follicular maturation via its direct effects on the GC in the late follicular phase [4]. Therefore, during recent years an increasing body of evidence has emerged examining the possible beneficial role of exogenous LH activity supplementation in stimulated ART cycles.

The purposes of this chapter are (1) to review the glycoprotein structure and action of luteinizing hormone (LH), (2) to examine the rationale of using luteinizing hormone (LH) supplementation during controlled ovarian stimulation, (3) to present the clinical evidence supporting LH supplementation during COS in different subset of infertility patients, (4) to describe the commercially available preparations containing LH activity, and lastly (5) to analyze the differences in LH activity provided by rec-hLH and hMG preparations.

16.2 Structure and Function of LH

LH is a protein covalently linked to a carbohydrate (glycoprotein). It is synthesized and secreted by gonadotrophs of the anterior pituitary gland under stimulation of the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus [5]. The LH molecule comprises two non-covalently linked protein subunits, alpha and beta. The three-dimensional structure and the active conformation of the subunits are maintained by internal disulfide bonds [6]. The alpha subunit contains 92 amino acids (AA) and is identical in all gonadotropins (i.e., LH, FSH, and hCG). The beta subunit differs in the aforementioned gonadotropins and confers unique receptor specificity as well as differential biological and immunological properties (Fig. 16.1) [7]. Protein subunits alone have no biologic activity; the latter is provided by glycosylation, which is achieved by the attachment of carbohydrate moieties forming heterodimers [3]. The extent and pattern of glycosylation convey the differential spectrum of charges, bioactivities, and half-lives of each gonadotropin [7, 8]. The LH molecule is further modified in vivo by the addition of a sialic acid (sialylation) or sulfonic group (sulfonation) to the carbohydrate moieties. Both sialylation and sulfonation are physiological processes with major roles in gonadotropin biological activity modulation [3, 7–9]. Elimination of LH from circulation is modulated by the number of glycosylation sites and sialic acid residues attached to the carbohydrate moieties [10]. LH beta subunits contain a single site of N-linked glycosylation (Asn 30) and few sialic acid residues (only 1 or 2); as such, native LH has a short half-life of only 20–30 min (Fig. 16.2) [8, 10]. LH shows physiologic fluctuations in isoform profile during the menstrual cycle. More

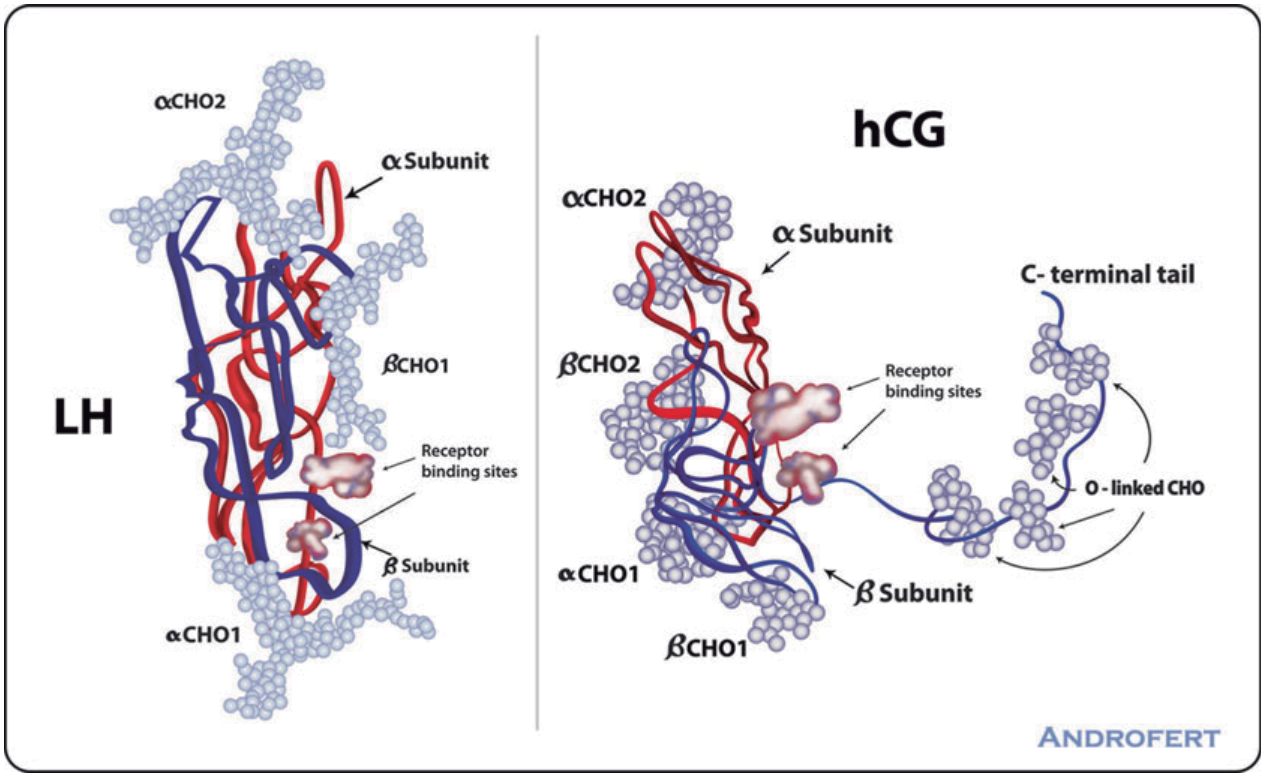

Fig. 16.1 Luteinizing hormone and human chorionic gonadotropin molecules. (**a**) LH is a glycoprotein with two subunits, the alpha subunit (*red*), similar to that of FSH and hCG with two carbohydrate attachment sites, and the beta subunit (*blue*), with only one carbohydrate attachment site. The light blue balls represent the carbohydrate chains. (**b**) hCG is similar in its structural attributes to LH. A notable exception is the presence of a long carboxyl terminal segment that is O-glycosylated (O-linked CHO), conferring longer half-life to hCG. The alpha and beta subunits are represented in *red* and *blue* strands, respectively, whereas the *light blue balls* represent the carbohydrate chains (Adapted from Leão and Esteves [3])

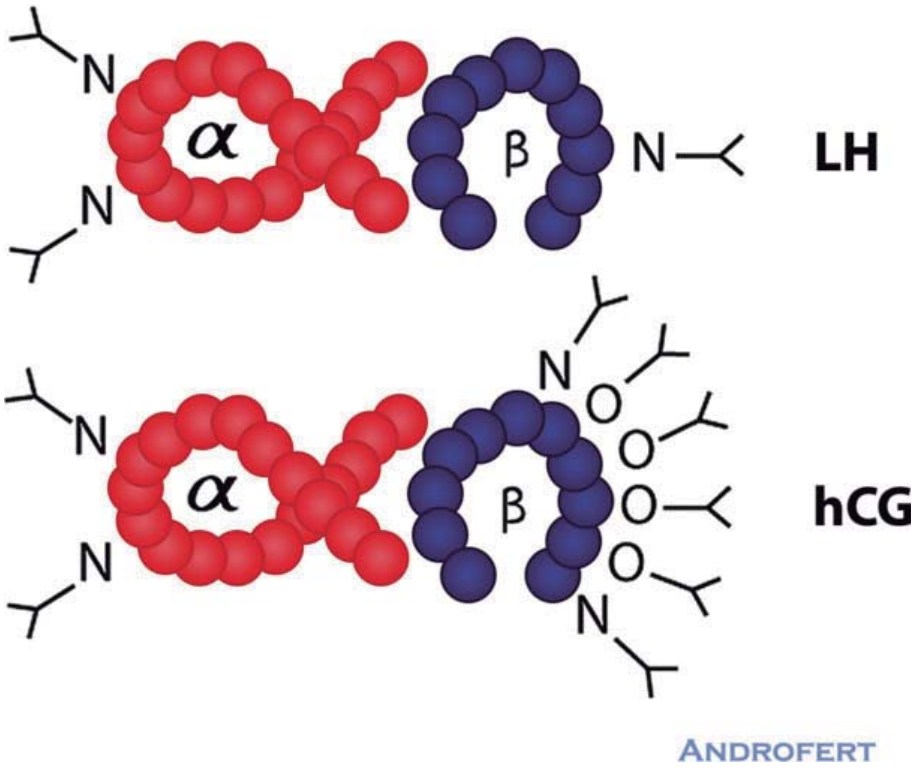

Fig. 16.2 Glycosylation patterns of LH and hCG. The alpha subunits of each hormone are identical in amino acid sequence and contain two sites of N-linked glycosylation. The beta subunit confers hormone specificity and contains variable amounts of N-linked glycosylation. LH beta subunit contains a single site of N-linked glycosylation, while hCG beta subunit contains two sites of N-linked glycosylation. In addition, hCG has an extended C-terminal that contains four sites of O-linked glycosylation

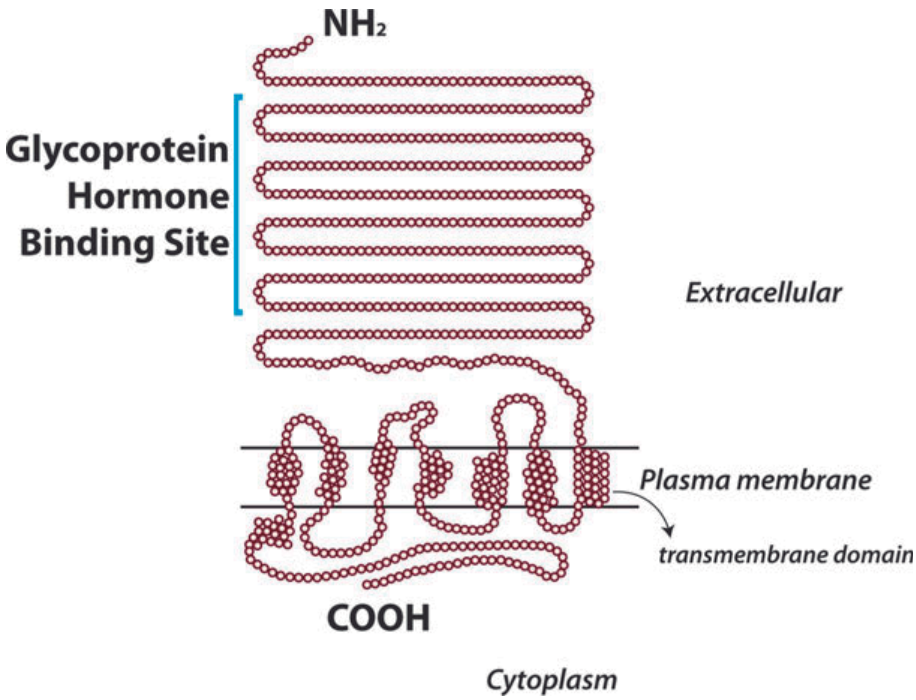

Fig. 16.3 LH/hCG receptor. LH receptors are included in the subgroup of G protein-coupled receptors. Such receptors are characterized by having 7-transmembrane domains and a large N-terminal extracellular region, which is the predominant site of hormone binding

basic LH isoforms are seen at midcycle due to considerably decreased sulfonation concomitant with slightly increased sialylation. Both changes increase LH half-life in the circulation, thus explaining the increased levels of serum LH at this period. This change in isoform profile seems to be physiologically important for ovulation triggering [11].

LH binds to a subgroup of G protein-coupled receptors with 7 transmembrane domains and a large N-terminal extracellular region (Fig. 16.3) [12, 13]. Receptor activation requires that hormones bind to the N-terminal region, thus leading to intramolecular signal transduction from the ligand–receptor complex to the transmembrane domains. Although the mechanism that underlies this intramolecular signaling pathway is not fully understood, it involves stimulation of adenyl cyclase via coupling to Gs proteins [12, 13]. Unlike FSH receptors that are expressed exclusively in the granulosa cells (GC), LH receptors are expressed in both GC and theca cells. The LH receptor expression is at its maximum in the GC of preovulatory follicles, but antral follicles with 3–10 mm in diameter have already expressed these receptors at approximately 10 % of the maximum [14].

16.2.1 The Role of LH on Ovarian Steroidogenesis

The two-cell system, first proposed by Falck in 1959, is based on the assumption that while FSH receptors are present only in the GC, LH receptors are present in the theca cells and absent in the GC during the early follicular stages [15–18]. Theca cells are characterized by exhibiting steroidogenic activity in response to LH stimulation. Specifically, cholesterol is converted into androgens (i.e., testosterone and androstenedione) by transcription activities of cholesterol side-chain cleavage enzyme (P450scc), P450c17, and 3β-hydroxysteroid dehydrogenase (3β-HSD) genes. The starting point of steroid biosynthesis is cholesterol, a carbon 27 (C27) steroid. Cholesterol is converted to pregnenolone (C21) by P450scc (CYP11A – cytochrome P450, family 11, subfamily A, polypeptide 1), whose regulation is mediated by steroidogenic acute regulatory protein (StAR). StAR facilitates the influx of cholesterol into the mitochondria where P450scc is located. StAR expression is enhanced by cAMP and by stimulation of GC with FSH and LH or hCG [3, 4, 18, 19].

The primary route of pregnenolone metabolism is via the delta 5 pathway, the first two steps of which

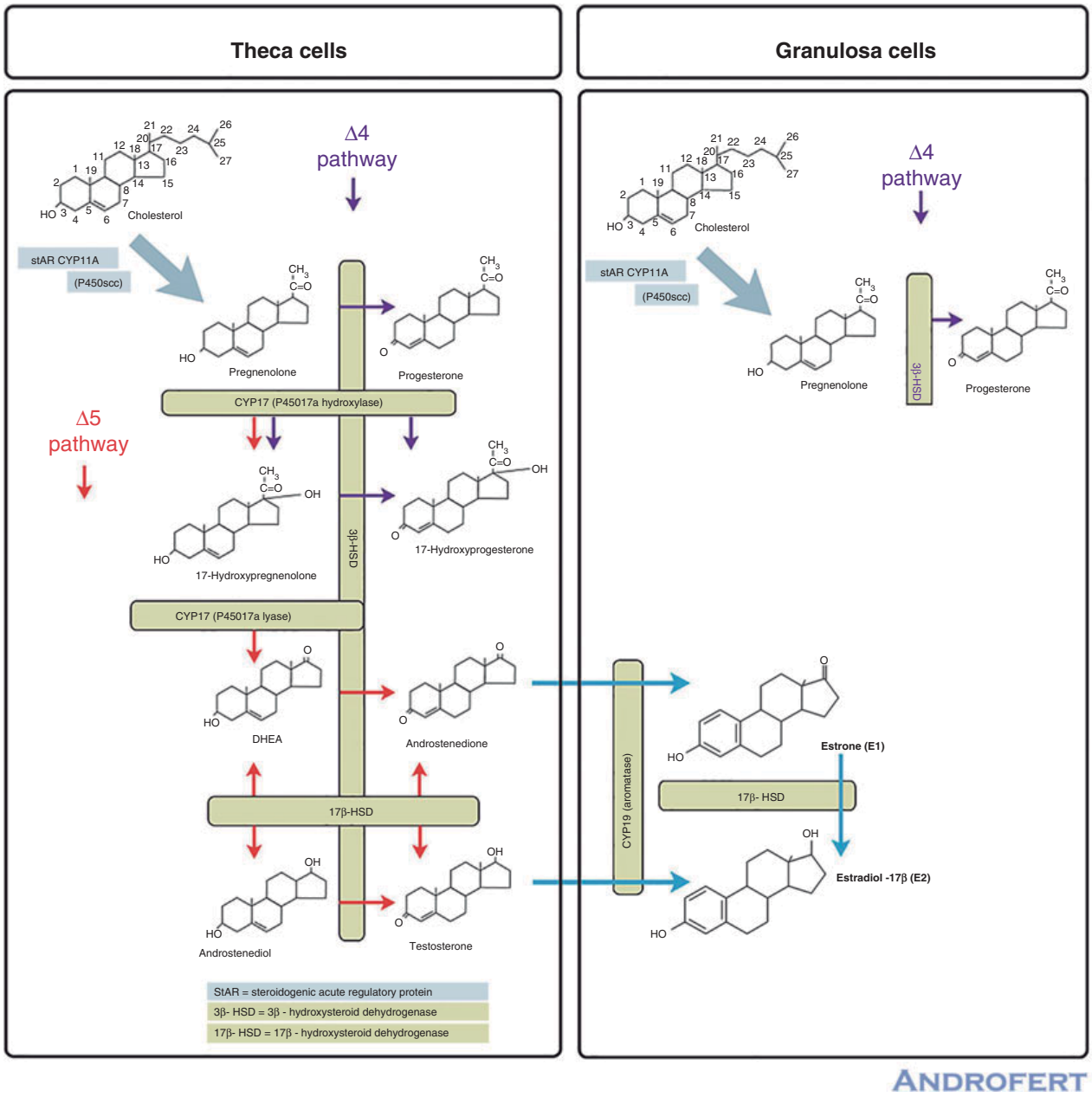

Fig. 16.4 Human ovarian steroidogenesis. The starting point for steroid biosynthesis is the conversion of cholesterol in pregnenolone by P450scc. One route of pregnenolone metabolism is the delta-5 pathway (*red arrows*) by the action of CYP17 (P450c17). Hydroxylation of pregnenolone at the C17a position forms 17-hydroxypregnenolone, and subsequent removal of the acetyl group forms the androgen precursor dehydroepiandrosterone (DHEA). Another route of pregnenolone metabolism is the delta-4 pathway (*purple arrows*) in which pregnenolone is converted to progesterone by the action of 3b-HSD (an irreversible conversion). Progesterone is then converted to 17-hydroxyprogesterone by CYP17. In humans, 17-hydroxyprogesterone cannot be further metabolized. Aromatization of androgens to estrogens is a distinct activity within the granulosa layer induced by FSH via activation of the P450 aromatase (P450arom) gene (From Leão and Esteves [3])

are driven by the same enzyme, CYP17 (P450c17). The hydroxylation of pregnenolone at the C17a position forms 17-hydroxypregnenolone, and the subsequent removal of the acetyl group forms the androgen precursor dehydroepiandrosterone (DHEA). Accordingly, CYP17 has both hydroxylase and lyase activity. Lastly, DHEA is converted to androstenedione by 3β-HSD [15, 19, 20]. A secondary route of metabolism involves the conversion of pregnenolone to progesterone by the action of 3β-HSD via the delta 4 pathway. Progesterone is then converted to 17-hydroxyprogesterone by CYP17 (Fig. 16.4) [20]. Importantly, CYP17 is located exclusively in thecal and interstitial cells, the

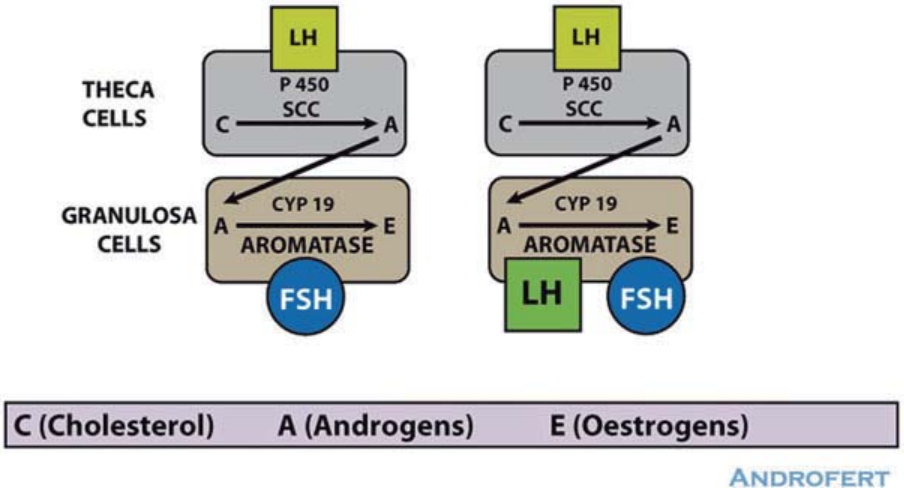

Fig. 16.5 The "two-cell" system. FSH receptors are present exclusively in the granulosa cells. LH receptors are present in the theca cells and initially absent in the granulosa cells. In response to LH, theca cells convert cholesterol to androgens (testosterone and androstenedione). CYP17 is located exclusively in thecal cells, whereas CYP19 (aromatase) is expressed only in the granulosa. Thus, androgens must diffuse into the granulosa layer to be converted to estrogen via aromatization induced by FSH. Both FSH and LH act via AMPc production. In the late follicular phase, FSH induces LH receptor formation in the granulosa cells, which acquire LH responsiveness. In the granulosa, LH enhances FSH action (increasing estrogen production)

extrafollicular compartment of the ovary, whereas CYP19 (aromatase), that converts androgens to estrogens, is expressed exclusively in GC, the intrafollicular compartment [20–22]. Thus, aromatization of androgens to estrogens is a distinct activity within the granulosa layer induced by FSH via activation of the P450 aromatase (P450arom) gene. Androgens produced in the theca layer must therefore diffuse into the granulosa layer to be converted to estrogens (Fig. 16.5). Hence, increasing levels of estradiol in the peripheral circulation during the follicular phase reflect the release of estrogen from granulosa cells into blood vessels [15, 22]. Theca and granulosa cells also secrete peptides that act as both autocrine and paracrine factors. Insulin-like growth factor (IGF) is secreted by theca cells and enhances LH-mediated androgen production within the thecal compartment as well as FSH-mediated aromatization in granulosa cells [15]. Inhibin and activin are produced in the granulosa cells in response to FSH and modulate the expression of steroidogenic enzymes, especially P450c17 in theca cells. While inhibin enhances androgen synthesis, activin has an opposite effect. Activin also has the important autocrine role of enhancing FSH action mainly by increasing the expression of FSH receptors [15] (Fig. 16.6).

LH also acts in GC to stimulate progesterone production. Most circulating progesterone (~95 %) is produced in the intrafollicular compartment by the granulosa cells via the action of 3b-HSD that catalyzes conversion of pregnenolone (delta-4 pathway) under the LH influence (see Fig. 16.4) [18, 22]. Despite a marked increase in progesterone levels measured at the veins of the active ovary in the mid-follicular phase, peripheral concentrations increase only slightly probably due to active liver metabolism [23]. Progesterone can be further converted to 17-hydroxyprogesterone by CYP17 (via delta-4 pathway). However, very little 17-hydroxyprogesterone is converted to androstenedione, since human CYP17 catalyzes this reaction at only 3 % of the rate for the conversion of 17-hydroxypregnenolone to DHEA [18, 22, 24]. Therefore, 17-hydroxyprogesterone is basically the final product of the delta-4 pathway in humans. Moreover, progesterone itself cannot be metabolized in the GCs because CYP17 is not expressed within this cell compartment; as such, progesterone is the final product of the delta-4 pathway in the intrafollicular compartment and cannot be converted to estradiol in the GC under the effect of LH [20]. The preovulatory rise in progesterone facilitates the positive feedback action of

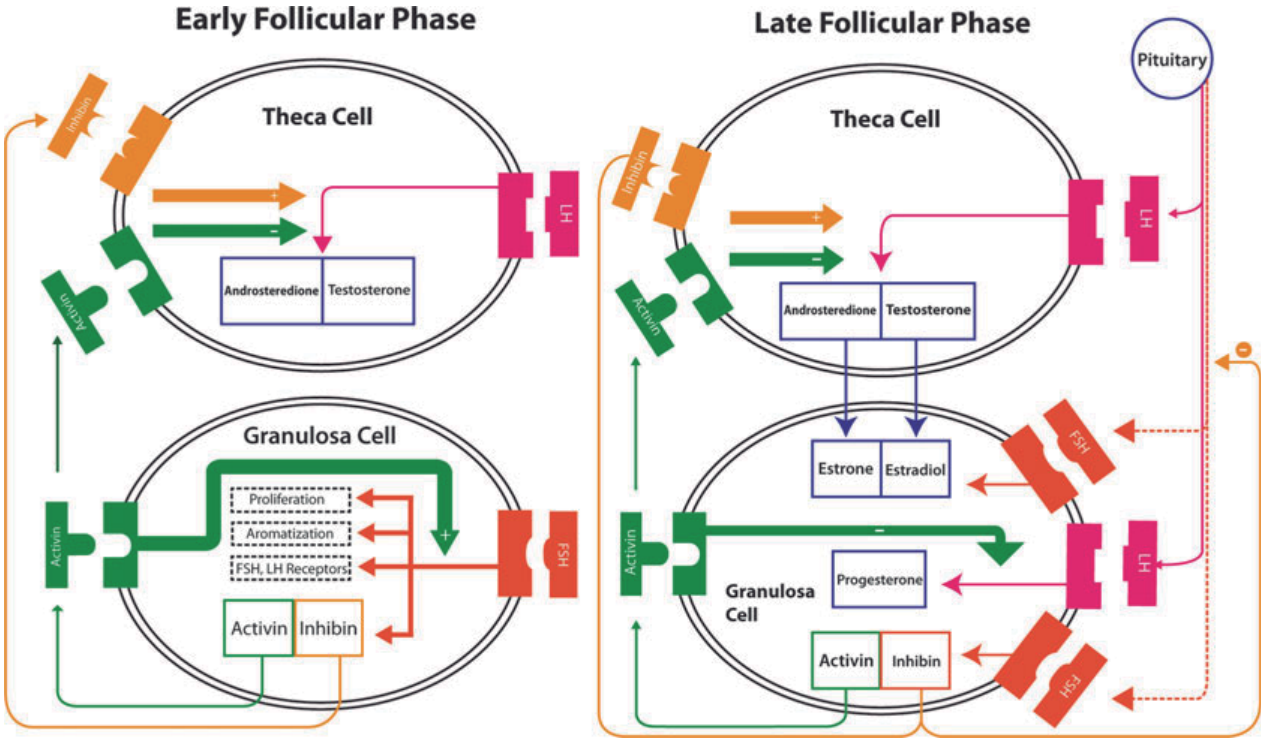

Fig. 16.6 Modulation of steroidogenic enzymes. In the early follicular phase, inhibin and activin are produced in the granulosa cells in response to FSH. They have important paracrine functions to modulate the expression of steroidogenic enzymes, especially P450c17 in theca cells. Inhibin enhances LH function, thus stimulating androgen synthesis to latter aromatization to estrogen in the granulosa, whereas activin suppresses androgen synthesis. Activin has also an important autocrine role of enhancing FSH action, especially by increasing the production of FSH receptors. Production of inhibin by the granulosa cells is increased in the late follicular phase while activin is decreased, with a positive effect on androgen production by theca cells. FSH induces LH receptor formation in the granulosa cells, which acquire LH responsiveness and therefore less FSH dependence. In granulosa, LH enhances FSH action that in turn increases estrogen production, initiates progesterone production (negatively modulated by activin), and control granulosa production of inhibin. The increase in inhibin, in turn, suppresses FSH secretion by the pituitary, important to ensure the dominance of the follicle

estrogen on the pituitary; the latter is the key factor to induce the midcycle LH peak in the natural cycle. Progesterone also stimulates a midcycle FSH surge, important to support the full expression of LH receptors at the granulosa layer [22, 25].

In summary, ovarian steroidogenesis is the result of combined LH and FSH stimulation of the two cell types, theca and granulosa, influenced by autocrine and paracrine factors.

16.2.2 The Role of LH on Follicular Maturation and Luteal Phase Support

In the mid-follicular phase, FSH induces LH receptor expression in the granulosa cells of developing follicles [26]. The action of LH on its receptors activates cyclic AMP-protein kinase A (cAMP/PKA) pathway, which represents an additional stimulus to follicular growth [27]. Thereby, the maturing follicle also reduces its dependency on FSH by acquiring LH receptors and LH responsiveness [26–30]. FSH and LH cooperate in inducing the local production of the soluble molecule inhibin B and growth factors. Among these, insulin growth factors (IGF) I and II, which are expressed by both granulosa and theca cells throughout folliculogenesis, are important in promoting follicular maturation [31, 32]. Furthermore, LH exerts an antiapoptotic effect on the GCs, mediated by the production of fibroblast growth factors that maintain calcium homeostasis and granulosa cell viability by stimulating calcium efflux via a protein kinase C (PKC) delta-dependent pathway [33]. Additional signaling

pathways (e.g., AKT and ERK1/2 pathways) involve the expression of EGF-like growth factors that influence GC proliferation, differentiation, and survival (apoptosis blockage) [34, 35]. Lastly, aromatase expression and steroidogenic function via LH receptor activation are likely to involve cAMP/PKA, extracellular signal-regulated (ERK) 1 and 2, and AKT pathways, all playing a crucial role in the final stages of maturation of human oocytes and follicles [36, 37].

LH activity during the luteal phase is totally responsible for the maintenance and the steroidogenic activity of the corpus luteum [38]. LH is responsible for the upregulation of growth factors like vascular endothelial growth factor A [39, 40], which plays a dynamic role in luteal angiogenesis, and epidermal growth factor-like ligands, amphiregulin and epiregulin, which regulate apoptosis in luteinized human granulosa cells [34, 41–44]. Furthermore, LH stimulates expression of extragonadal LH receptors in the endometrium [45, 46] and production of cytokines involved in implantation [47].

Therefore, LH regulates both granulosa and theca cells and has a pivotal role in follicular development and maturation. In light of the aforementioned findings, we can conclude that (1) both gonadotropins contribute (via granulosa) to maintain the autocrine–paracrine system governing dominant follicle's growth; (2) LH is crucial in sustaining FSH activity in the granulosa during intermediate–late stages of folliculogenesis; and (3) LH is critical for maintaining corpus luteum function during the luteal phase.

16.3 Rationale of LH Supplementation in Stimulated Cycles

The "LH window" concept, as outlined by Shoham in 2002, proposes that in the absence of a threshold level of serum LH, estradiol production will be insufficient for follicular development, endometrial proliferation, and corpus luteum formation [48]. This concept can be clearly observed in patients with hypogonadotrophic hypogonadism who do not achieve adequate steroidogenesis by stimulation with FSH alone, but resume sufficient estradiol production by LH supplementation [49]. Evidence therefore suggests that in reproductive cycles optimal follicular development occurs within a "LH window," that is, above an LH threshold of 1.1 and below an LH ceiling of 5.1 IU/L [48, 49].

After pituitary suppression, still widely used in association with COS, residual circulating levels of endogenous LH are usually adequate to support multiple follicular growth and oocyte development in COS with gonadotropins devoid of LH activity [50, 51]. In fact, only 1 % of LH receptors need to be occupied to drive adequate ovarian steroidogenesis. Fair evidence indicates that most normogonadotropic women have sufficient levels of endogenous LH and do not require exogenous LH supplementation [52–54]. Despite of that, a recent large meta-analysis including a total of 40 RCTs and 6443 women aged 18–45 years found a small relative increase (estimate of 9 %) in clinical pregnancy rate in patients treated with of rec-hFSH plus rec-hLH versus rec-hFSH alone (RR 1.09; 95 % CI 1.01–1.18) [55]. More importantly, ovarian response to COS with FSH-only-containing gonadotropins has shown to be suboptimal in subsets of normogonadotropic women, including those with advanced reproductive age (≥35 years old) [56, 57], diminished ovarian reserve [54, 58], and highly suppressed levels of endogenous LH, in whom LH activity falls below the LH threshold [59–63]. In addition, a subgroup of normogonadotropic patients who had normal estimated ovarian reserve but suboptimal responses to FSH-alone stimulation has also been identified and termed "hyporesponders" [64–67].

Clinical evidence indicates that the aforementioned subgroups have less responsive ovaries in stimulated cycles with FSH, which could be explained by a wide range of factors, including reduced paracrine ovarian activity [68], genetically determined reduced LH bioactivity [69], reduced androgen secretory capacity [70], and decreased number of functional LH receptors [71]. Serum androgen levels, especially total testosterone (T), calculated free T, dehydroepiandrosterone sulfate, and androstenedione, decline

steeply with age, with the decline of each being greater in the early reproductive years than the later decades [72, 73]. Hence, it has been hypothesized that such women would benefit from LH-containing gonadotropin preparations. Action of LH at the follicular level could promote an increase in ovarian steroidogenesis and androgen production for its later aromatization into estrogens, with a positive impact on the follicular milieu. Furthermore, LH has also a direct effect on follicular growth and maturation via different signaling pathways that positively impact oocyte quality [74].

16.4 Clinical Evidence Supporting LH Supplementation During COS in Selected Patients

16.4.1 Hypogonadotropic Hypogonadism

The European Recombinant Human LH Study Group investigated the efficacy of rec-hLH for supporting FSH-induced follicular development in hypogonadotropic hypogonadal women (LH levels of <1.2 IU/l; WHO group I anovulation) [49]. Thirty-eight patients were randomized to receive 0, 25, 75, or 225 IU/day of rec-hLH in addition to a fixed dose of rec-hFSH (150 IU/day). The authors found that rec-hLH was able to promote a dose-related increase in estradiol and androstenedione secretion by rec-hFSH-induced follicles. Serum concentrations on the last day of FSH administration were 65 ± 4, 195 ± 94, 1392 ± 585, and 2441 ± 904 pmol/L for E2 and 3.6 ± 0.9, 5.1 ± 1.3, 6.4 ± 1.3, and 6.7 ± 1.3 nmol/L for androstenedione in the patients treated with 0, 25, 75, and 225 IU rec-hLH, respectively. LH supplementation also increased ovarian sensitivity to FSH, as shown by the proportion of patients who developed follicles after the administration of a defined dosage of FSH. While only 12.5 % of the patients treated with FSH alone developed follicles, the proportion substantially increased, according to the varying doses of rec-hLH (42.8 % in 25 IU and 77.8 % and 80 % in 75 IU and 225 IU, respectively). Furthermore, follicles that had been exposed to rec-hLH showed an increased ability to luteinize after hCG exposure.

In the aforementioned study, a daily dose of 75 IU rec-hLH was effective in the majority of women in promoting optimal follicular development (defined as >or=1 follicle >or=17 mm; E2, >or=400 pmol/L; midluteal phase progesterone, >or=25 nmol/L) and maximal endometrial growth. Lastly, rec-hLH was shown not to be immunogenic and was well tolerated by the patients.

In conclusion, exogenous LH activity supplementation is mandatory in stimulation protocols applied to women with hypogonadotropic hypogonadism.

16.4.2 Older Women (>35 Years Old)

The impact of luteinizing hormone administration in ovarian stimulation with gonadotropin-releasing hormone (GnRH) antagonist cycles was examined by Bosch and colleagues in a randomized controlled trial (RCT) involving 720 women undergoing their first or second IVF [75]. The authors compared cycle outcome, according to the use of rec-hFSH or rec-hFSH + rec-hLH in an age-adjusted analysis. For the patients <36 years old, the total starting dose of gonadotropins was 225 IU/d for both stimulation protocols. In the rec-hFSH-alone group, 225 IU/d SC of rec-hFSH was administered, and the starting dose for the rec-hFSH + rec-hLH group was 150 IU/d of rec-hFSH and 75 IU/day of rec-hLH. As noted, LH supplementation was started on stimulation day 1. For those patients aged 36–39 years, the total starting dose of gonadotropins was 300 IU/d for both study groups: rec-hFSH-alone, 300 IU/d SC of rec-hFSH was administered, and for the rec-hFSH + rec-hLH group, 225 IU/d of rec-hFSH + 75 IU/d rec-hLH. The rec-hLH dose remained fixed across the cycle. In the younger population (up to 35 years old), implantation rates were similar, 27.8 % versus 28.6 %, odds ratio (OR) 1.03 (95 % confidence interval [CI] 0.73–1.47), as was the ongoing pregnancy rate per started cycle, 37.4 % versus 37.4 %, OR 1.0 (95 % CI 0.66–1.52). In older patients

(36–39 yrs.), the implantation rate was significantly higher in the rec-hFSH + rec-LH group: 26.7 % versus 18.6 %, OR 1.56 (95 % CI 1.04–2.33). Ongoing pregnancy rates per started cycle were not statistically different: 33.5 % versus 25.3 %, OR 1.49 (95 % CI 0.93–2.38).

Contrary results have been reported by Konig et al. in an RCT involving 253 couples undergoing IVF/ICSI [76]. In their study, women were 35 years or older and received ovarian stimulation in a GnRH antagonist protocol with either rec-hFSH 225 IU/day or rec-hFSH + rec-hLH 150 IU/d starting on stimulation day 6. The intention-to-treat analysis revealed implantation rates (18.8 % vs. 20.7 %; mean difference −1.9 %, 95 % confidence interval [CI] −8.0 to 11.7) and clinical pregnancy rates (28.0 % vs. 29.7 %; mean difference −1.5 %, 95 % CI −9.4 to 12.7).

A systematic review and meta-analysis of the studies examining the age-related effects of LH supplementation in COS were conducted by Hill and colleagues [57]. The authors demonstrated that LH supplementation in women aged >34 years old undergoing COS with rec-hFSH was beneficial. Their study included 7 RCTs (902 women) and compared COS using rec-hFSH alone or in combination with rec-hLH. GnRH-agonist downregulation was used in five trials, while GnRH antagonist and GnRH-agonist micro-flare were used in the remaining trials. The dose and day of starting rec-hLH supplementation varied among trials. In five of them a fixed dose of 150 IU rec-hLH, which started either on the sixth or seventh stimulation day, was used. One trial used a fixed 2:1 ratio of rec-hFSH and rec-hLH, while another used a fixed dose of 75 IU rec-hLH regardless of the FSH dose; in both of them LH supplementation was given from the first day of stimulation on. Implantation (OR = 1.36; 95 % CI: 1.05–1.78, $I^2 = 12$ %) and clinical pregnancy rates (OR = 1.37; 95 % CI: 1.03–1.83, $I^2 = 28$ %) were significantly higher for women who received rec-hLH in addition to rec-hFSH compared with those in whom rec-hFSH was administered alone.

The meta-analysis by Hill et al. was subsequently reexamined by Konig and colleagues, who replaced the study of Bosch and cols. by their own and concluded that no effect whatsoever could be observed by adding LH to older patients [77]. Nevertheless, a methodological bias could have been produced by replacing the Bosch and colleagues' study, which represented 36 % of the weight of all studies pooled in the aforementioned meta-analysis, by the one of Konig and cols. because the protocols of COS differed with regard to the day LH supplementation has started; LH supplementation was started in the mid-follicular phase in the latter in contrast to the former, in which rec-LH was administered since the first day of stimulation. It has been suggested that LH supplementation should be initiated on the beginning day of stimulation to get total advantage of the LH effects on both theca and granulosa cells [78].

In conclusion, evidence suggests that rec-hLH supplementation has a positive effect on cycle outcome of older women (>35 years old), particularly when used from the start of COS. Nevertheless, given the heterogeneity of the published data, additional large RCTs examining the impact of rec-hLH supplementation from the early phases of COS are needed to draw a conclusive recommendation about the routine incorporation of rec-hLH in older women undergoing IVF/ICSI.

16.4.3 Poor Responders

Mochtar et al., evaluating poor responders, have demonstrated the usefulness of adding rec-hLH to COS. These authors pooled three RCTs including 310 participants and showed that higher ongoing pregnancy rates (OR = 1.85; 95 % CI: 1.1–3.11) were obtained in patients treated with the combination of rec-hFSH and rec-hLH compared with rec-hFSH alone [54]. In another meta-analysis of Bosdou et al., which included 7 RCTs and 603 patients classified as poor responders, differences in clinical pregnancy were not detected in the group of patients receiving LH supplementation [58]. Nevertheless, the definition criteria for poor responders were not uniform among the included studies, and two of the RCTs evaluated slow/hyporesponders rather than poor responders.

The protocols of stimulation also varied as GnRH antagonists and agonists were applied in two trials each, and GnRH-agonist short protocol was used in three subjects. The way rec-hLH supplementation was given also varied as daily doses of either 75 IU or 150 IU were used, and the starting day differed or was not traced. Rec-hLH was added to rec-hFSH from the first stimulation day in one trial, at stimulation day 7 in three trials, at day 8 in one trial, and on the day of the first GnRH antagonist injection in another trial. Although statistical significance was not reached, the magnitude of the effect size and the width of the 95 % CI regarding the clinical pregnancy rates (RD = +6 %; 95 % CI: −0.3 to +13 %; $p = 0.06$) suggested a potential clinical benefit of LH supplementation. Nevertheless, the authors of the aforesaid meta-analyses did find that rec-hLH supplementation was beneficial in terms of live birth rates after IVF (RD = +19 %; CI: +1 to +36 %), but their results were derived from a single RCT.

Lately, a large meta-analysis assessed the outcomes of rec-hFSH plus rec-hLH or rec-hFSH alone for ovarian stimulation in association with GnRH analogues during ART [55]. A total of 40 RCTs involving 6443 women aged 18–45 years were included, of which 14 studies (1129 patients) specifically investigated poor responders. Poor response (POR) was defined according to study authors' criteria and although the studies were published prior to the European Society of Human Reproduction and Embryology (ESHRE) consensus definition of POR [79], in 10 of the 14 studies reporting POR data, the definition of POR employed was aligned with the subsequently reported ESHRE definition. According to the ESHRE consensus, POR is defined by the presence of at least two of the following three features: (1) advanced maternal age (≥40 years) or any other risk factor for POR, (2) a previous POR (≤3 oocytes with conventional stimulation), and (3) an abnormal ovarian reserve test (antral follicle count [AFC] <5–7; anti-Mullerian hormone [AMH] <0.5–1.1 ng/mL), but two episodes of POR after maximal COS per se are sufficient to define a patient as poor responder. Patients of advanced age with an abnormal ORT may be classified as POR since these features indicate reduced ovarian reserve and act as a surrogate of ovarian stimulation cycle outcome. In this case, the patients should be defined as "expected poor responder" [79]. In the aforementioned study by Lehert and colleagues, significantly more oocytes were retrieved with rec-hFSH plus rec-hLH versus rec-hFSH alone in poor responders (12 studies, $n = 1077$; weighted mean difference +0.75 oocytes; 95 % CI 0.14–1.36). Also, significantly higher clinical pregnancy rates were observed in this patient category with rec-hFSH plus rec-hLH versus rec-hFSH alone (14 studies, $n = 1179$; RR 1.30; 95 % CI 1.01–1.67; ITT population).

In conclusion, current evidence suggests that there is an increase in both the number of oocytes retrieved and clinical pregnancy rates in poor responders treated with rec-hLH in addition to rec-hFSH.

16.4.4 Hyporesponders

The concept of "hypo-response" to COS has been proposed to identify those at first hand good prognosis normogonadotropic young women with normal ovarian reserve who turn out to require high amounts of rec-hFSH (e.g., >2500 IU total dose) to obtain an adequate number (i.e., >4) of oocytes retrieved [64–67]. Such normoovulatory and normogonadotropic women differ from classical poor responders because they are usually young (<39 years) and ovarian biomarkers (AMH/AFC) are within normal ranges. Although the pathogenesis of hyporesponsiveness to FSH is unknown, it has been speculated that hypo-response is a genetically determined condition (*see Chapter 14: Pharmacogenomics Approach to Controlled Ovarian Stimulation*). More specifically, ovarian resistance to exogenous FSH has been associated with the presence of at least two genetic variations, including a polymorphic allele of the LH beta-subunit gene (v-betaLH), which has been shown to have altered in vitro and in vivo activities, and FSH receptor (FSH-R) Ser/680 variant [69, 80–83]. Given a possible association between ovarian resistance to FSH stimulation (hypo-response)

and a genetically determined less bioactive LH molecule or FSH-R dysfunction, several investigators have examined the roles of exogenous LH activity supplementation and increased FSH doses on ART cycle outcome.

The role of LH supplementation and increased FSH doses in hyporesponders were evaluated by Ferraretti and colleagues, who conducted an RCT involving 184 patients (age <38 years) undergoing COS for IVF after pituitary desensitization [67]. Hyporesponsiveness to rec-hFSH was defined by the observation of a steady follicular growth (>10 antral follicles ≥8 mm in diameter) and estradiol levels (≥100 pg/mL) between stimulation days 7–10 despite continuous rec-hFSH administration. Upon reaching this stage, patients were randomized to receive (1) an increased rec-hFSH dose alone (max 450 IU/daily; $n=54$), (2) LH activity supplementation with rec-hLH (75 IU/day or 150 IU/day) in addition to an increased FSH dose ($n=54$), and (3) LH activity supplementation with hMG in addition to an increased rec-hFSH dose ($n=26$). Fifty-four age-matched women with normal responses to COS were included as a control group. The average number of oocytes retrieved was significantly lower in hyporesponders treated with rec-hFSH step-up (8.2) versus the other three groups (11.1, 10.9, 9.8), respectively. Pregnancy rates were significantly higher in the group treated with rec-hLH plus increased rec-hFSH dose (54.4 %) compared with both the patients receiving rec-hFSH alone (24.4 %) and hMG (11 %; $p<0.05$). Pregnancy rates in the group of women receiving rec-hLH supplementation and an increased rec-hFSH dose were not different from controls (41 %). Although live birth rates in both rec-hLH (40.7 %) and control (37 %) groups were twofold higher than the other two groups (22 % and 18 %, respectively), the difference did not reach statistical significance.

The role of rec-hLH supplementation per se in hyporesponders was evaluated by De Placido and colleagues, who conducted a multicenter RCT involving a total of 117 IVF/ICSI cycles [66]. Hyporesponders (age <37 years, basal FSH ≤10 IU/l) were defined by the presence of low serum estradiol levels (below 180 pg/mL) and at least 6 follicles ranging between 6 and 10 mm, but no follicles over 10 mm on stimulation day 8 with rec-hFSH. After pituitary desensitization and stimulation with a fixed dose (225 IU/day) of rec-hFSH for the first 8 days, the patients were randomized to receive either an additional 150 IU/day of rec-hLH supplementation ($n=65$) or an increase in the daily dose of rec-hFSH by 150 IU/day ($n=65$; rec-hFSH "step-up" protocol). An age/BMI-matched population of "normal responders" (i.e., tripling E_2 levels between stimulation days 5 and 8 and more than 4 follicles >10 mm on stimulation day 8) was selected as a control group ($n=130$). The number of oocytes retrieved was significantly higher in the patients who received rec-LH supplementation (9.0 ± 4.3) compared with those in whom an increased dose of rec-hFSH was administered (6.1 ± 2.6; $p<0.01$), and the results of both aforementioned groups were lower than those obtained in the control group (10.49 ± 3.7; $p<0.05$). Implantation and ongoing pregnancy rates were similar in "hyporesponders" treated with rec-hLH and "normal responders" (14.2 % and 32.5 % vs. 18.1 % and 40.2 %, respectively). Conversely, both parameters were significantly lower ($p<0.05$) in "hyporesponders" treated with step-up rec-hFSH (10.0 and 22.0 %).

In conclusion, these studies reinforced the idea that hyporesponders benefit from LH supplementation and that hyporesponsiveness to rec-hFSH could be related to the presence of less bioactive LH due to specific genetic-determined variations in the beta subunit of the LH molecule.

16.4.5 Deeply Suppressed LH Levels

Profound suppression of LH concentrations as a consequence of GnRH-agonist downregulation has been found in 7–48 % of normogonadotropic women undergoing controlled ovarian stimulation [60, 61, 84, 85]. This wide variation might be justified by the type and mode of GnRH-agonist action. Intranasal administration of buserelin resulted in significantly less depressed levels of mid-follicular LH levels compared with the subcutaneous route. Moreover, the inhibitory

effect on ovarian steroidogenesis and follicular development is more evident with the more potent buserelin than with leuprolide acetate [61, 85].

Early studies have suggested that ovarian response and IVF cycle outcome are negatively impacted when mid-follicular serum LH levels are below a certain threshold (between 0.5 and 0.7 UI/L) after downregulation with GnRH agonists and ovarian stimulation with FSH monotherapy [59–63, 84]. Westergaard and colleagues, retrospectively analyzing 200 normogonadotropic women, reported that as many as 49 % of those stimulated with rec-hFSH under pituitary suppression with buserelin acetate (0.5 mg SC daily) for 14 days achieved very low concentrations of LH (<0.5 IU/L) in the mid-follicular phase, albeit GnRH-a dose was reduced to 0.2 mg SC per day during ovarian stimulation [60]. In comparison with the normal LH group, these women had serum estradiol concentrations significantly lower on Sd8 (1349 ± 101 vs. 2908 ± 225 pmol/L; $p < 0.001$). Although the proportion of patients with a positive pregnancy test was similar in the two groups (30 % vs. 34 % per started cycle in the low and normal LH groups, respectively), a fivefold higher risk of early pregnancy loss was observed in the low LH group (45 % vs. 9 %; $p < 0.005$). In another study, Fleming and colleagues found that 26 % of women treated with highly purified or recombinant FSH and GnRH agonist (type and dose not reported) had suppressed LH concentration (≤0.7 IU/l) on Sd7 [84]. Patients with suppressed LH had lower estradiol concentrations ($p = 0.001$) irrespective of whether the FSH is derived from purified urinary or recombinant sources. However, the negative impact on cycle outcome (longer treatment duration combined with a reduced oocyte yield) was observed only in women treated with urinary FSH, thus indicating that the more potent recombinant FSH treatment could overcome the impact of LH suppression upon gross ovarian response. Furthermore, no effect upon pregnancy outcome was observed irrespective of the level of LH suppression and type of FSH preparation. Humaidan and colleagues also studied the effect of LH levels on stimulation day 8 on ovarian response and pregnancy outcome [61]. The authors retrospectively analyzed 207 normogonadotropic women receiving pituitary downregulation with buserelin acetate (0.8 mg SC daily until pituitary downregulation and 0.4 mg/day during ovarian stimulation) and ovarian stimulation with rec-hFSH. LH levels on Sd8 were directly related to estradiol levels and inversely related to the total consumption of exogenous FSH and duration of gonadotropin stimulation ($p < 0.002$). In their study, however, only 12 % of the patients showed LH levels <0.5 IU/L. While the number of retrieved oocytes was not affected by LH suppression, the frequency of fertilized oocytes was significantly lower in the group with profound LH suppression ($p < 0.05$). Likewise in the study of Fleming and cols., pregnancy and implantation rates were not significantly affected by profound mid-follicular LH suppression. Taken together, these aforementioned studies indicate that deeply suppressed LH levels in GnRH agonist-treated women have a significant impact on the ovarian response during ovarian stimulation, but its impact on pregnancy outcome is controversial.

Contrary results have been reported by Balasch and colleagues studying 144 infertile women undergoing IVF/intracytoplasmic sperm injection (ICSI) treatment, in whom pituitary desensitization was carried out by the administration of leuprolide acetate (1 mg SC daily, then reduced to 0.5 mg after downregulation was confirmed) [85]. Using a receiver-operating characteristic (ROC) analysis, the authors showed that the serum LH concentration on Sd7 was unable to discriminate between conception and non-conception cycles (AUC = 0.52; 95 % CI: 0.44–0.61). In this study, only 7 % of the patients had mid-follicular LH serum concentration <0.5 IL/L, and no significant differences were found with respect to ovarian response, number of oocytes retrieved, IVF/ICSI outcome, implantation, and the outcome of pregnancy between these patients and those with normal LH on Sd7.

In conclusion, conflicting evidence exists regarding the impact of deeply suppressed levels of mid-follicular serum LH levels on ovarian response to stimulation with rec-FSH. Current data indicate that the choice of GnRH-a plays a role in the frequency of patients exhibiting profound mid-follicular LH suppression. Whether these women would benefit from supplementation with LH activity during ovarian stimulation remains to be proven.

16.5 Gonadotropin Preparations Containing LH Activity

Currently, there are three commercially available gonadotropin preparations containing LH activity: (1) urinary hMG, in which LH activity is dependent on hCG rather than LH, (2) pure LH glycoprotein produced by recombinant technology (lutropin alfa), and (3) a combination of FSH (follitropin alfa) and LH (lutropin alfa) in a fixed ratio of 2:1 also manufactured by recombinant technology (Sect. 16.1) [3].

16.5.1 Menotropin

Menotropin, or human menopausal gonadotropin (hMG), was first extracted from the urine of postmenopausal women in 1949 [2]. Early preparations contained varying amounts of FSH, LH, and hCG in only 5 % pure forms [1–3]. Improvements in the purification techniques standardized FSH and LH activities to 75 IU for each type of gonadotropin in 1963, as measured by standard in vivo bioassays (Steelman–Pohley assay). Human menopausal gonadotropin preparations have both FSH and LH activity, but the latter is primarily derived from the hCG component present in postmenopausal urine and concentrated during purification [2, 86, 87, 88]. Sometimes hCG is added to achieve the desired amount of LH-like biological activity [2]. In 1999, purified hMG gonadotropins were introduced, allowing its subcutaneous (SC) administration [2, 3]. At present, both conventional hMG and highly purified hMG (HP-hMG) are commercially available in vials containing lyophilized powder of FSH and LH at 1:1 ratio [3]. The enhanced purity of HP-hMG enabled subcutaneous delivery [3].

16.5.2 Lutroprin Alfa

Lutroprin alfa was introduced in the market in the year 2000 intended for promoting ovarian stimulation in women with WHO type I anovulation. The manufacturing process of lutropin alfa involves recombinant technology in which the genes coding for human LH alpha and beta subunits are incorporated into the nuclear DNA of Chinese hamster ovary (CHO) cells via a plasmid vector [2, 3, 88]. As a result, a master LH-producing cell bank is built [88, 89]. A working cell bank is then made by growing cells in culture flasks, which are afterwards combined with a suspension of microcarrier beads and transferred to a bioreactor vessel with continuous culture media infusion. The cell culture supernatant medium, containing the proteins secreted by the cells, is collected from the bioreactor. The harvested "crude LH" is then purified by chromatography, followed by ultrafiltration. Each purification step is rigorously controlled in order to ensure batch-to-batch consistency of the final purified product that is the recombinant human LH (rec-hLH) [3].

Lutropin alfa is highly pure and has high biological activity (9000 IU/mg protein) [3, 90]. It is presented in vials of 82.5 IU lyophilized pure glycoprotein powder to be reconstituted with diluent before administration using a conventional syringe and needle (75 IU of lutropin alfa is delivered per vial). Lutropin alfa is intended for subcutaneous daily injections, which represents an important gain for patients as better tolerability (lower pain at injection site) has been reported with SC injections compared with the intramuscular route. Importantly, SC injections allow self-administration that is more convenient and less time consuming as patients need fewer visits to the clinic or hospital for injections [91, 92]. Due to the relatively short half-life of LH, daily injections of lutropin alfa are needed during the stimulation period [3, 90]. After each injection, terminal half-life is reached within 10–12 h, and then LH levels decline until the next injection.

16.5.3 Follitropin Alfa in Combination with Lutropin Alfa

A preparation containing both rec-hFSH (follitropin alfa) and rec-hLH (lutropin alfa) at 2:1 ratio was launched in 2007 [93]. The 2:1 ratio of FSH and LH in a fixed dose combination was obtained by recombinant technology and vial

Table 16.1 Differences in LH activity among gonadotropin preparations

	Purity (LH content)	FSH activity (IU/vial)	LH activity (IU/vial)	hCG content (IU/vial)	Specific activity (LH/mg protein)
Lutropin alfa	>99 %	0	75[a]	–	9000
Follitropin alfa + lutropin alfa 2:1 ratio	>99 %	150	75	–	9000
HP-hMG	Unknown[b]	75	75[b]	~8	–

[a]1 μg of lutropin alfa = 22 IU
[b]Derives primarily from the hCG component, which preferentially is concentrated during the purification process and sometimes was added to achieve the desired amount of LH-like biological activity
HP-hMG highly purified human menopausal gonadotropin

filling using protein mass (FbM). The use of FbM as opposed of filled-by-bioassay was possible due to the specific activity; isoform distribution and sialylation profile of both gonadotropins are highly consistent among manufactured batches [88]. It is intended for subcutaneous daily injections and is presented in vials of lyophilized pure glycoprotein powder to be reconstituted with diluent before administration using a conventional syringe and needle (150 IU of follitropin alfa and 75 IU of lutropin alfa is delivered per vial). The results of two phase I, randomized, crossover studies demonstrated bioequivalence between rec-hFSH and rec-hLH administered alone or in fixed 2:1 combination, thus allowing administration of both recombinant gonadotropins in a single injection [94].

16.6 Differences in LH Activity Between rec-hLH and hMG Preparations

Recombinant LH has three major differences compared with hMG preparations. First, rec-hLH has a better quality and safety profile compared with hMG [3, 90]. High purity and specific activity are common features of gonadotropin preparations manufactured using recombinant technology. Each product batch of recombinant LH is routinely characterized and controlled using physicochemical techniques, including size-exclusion high-performance liquid chromatography (SE-HPLC), which allows assessment of both the integrity and the amount of glycoproteins, and isoelectric focusing (IEF) and glycan mapping, which characterize protein glycoforms present in each preparation [95, 96]. Conversely, the manufacturing process of urine-derived gonadotropins is less stringent, as urine is pooled and the donor source cannot be fully traced. As the pool is constantly changing, standardization is difficult to ascertain [97–99]. Although sophisticated purification techniques are currently available, which allow the safe clinical use of urinary formulations, extraneous urinary proteins may account for more than 30 % of the protein content in high-purified hMG products (Table 16.1) [3, 100].

Second, rec-hLH is associated with better dose precision due to fill-by-mass (FbM) technology that virtually eliminates batch-to-batch variation [2, 3, 100, 101]. The conventional method used to quantify the glycoprotein activity in gonadotropin products is the Steelman–Pohley assay, which is an in vivo rat bioassay. As well as being costly and subject to ethical concerns related to the use of animals, this technique has an inherent variability of up to 20 % [101, 102]. In 2003, Driebergen and Baer demonstrated the batch-to-batch consistency of follitropin alfa in terms of specific activity, isoform pattern, and sialylation profile [101]. The authors showed that there was a constant relationship between FSH mass and its biological activity. Following these observations, a new method was developed to calibrate each batch of follitropin alfa, and also lutropin alfa, using SE-HPLC, which measures glycoprotein content by protein mass. This technique has enabled lutropin alfa to be filled and released on the basis of mass (75 IU of

Table 16.2 Structural characteristics, half-life in serum, and downstream effects of LH and hCG following receptor binding

	LH	hCG
Amino acid number		
Alpha subunit	92	92
Beta subunit	121	145
N-linked glycosylation sites		
Alpha subunit	2	2
Beta subunit	1	2
O-linked glycosylation sites	–	4
Carboxyl terminal segment	Nonexistent	Present
Half-life (hours)		
Initial, range of mean	0.6–1.3	3.9–5.5
Terminal, range of mean	9–12	23–31
Response		
ED50 (pM)[a]	530.0 ± 51.2	107.1 ± 14.3
Time to maximal cAMP accumulation[a]	10 min	1 h
ERK 1/2 activation[b]	Strong	Weak
AKT activation[b]	Strong	Minimal
CYP19A1 expression in presence of ERK1/2 pathway blockade[b]	Increased	Unaffected

Initial half-life (distribution): time for the plasma concentration to decrease steeply because of the distribution into tissues

Terminal (elimination) half-life = time that it takes for the concentration in blood plasma of a substance to reach one-half of its steady-state value

ERK extracellular signal-regulated kinases, *AKT* protein kinase B, *CYP19A1*0 cytochrome P450, family 19, subfamily A1

[a]Median effective dose to produce a response 50 % of the COS-7/LHCGR cells, which constitutively express LH receptors

[b]Effect on human granulosa cells

LH assessed by the Steelman–Pohley assay corresponds to 3.4 μg of lutropin alfa), with dose variability of only 2 % [90, 101].

Third, LH activity is derived from pure LH glycoprotein unlike hMG, in which hCG is concentrated during purification or added to achieve the desired amount of LH-like biological activity [2, 3, 90]. LH and hCG differ in the composition of their carbohydrate moieties which, in turn, affect bioactivity and half-life. Although hCG amino acid sequence is similar to that of LH, a notable difference is the presence of a long carboxyl terminal segment with 24 AA containing four sites of O-linked oligosaccharides [3, 103] (see Fig. 16.1). Furthermore, hCG beta subunits contain two sites of N-linked glycosylation compared with a single site in LH. Due to the higher number of both glycosylation sites and sialic acid residues (approximately 20) than LH, hCG exhibit a markedly longer terminal half-life. After administration, recombinant human LH is eliminated with a terminal half-life of 10–12 h in contrast to 23–31 h of hCG [10, 104] (Table 16.2).

16.6.1 Differences Between LH and hCG: Evidence from In Vitro Studies

Although both human LH and hCG act on the same LH/hCG receptor (LH-R), evidence from in vitro models indicates that LH receptors differentiate between LH and hCG coupling. While LH exclusively stimulate the targeted LH-R by cis-activation, hCG is also capable of inducing transactivation, thus affecting the kinetics of cAMP production and downstream ERK1/2- and AKT-pathway activation [105, 106]. Using equimolar concentrations of LH and hCG in human granulosa cells obtained from women undergoing oocyte retrieval for ART, Casarini and colleagues have shown that hCG is fivefold more potent in vitro than LH at the receptor

level based on the measurement of intracellular cAMP [105]. Using equipotent doses of LH and hCG, however, the aforementioned authors showed that accumulation of intracellular cAMP by LH was significantly faster, with maximal activation achieved in 10 minutes, while by hCG the same levels of the maximal stimulation were attained only after 60 min of stimulation (see Table 16.2). Interestingly, LH and hCG were equipotent in terms of progesterone production in spite of overall lower cAMP levels after LH stimulation. Despite being mainly dependent on the cAMP/PKA-pathway [107], progesterone production in preovulatory GCs may involve other signaling pathways modulated by ERK1/2 and AKT [108], including [109] molecules of the EGF family such as neuregulin 1 and amphiregulin [34, 110].

In additional experiments using the same in vitro model, Casarini and cols. also evaluated the effects of LH and hCG at activating the ERK1/2 and AKT pathways. ERK and AKT are cell cycle regulators; while AKT is involved in cell signaling leading to cell survival (by blocking apoptosis), ERK represents a range of cell proteins that communicate a signal from a receptor on the cell surface to the DNA in the nucleus. Stimulation with equimolar concentrations of LH resulted in a strong, rapid (10 min), and sustained (45 min) activation of ERK1/2, while hCG induced a much weaker and short-lived stimulation, reaching significance only at 10 min. As far as AKT is concerned, LH provoked a substantial increase in AKT between 10 and 30 min, while hCG stimulation at same doses was virtually nonexistent. Given the different intracellular signaling of hLH and hCG on acute ERK and AKT activation, these authors assessed whether the expression of genes known to be under LH and/or hCG control, epiregulin (AREG) and neuregulin 1 (NRG1) and of CYP19A1 (aromatase), would be differentially affected by the type of gonadotropin. While both LH or hCG stimulation resulted in a marked stimulation of the expression of such genes, LH was significantly more potent than hCG on AREG. Epiregulin may play a role in the ovulatory process and oocyte maturation [108, 111] exerted via both ERK- and AKT-pathway activation [34, 112]. In conclusion, LH and hCG action on the same receptor results in quantitatively and qualitatively different intracellular signaling. While equimolar concentrations of LH and hCG possess different in vitro potency (in terms of cAMP), equipotent concentrations of LH and hCG stimulate intracellular cAMP accumulation with significantly different kinetics. Moreover, LH is more potent than hCG on the ERK and AKT pathways and elicits different kinetic response.

The investigation of the functional role of the cAMP, ERK, and AKT signaling pathways in human fertility has revealed that LH/hCG stimulation of the same receptor results in activation of different, complex signal transduction pathways and molecules [111–113]. In vitro activation of cAMP-pathway by gonadotropins is traditionally associated with structural changes, consisting in cell-rounding [114, 115], apoptotic events [115–117] and in the prevention of meiosis resumption of the oocyte [118]. In contrast, gonadotropin-dependent activation of antiapoptotic pathways [34, 119] and proliferative effects [120] seems to be mediated by ERK1/2 and AKT, and reduction of ERK1/2 signaling activates apoptotic signals in the GCs [121]. Taken together, these results indicate that hCG and hLH action on the regulation of cell cycle and apoptosis in granulosa cell might be divergent and/or dependent on which signal transduction pathway is activated. This is especially relevant in influencing the cell fate during folliculogenesis, when the activation of different signal transduction pathways mediates a delicate balance between pro- and antiapoptotic signals [122]. While the in vivo effects of the differential activation of the various pathways remain to be investigated, the nonequivalence of LH and hCG deserves consideration in the application of therapeutic strategies involving LH activity supplementation in COS protocols.

16.6.2 Differences Between LH and hCG: Evidence from Clinical Studies

It has been shown that the expression of the LH receptor gene, as well as genes involved in the biosynthesis of cholesterol and steroids in granulosa

cells (CYP11A activity decreased by 2.4-fold), is lower in patients treated with hMG preparations compared with those treated with FSH preparations [123]. Such effects are caused by a constant ligand exposure during the follicular phase due to long half-life and high receptor binding affinity of hCG. In animal models, downregulation of LH receptors is maintained for up to 48 h after hMG administration [124]. These findings indicate that the GCs have lower LH-induced cholesterol uptake, a reduction in the de novo cholesterol synthesis, and a reduction in steroid synthesis and thus could explain the observed lower serum progesterone levels achieved in patients treated with hMG compared with FSH [123, 125].

In a study prospectively evaluating 60 normogonadotropic women who, when undergoing induction of multiple follicular growth, showed an insufficient ovarian response in terms of follicular growth (defined as "low responders"), Ruvolo and colleagues examined the impact of LH supplementation on cumulus cell apoptosis [126]. On stimulation day 8, one group was treated with rec-hFSH combined with rec-hLH, while the other was stimulated with rec-hFSH alone. Terminal deoxynucleotidyl transferase-mediated digoxigenin-deoxyuridine-triphosphate (dUTP) nick-end labeling (TUNEL) assay and anti-caspase-3 cleaved immunoassay were used to measure apoptosis in the cumulus cells. A statistically significant increase in the number of immature oocytes was observed in the group treated with rec-hFSH alone (2.33 in the rec-hFSH group vs. 0.58 in the rec-hLH group; $p<0.01$). In contrast, apoptosis markers were lower in the group who received LH supplementation by rec-hLH. A lower rate of cells with chromatin fragmentation (TdT, 18.2 % vs. 12.1 %) and lower presence of caspase-3 cleaved (17.0 % vs. 11.0 %) were observed in the rec-hLH group compared with the rec-hFSH group. Implantation rates were significantly higher in the rec-hLH group (15.6 %) compared with the rec-hFSH group (12.5 %, $p<0.01$). The authors concluded that supplementation with rec-hLH reduced the number of immature oocytes collected after pickup. Furthermore, they speculated that the increase in implantation rate might be correlated with the reduction of apoptosis seen in the cumulus cells of patients treated with rec-hLH, due to a direct action of rec-hLH on the cumulus and granulosa cells, or because of the paracrine effect mediated by secreting factors in the theca and oocyte cells. Thus, maintaining their physiological function for a longer time, cumulus cells are better able to support nuclear and cytoplasmic maturation of the oocyte until ovulation, thus allowing the collection of oocytes with better "intrinsic" qualities that are necessary for sustaining fertilization and the early phases of embryogenesis. Hence, if cumulus cells are preserved from apoptotic processes, the oocyte receives no molecular signal able to activate apoptotic pathways [126].

The clinical implications of the aforementioned observations have been investigated by "tail to tail" comparison between hMG and rec-LH preparations. An open-label RCT in 2012 compared HP-hMG and a fixed combination of rec-FSH and rec-hLH in 35 women with hypogonadotropic hypogonadism. Eighteen patients received 150 IU hMG-HP (150 IU FSH + 150 IU LH-like activity) and seventeen received 150 IU rec-hFSH/75 IU rec-hLH daily for a maximum of 16 days. The proportion of patients reaching ovulation did not differ between the groups (70 % vs. 88 %, respectively), but the pregnancy rate was significantly higher in those treated with the combination of recombinant gonadotropins (55.6 % vs. 23.3 %; $p=0.01$) [127]. In another RCT, Pacchiarotti and colleagues enrolled 122 women with low baseline endogenous LH levels (<1.2 IU/L) in the presence of normal FSH levels undergoing IVF. The patients were treated with a downregulation protocol consisting of triptorelin 0.1 mg at day 21 of the cycle and were randomized to receive an ovarian stimulation with 225 IU/day of either HP-hMG or rec-FSH plus rec-hLH in a 2:1 ratio. Fewer days of stimulation (10.9 ± 1.1 vs. 14.1 ± 1.6; $p=0.013$) and a higher number of retrieved oocytes (7.8 ± 1.1 vs. 4.1 ± 12; $p=0.002$) were noted in the group that received follitropin alfa + lutropin alfa 2:1 compared with the group who received HMG. However, differences were not observed in estradiol levels on hCG day (1987 ± 699 pg/mL vs. 2056 ± 560 pg/mL),

pregnancy rates per cycle (28.3 % vs. 29.3 %), and implantation rates (12.1 % vs. 12.2 %), despite higher cancelation rates due to excessive response in women receiving follitropin + lutropin alfa (11.1 % vs. 1.7 %; $p = 0.042$), which therefore indicates that the latter is a more potent preparation for COS [128].

Furthermore, the German experience with the use of rec-hLH compared with hMG in daily ART practice was recently reported by analyzing data from the National IVF Registry (DIR), in which patients undergoing IVF from approximately 85 % of the German IVF centers are prospectively enrolled [129]. A total of 4719 women, 1573 per group, matched by age, body mass index, indication, and number of previous ART cycles, treated with either rec-hFSH and rec-hLH in a fixed 2:1 ratio or hMG, either alone or in combination with rec-hFSH, after down-regulation in a long GnRH-agonist protocol, was analyzed. The mean gonadotropin consumption (in ampoules of 75 IU) was significantly lower in the group treated with the fixed combination of rec-hFSH and rec-hLH (34.3) compared with the two hMG groups (hMG alone: 36.4, $p < 0.001$; hMG in combination with rec-hFSH: 46.3, $p < 0.001$). Pregnancy rates per cycle (25.5 % vs. 21.5 %, $p = 0.006$; 25.5 % vs. 21.7 %, $p = 0.02$) and per embryo transfer (31.3 % vs. 26.0 %, $p = 0.02$; 31.3 % vs. 25.6 %, $p = 0.008$) and implantation rate per embryo transferred (19.0 % vs. 14 % in both pairwise comparisons, $p < 0.001$) were higher in the group treated with the fixed combination of rec-hFSH and rec-hLH compared with the aforesaid hMG groups, respectively. Lastly, in 2013, a crossover study evaluated 33 patients using HP-hMG in their first IVF cycle and 2:1 rec-hFSH plus rec-hLH in their second IVF attempt [130]. Estradiol levels on the day of hCG (2633 ± 871 vs. 2101 ± 816; $p < 0.05$) and the number of oocytes retrieved (9.8 ± 3.3 vs. 7.3 ± 3.1; $p < 0.01$) were higher in the group that received the 2:1 rec-hFSH plus rec-hLH formulation. Despite implantation and clinical pregnancy rates per started cycle were not different between the groups (29.6 % and 48.4 %, respectively, for hMG and 28.4 % and 48.4 % for rec-hFSH plus rec-hLH), 2/3 of the patients in rec-FSH + rec-LH group (vs. 1/3 hMG group) would have frozen embryos to transfer if a fresh transfer failed.

Despite being developed for stimulation of follicular growth in women with severe LH and FSH deficiency, the 2:1 formulation of follitropin alfa and lutropin alfa has expanded to normogonadotropic women undergoing ART. In a 3-year, multicenter, open-label, observational, post-marketing surveillance study involving 2200 German women (21–45 years) undergoing ART, the most common reasons for physicians to prescribe the 2:1 formulation of follitropin alfa and lutropin alfa were poor ovarian response (39.4 %), low baseline LH level (17.8 %), age (13.8 %), and low baseline E_2 level (7.3 %) [131]. Recently, a cost-effectiveness model compared rec-FSH + rec-hLH and HP-hMG for ovulation induction in hypogonadotropic hypogonadal women, according to the Italian Health Service perspective, in which only direct costs (drugs, specialist visits, patient examinations, and hospitalizations) are included [132]. A Markov model was developed, considering the probability of pregnancy and miscarriage in three cycles of therapy. In that model, the patients started the therapy with recombinant or urinary gonadotropins following pregnancy evaluation. If a woman became pregnant, the possibility of miscarriage was considered. Women who did not become pregnant during the first series of treatment or had a miscarriage underwent a second cycle of therapy, maintaining the same treatment of the previous cycle. The same process was applied to the third cycle. Consumption of gonadotropins and outcome of HP-hMG and rec-hFSH + rec-hLH cycles were based on the study by Carone and cols [127]. Rec-hFSH + rec-hLH was associated with a higher acquisition cost (€3453.50) and higher efficacy (0.87) compared with HP-hMG (€2719.70 and 0.50). The average cost per pregnancy was estimated to be €3990.00 for recombinant strategy and €5439.80 for urinary strategy, thus indicating that the combination therapy with rec-hFSH + rec-hLH is associated with a better cost-effectiveness compared to HP-hMG in the treatment of infertility in hypogonadotropic hypogonadal women.

In conclusion, evidence from experimental and clinical studies indicates that LH activity driven by hCG and rec-hLH is not equivalent neither at the molecular level nor at the functional level. COS protocols involving LH supplementation with rec-hFSH appear to be more effective and efficacious than those with hMG (hCG activity). Nevertheless, large RCTs are needed to confirm these observations.

Conclusions

- Ovarian steroidogenesis is the result of combined LH and FSH stimulation of the two cell types, theca and granulosa, influenced by autocrine and paracrine factors.
- LH has a pivotal role in follicular development and maturation. LH is crucial in sustaining FSH activity in the granulosa during intermediate–late stages of folliculogenesis and is critical for maintaining corpus luteum function during the luteal phase.
- Exogenous LH activity supplementation is mandatory in stimulation protocols applied to women with hypogonadotropic hypogonadism.
- Review of the studies exploring the supplementation of rec-hLH to COS regimens indicates that, at present, the addition of rec-hLH to the general population of infertile women undergoing IVF/ICSI cycles remains controversial.
- Fair evidence indicates that rec-hLH supplementation has a positive effect on cycle outcome of older women (>35 years old), particularly when used from the start of COS.
- Fair evidence indicates that both the number of oocytes retrieved as well as the clinical pregnancy rates are increased in poor responders undergoing COS with a combination of rec-hLH and rec-hFSH.
- Hyporesponsiveness to rec-hFSH could be related to the presence of less bioactive LH due to specific genetic-determined variations in the beta subunit of the LH molecule. Limited data indicate that hyporesponders benefit from LH supplementation during COS.
- Conflicting evidence exists regarding the impact of deeply suppressed levels of mid-follicular serum LH levels on ovarian response to stimulation with rec-FSH. Current data indicates that the choice of GnRH-a plays a role on the frequency of patients exhibiting profound mid-follicular LH suppression. Whether these women would benefit from supplementation with LH activity during ovarian stimulation remains to be proven.
- Evidence from experimental and clinical studies indicate that LH activity driven by hCG and rec-hLH is not equivalent neither at the molecular level nor at the functional level. COS protocols involving LH supplementation with rec-hLH, particularly using a fixed 2:1 combination of rec-hFSH and rec-hLH, appear to be more effective and efficacious than those with hMG (hCG activity). Nevertheless, large RCTs are needed to confirm these observations.

References

1. Beall SA, DeCherney A. History and challenges surrounding ovarian stimulation in the treatment of infertility. Fertil Steril. 2012;97(4):785–801.
2. Lunenfeld B. Historical perspectives in gonadotrophin therapy. Hum Reprod Update. 2004;10(6): 453–67.
3. Leão RB, Esteves SC. Gonadotropin therapy in assisted reproduction: an evolutionary perspective from biologics to biotech. Clinics (Sao Paulo). 2014;69(4):279–93.
4. Alviggi C, Mollo A, Clarizia R, De Placido G. Exploiting LH in ovarian stimulation. Reprod Biomed Online. 2006;12(2):221–33.
5. Speroff L, Fritz MA. Chapter 5: Neuroendocrinology. In: Speroff L, Fritz MA, editors. Clinical gynecologic endocrinology and infertility. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 295–6.
6. Vaitukaitis JL, Ross GT, Braunstein GD, Rayford PL. Gonadotropins and their subunits: basic and clinical studies. Recent Prog Horm Res. 1976;32: 289–331.
7. Ulloa-Aguirre A, Espinoza R, Damian-Matsumura P, Chappel SC. Immunological and biological potencies of the different molecular species of gonadotrophins. Hum Reprod. 1988;3(4):491–501.
8. Wide L, Naessén T, Sundström-Poromaa I, Eriksson K. Sulfonation and sialylation of gonadotropins in women during the menstrual cycle, after menopause, and with polycystic ovarian syndrome and in men. J Clin Endocrinol Metab. 2007;92(11):4410–7.

9. Ulloa-Aguirre A, Midgley Jr AR, Beitins IZ, Padmanabhan V. Follicle-stimulating isohormones: characterization and physiological relevance. Endocr Rev. 1995;16(6):765–87.
10. Campbell RK. Molecular pharmacology of gonadotropins. Endocrine. 2005;26(3):291–6.
11. Combarnous Y. Molecular basis of the specificity of binding of glycoprotein hormones to their receptors. Endocr Rev. 1992;13(4):670–91.
12. Costagliola S, Urizar E, Mendive F, Vassart G. Specificity and promiscuity of gonadotropin receptors. Reproduction. 2005;130(3):275–81.
13. Hsueh AJ, Adashi EY, Jones PB, Welsh Jr TH. Hormonal regulation of the differentiation of cultured ovarian granulosa cells. Endocr Rev. 1984;5(1):76–127.
14. Jeppesen JV, Kristensen SG, Nielsen ME, et al. LH-receptor gene expression in human granulosa and cumulus cells from antral and preovulatory follicles. J Clin Endocrinol Metab. 2012;97(8):E1524–31.
15. Speroff L, Fritz MA. Chapter 2: Hormone biosynthesis, metabolism, and mechanisms of action. Steroidogenesis. In: Speroff L, Fritz MA, editors. Clinical gynecologic endocrinology and infertility. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 109–16.
16. Young JM, McNeilly AS. Theca: the forgotten cell of the ovarian follicle. Reproduction. 2010;140(4): 489–504.
17. Falck B. Site of production of oestrogen in rat ovary as studied in microtransplants. Acta Physiol Scand Suppl. 1959;47(163):1–101.
18. Miller WL. Steroidogenic enzymes. Endocr Dev. 2008;13:1–18.
19. Wickenheisser JK, Nelson-DeGrave VL, McAllister JM. Human ovarian theca cells in culture. Trends Endocrinol Metab. 2006;17(2):65–71.
20. Smyth CD, Miró F, Whitelaw PF, Howles CM, Hillier SG. Ovarian thecal/interstitial androgen synthesis is enhanced by a follicle-stimulating hormone-stimulated paracrine mechanism. Endocrinology. 1993;133(4):1532–8.
21. Whitelaw PF, Smyth CD, Howles CM, Hillier SG. Cell-specific expression of aromatase and LH receptor mRNAs in rat ovary. J Mol Endocrinol. 1992;9(3):309–12.
22. Speroff L, Fritz MA. Chapter 6: Regulation of the menstrual cycle. In: Speroff L, Fritz MA, editors. Clinical gynecologic endocrinology and infertility. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 348–83.
23. Coutts JRT, Gaukroger JM, Kader AS, et al. Steroidogenesis by the human Graafian follicle. In: Coutts JRT, editor. Functional morphology of the human ovary. Lancaster: MTP; 1981. p. 56–72.
24. Filicori M, Cognigni GE, Taraborrelli S, et al. Luteinizing hormone activity supplementation enhances follicle-stimulating hormone efficacy and improves ovulation induction outcome. Clin Endocrinol Metab. 1999;84(8):2659–63.
25. Couzinet B, Brailly S, Bouchard P, Schaison G. Progesterone stimulates luteinizing hormone secretion by acting directly on the pituitary. J Clin Endocrinol Metab. 1992;74(2):374–8.
26. Zeleznik AJ, Hillier SG. The role of gonadotropins in the selection of the preovulatory follicle. Clin Obstet Gynecol. 1984;27(4):927–40.
27. Goff AK, Armstrong DT. Stimulatory action of gonadotropins and prostaglandins on adenosine-3′,5′-monophosphate production by isolated rat granulosa cells. Endocrinology. 1977;101(5):1461–7.
28. Campbell BK, Dobson H, Baird DT, Scaramuzzi RJ. Examination of the relative role of FSH and LH in the mechanism of ovulatory follicle selection in sheep. J Reprod Fertil. 1999;117(2):355–67.
29. Sullivan MW, Stewart-Akers A, Krasnow JS, Berga SL, Zeleznik AJ. Ovarian responses in women to recombinant follicle-stimulating hormone and luteinizing hormone (LH): a role for LH in the final stages of follicular maturation. J Clin Endocrinol Metab. 1999;84(1):228–32.
30. Vegetti W, Alagna F. FSH and folliculogenesis: from physiology to ovarian stimulation. Reprod Biomed Online. 2006;12(6):684–94.
31. Huang ZH, Clayton PE, Brady G, Morris ID. Insulin-like growth factor-I gene expression in human granulosa-lutein cells. J Mol Endocrinol. 1994;12(3): 283–91.
32. Zhou J, Bondy C. Anatomy of the human ovarian insulin-like growth factor system. Biol Reprod. 1993;48(3):467–82.
33. Peluso JJ, Pappalardo A, Fernandez G. Basic fibroblast growth factor maintains calcium homeostasis and granulosa cell viability by stimulating calcium efflux via a PKC delta-dependent pathway. Endocrinology. 2001;142(10):4203–11.
34. Ben-Ami I, Armon L, Freimann S, Strassburger D, Ron-El R, et al. EGF- like growth factors as LH mediators in the human corpus luteum. Hum Reprod. 2009;24(1):176–84.
35. Park J-Y, Su Y-Q, Ariga M, Law E, Jin S-LC, et al. EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science. 2004;303(5658): 682–4.
36. Donadeu FX, Ascoli M. The differential effects of the gonadotropin receptors on aromatase expression in primary cultures of immature rat granulosa cells are highly dependent on the density of receptors expressed and the activation of the inositol phosphate cascade. Endocrinology. 2005;146(9):3907–16.
37. Lindeberg M, Carlström K, Ritvos O, Hovatta O. Gonadotrophin stimulation of non-luteinized granulosa cells increases steroid production and the expression of enzymes involved in estrogen and progesterone synthesis. Hum Reprod. 2007;22(2): 401–6.
38. Casper RF, Yen SS. Induction of luteolysis in the human with a long-acting analog of luteinizing hormone-releasing factor. Science. 1979;205(4404): 408–10.

39. Sugino N, Kaskida S, Takiguchi S, Karube A, Kato H. Expression of vascular endothelial growth factor and its receptors in the human corpus luteum during the menstrual cycle and in early pregnancy. J Clin Endocrinol. 2000;85(10):3919–24.
40. Wang TH, Horng SG, Chang CL, Wu HM, Tsai YJ, Wang HS, Soong YK. Human chorionic gonadotropin-induced hyperstimulation syndrome is associated with up-regulation of vascular endothelial growth factor. J Clin Endocrinol Metab. 2002;87(7):3300–8.
41. Robinson RS, Nicklin LT, Hammond AJ, Schams D, Hunter MG, Mann GE. Fibroblast growth factor 2 is more dynamic than vascular endothelial growth factor A during the follicle-luteal transition in the cow. Biol Reprod. 2007;77(1):28–36.
42. Stirling D, Waterman MR, Simpson ER. Expression of mRNA encoding basic fibroblast growth factor (bFGF) in bovine corpora lutea and cultured luteal cells. J Reprod Fertil. 1991;91(1):1–8.
43. Fraser HM, Dickson SE, Lunn SF, Wulff C, Morris KD, Caroll VA, Bicknell R. Suppression of luteal angiogenesis in the primate after neutralization of vascular endothelial growth factor. Endocrinology. 2000;141(3):995–1000.
44. Berisha B, Steffl M, Amselgruber W, Schams D. Changes in fibroblast growth factor 2 and its receptors in bovine follicles before and after GnRH application and after ovulation. Reproduction. 2006;131(2):319–29.
45. Rao CV. Multiple novel roles of luteinizing hormone. Fertil Steril. 2001;76(6):1097–100.
46. Tesarik J, Hazout A, Mendoza C. Luteinizing hormone affects uterine receptivity independently of ovarian function. Reprod Biomed Online. 2003;7(1):59–64.
47. Licht P, Russu V, Wildt L. On the role of human chorionic gonadotropin (hCG) in the embryo-endometrial microenvironment: implications for differentiation and implantation. Semin Reprod Med. 2001;19(1):37–47.
48. Shoham Z, Smith H, Yeko T, O'Brien F, Hemsey G, O'Dea L. Recombinant LH (lutropin alfa) for the treatment of hypogonadotrophic women with profound LH deficiency: a randomized, double-blind, placebo-controlled, proof-of-efficacy study. Clin Endocrinol (Oxf). 2008;69(3):471–8.
49. European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: a dose-finding study. J Clin Endocrinol Metab. 1998;83(5):1507–14.
50. Loumaye E, Engrand P, Howles CM, O'Dea L. Assessment of the role of serum luteinizing hormone and estradiol response to follicle-stimulating hormone on in vitro fertilization treatment outcome. Fertil Steril. 1997;67(5):889–99.
51. Sills ES, Levy DP, Moomjy M, et al. A prospective, randomized comparison of ovulation induction using highly purified follicle- stimulating hormone alone and with recombinant human luteinizing hormone in in-vitro fertilization. Hum Reprod. 1999;14:2230–5.
52. Esteves SC, Schertz JC, Verza Jr S, Schneider DT, Zabaglia SF. A comparison of menotropin, highly-purified menotropin and follitropin alfa in cycles of intracytoplasmic sperm injection. Reprod Biol Endocrinol. 2009;7:111.
53. Kolibianakis EM, Kalogeropoulou L, Griesinger G, et al. Among patients treated with FSH and GnRH analogues for in vitro fertilization, is the addition of recombinant LH associated with the probability of live birth? A systematic review and meta-analysis. Hum Reprod Update. 2007;13(5):445–52.
54. Mochtar MH, Van der Veen, Ziech M, van Wely M. Recombinant Luteinizing Hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. Cochrane Database Syst Rev 2007;(2):CD005070.
55. Lehert P, Kolibianakis EM, Venetis CA, Schertz J, Saunders H, Arriagada P, et al. Recombinant human follicle-stimulating hormone (r-hFSH) plus recombinant luteinizing hormone versus r-hFSH alone for ovarian stimulation during assisted reproductive technology: systematic review and meta-analysis. Reprod Biol Endocrinol. 2014;12:17.
56. Marrs R, Meldrum D, Muasher S, Schoolcraft W, Werlin L, Kelly E. Randomized trial to compare the effect of recombinant human FSH (follitropin alfa) with or without recombinant human LH in women undergoing assisted reproduction treatment. Reprod Biomed Online. 2004;8(2):175–82.
57. Hill MJ, Levens ED, Levy G, et al. The use of recombinant luteinizing hormone in patients undergoing assisted reproductive techniques with advanced reproductive age: a systematic review and meta-analysis. Fertil Steril. 2012;97(5):1108–14.
58. Bosdou JK, Venetis CA, Kolibianakis EM, et al. The use of androgens or androgen-modulating agents in poor responders undergoing in vitro fertilization: a systematic review and meta-analysis. Hum Reprod Update. 2012;18(2):127–45.
59. Esposito MA, Barnhart KT, Coutifaris C, Patrizio P. Role of periovulatory luteinizing hormone concentrations during assisted reproductive technology cycles stimulated exclusively with recombinant follicle-stimulating hormone. Fertil Steril. 2001;75(3):519–24.
60. Westergaard LG, Laursen SB, Andersen CY. Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. Hum Reprod. 2000;15(5):1003–8.
61. Humaidan P, Bungum L, Bungum M, Andersen CY. Ovarian response and pregnancy outcome related to mid-follicular LH levels in women undergoing assisted reproduction with GnRH agonist down-regulation and recombinant FSH stimulation. Hum Reprod. 2002;17(8):2016–21.
62. Laml T, Obruca A, Fischl F, Huber JC. Recombinant luteinizing hormone in ovarian hyperstimulation

after stimulation failure in normogonadotrophic women. Gynecol Endocrinol. 1999;13(2):98–103.
63. Nakagawa K, Ohgi S, Nakashima A, Horikawa T, Sugiyama R, Saito H. The ratio of late-follicular to mid-follicular phase LH concentrations efficiently predicts ART outcomes in women undergoing ART treatment with GnRH-agonist long protocol and stimulation with recombinant FSH. J Assist Reprod Genet. 2008;25(8):359–64.
64. De Placido G, Mollo A, Alviggi C, et al. Rescue of IVF cycles by HMG in pituitary down-regulated normogonadotrophic young women characterized by a poor initial response to recombinant FSH. Hum Reprod. 2001;16(9):1875–9.
65. De Placido G, Alviggi C, Mollo A, et al. Effects of recombinant LH (rLH) supplementation during controlled ovarian hyperstimulation (COH) in normogonadotrophic women with an initial inadequate response to recombinant FSH (rFSH) after pituitary downregulation. Clin Endocrinol (Oxf). 2004;60(5):637–43.
66. De Placido G, Alviggi C, Perino A, et al. Recombinant human LH supplementation versus recombinant human FSH (rFSH) step-up protocol during controlled ovarian stimulation in normogonadotrophic women with initial inadequate ovarian response to rFSH. A multicentre, prospective, randomized controlled trial. Hum Reprod. 2005;20(2):390–6.
67. Ferraretti AP, Gianaroli L, Magli MC, D'angelo A, Farfalli V, Montanaro N. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. Fertil Steril. 2004;82(6):1521–6.
68. Hurwitz JM, Santoro N. Inhibins, activins, and follistatin in the aging female and male. Semin Reprod Med. 2004;22(3):209–17.
69. Alviggi C, Clarizia R, Pettersson K, et al. Suboptimal response to GnRHa long protocol is associated with a common LH polymorphism. Reprod Biomed Online. 2009;18(1):9–14.
70. Piltonen T, Koivunen R, Ruokonen A, Tapanainen JS. Ovarian age-related responsiveness to human chorionic gonadotropin. J Clin Endocrinol Metab. 2003;88(7):3327–32.
71. Vihko KK, Kujansuu E, Mörsky P, Huhtaniemi I, Punnonen R. Gonadotropins and gonadotropin receptors during the perimenopause. Eur J Endocrinol. 1996;134(3):357–61.
72. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. J Clin Endocrinol Metab. 2005;90(7):3847–53.
73. Marrama P, Montanini V, Celani MF, et al. Decrease in luteinizing hormone biological activity/immunoreactivity ratio in elderly men. Maturitas. 1984;5(4): 223–31.
74. Lévy DP, Navarro JM, Schattman GL, Davis OK, Rosenwaks Z. The role of LH in ovarian stimulation: exogenous LH: let's design the future. Hum Reprod. 2000;15(11):2258–65. Review. Erratum in: Hum Reprod. 2001;16(3):598.
75. Bosch E, Labarta E, Crespo J, Simón C, Remohí J, Pellicer A. Impact of luteinizing hormone administration on gonadotropin-releasing hormone antagonist cycles: an age-adjusted analysis. Fertil Steril. 2011;95(3):1031–6.
76. König TE, van der Houwen LE, Overbeek A, Hendriks ML, Beutler-Beemsterboer SN, Kuchenbecker WK, et al. Recombinant LH supplementation to a standard GnRH antagonist protocol in women of 35 years or older undergoing IVF/ICSI: a randomized controlled multicentre study. Hum Reprod. 2013;28(10):2804–12.
77. König TE, van der Houwen LE, Lambalk CB. Recombinant LH supplementation in women of 35 years and older undergoing IVF? Fertil Steril. 2012;98(1):e10–1.
78. Bosch E. Comment on 'Recombinant LH supplementation to a standard GnRH antagonist protocol in women of 35 years old or older undergoing IVF/ICSI: a randomized controlled multicentre study'. Hum Reprod. 2014;29(3):636–7.
79. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, ESHRE Working Group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011;26(7):1616–24.
80. Alviggi C, Clarizia R, Pettersson K, et al. Mutation LH beta subunit is associated with higher exogenous FSH consumption of FSH during ovarian stimulation. Hum Reprod. 2009;24 Suppl 1:i31.
81. Pettersson K, Söderholm JR. Individual differences in lutropin immunoreactivity revealed by monoclonal antibodies. Clin Chem. 1991;37(3):333–40.
82. Themmen APN, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. Endocr Rev. 2000;21(5):551–83.
83. Alviggi C, Pettersson K, Longobardi S, Andersen CY, Conforti A, De Rosa P, et al. A common polymorphic allele of the LH beta-subunit gene is associated with higher exogenous FSH consumption during controlled ovarian stimulation for assisted reproductive technology. Reprod Biol Endocrinol. 2013;11(1):51.
84. Fleming R, Rehka P, Deshpande N, Jamieson ME, Yates RW, Lyall H. Suppression of LH during ovarian stimulation: effects differ in cycles stimulated with purified urinary FSH and recombinant FSH. Hum Reprod. 2000;15(7):1440–5.
85. Balasch J, Vidal E, Peñarrubia J, Casamitjana R, Carmona F, Creus M, et al. Suppression of LH during ovarian stimulation: analysing threshold values and effects on ovarian response and the outcome of assisted reproduction in down-regulated women stimulated with recombinant FSH. Hum Reprod. 2001;16(8):1636–43.
86. Cole LA, Khanlian SA, Muller CY. Detection of perimenopause or postmenopause human chorionic gonadotropin: an unnecessary source of alarm. Am J Obstet Gynecol. 2008;198(3):275.e1–7.

87. Cole LA, Khanlian SA, Muller CY. Normal production of human chorionic gonadotropin in perimenopausal and menopausal women and after oophorectomy. Int J Gynecol Cancer. 2009;19(9):1556–9.
88. Howles CM. Genetic engineering of human FSH (Gonal-F). Hum Reprod Update. 1996;2(2):172–91.
89. Olijve W, de Boer W, Mulders JW, van Wezenbeek PM. Molecular biology and biochemistry of human recombinant follicle stimulating hormone (Puregon). Mol Hum Reprod. 1996;2(5):371–82.
90. Practice Committee of American Society for Reproductive Medicine, Birmingham, Alabama. Gonadotropin preparations: past, present, and future perspectives. Fertil Steril. 2008;90(5 Suppl):S13–20.
91. Alviggi C, Revelli A, Anserini P, et al. A prospective, randomised, controlled clinical study on the assessment of tolerability and of clinical efficacy of Merional (hMG-IBSA) administered subcutaneously versus Merional administered intramuscularly in women undergoing multifollicular ovarian stimulation in an ART programme (IVF). Reprod Biol Endocrinol. 2007;5:45.
92. Platteau P, Laurent E, Albano C, et al. An open, randomized single-centre study to compare the efficacy and convenience of follitropin beta administered by a pen device with follitropin alpha administered by a conventional syringe in women undergoing ovarian stimulation for IVF/ICSI. Hum Reprod. 2003;18(6):1200–4.
93. Bosch E. Recombinant human follicular stimulating hormone and recombinant human luteinizing hormone in a 2:1 ratio combination. Pharmacological characteristics and clinical applications. Expert Opin Biol Ther. 2010;10(6):1001–9.
94. Picard M, Rossier C, Papasouliotis O, Lugan I. Bioequivalence of recombinant human FSH and recombinant human LH in a fixed 2:1 combination: two phase I, randomised, crossover studies. Curr Med Res Opin. 2008;24(4):1199–208.
95. Gervais A, Hammel YA, Pelloux S, et al. Glycosylation of human recombinant gonadotrophins: characterization and batch-to-batch consistency. Glycobiology. 2003;13(3):179–89.
96. Hugues JN, Barlow DH, Rosenwaks Z, et al. Improvement in consistency of response to ovarian stimulation with recombinant human follicle stimulating hormone resulting from a new method for calibrating the therapeutic preparation. Reprod Biomed Online. 2003;6(2):185–90.
97. Giudice E, Crisci C, Eshkol A, Papoian R. Composition of commercial gonadotrophin preparations extracted from human post-menopausal urine: characterization of non-gonadotrophin proteins. Hum Reprod. 1994;9(12):2291–9.
98. van de Weijer BH, Mulders JW, Bos ES, Verhaert PD, van den Hooven HW. Compositional analyses of a human menopausal gonadotrophin preparation extracted from urine (menotropin). Identification of some of its major impurities. Reprod Biomed Online. 2003;7(5):547–57.
99. Kuwabara Y, Mine K, Katayama A, Inagawa T, Akira S, Takeshita T. Proteomic analyses of recombinant human follicle-stimulating hormone and urinary-derived gonadotropin preparations. J Reprod Med. 2009;54(8):459–66.
100. Bassett RM, Driebergen R. Continued improvements in the quality and consistency of follitropin alfa, recombinant human FSH. Reprod Biomed Online. 2005;10(2):169–77.
101. Driebergen R, Baer G. Quantification of follicle stimulating hormone (follitropin alfa): is in vivo bioassay still relevant in the recombinant age? Curr Med Res Opin. 2003;19(1):41–6.
102. Steelman SL, Pohley FM. Assay of follicle stimulating hormone based on the augmentation with human chorionic gonadotropin. Endocrinology. 1953;53(6):604–16.
103. Rozell TG, Okrainetz RJ. FSH: one hormone with multiple forms, or a family of multiples hormones. In: Chedrese PJ, editor. Reproductive endocrinology: a molecular approach. New York: Springer Science + Business Media, LLC; 2009. p. 145–60.
104. Le Cotonnec JY, Porchet HC, Beltrami V, Munafo A. Clinical pharmacology of recombinant human luteinizing hormone: Part I. Pharmacokinetics after intravenous administration to healthy female volunteers and comparison with urinary human luteinizing hormone. Fertil Steril. 1998;69(2):189–94.
105. Casarini L, Lispi M, Longobardi S, Milosa F, La Marca A, Tagliasacchi D, et al. LH and hCG action on the same receptor results in quantitatively and qualitatively different intracellular signalling. PLoS One. 2012;7(10), e46682.
106. Grzesik P, Teichmann A, Furkert J, Rutz C, Wiesner B, Kleinau G, et al. Differences between lutropin-mediated and choriogonadotropin-mediated receptor activation. FEBS J. 2014;281(5):1479–92.
107. Karlsson AB, Maizels ET, Flynn MP, Jones JC, Shelden EA, et al. Luteinizing hormone receptor-stimulated progesterone production by preovulatory granulosa cells requires protein kinase A-dependent activation/dephosphorylation of the actin dynamizing protein cofilin. Mol Endocrinol. 2010;24(9):1765–81.
108. Chin EC, Abayasekara DRE. Progesterone secretion by luteinizing human granulosa cells: a possible cAMP-dependent but PKA-independent mechanism involved in its regulation. J Endocrinol. 2004;183(1):51–60.
109. Ryan KE, Glister C, Lonergan P, Martin F, Knight PG, et al. Functional significance of the signal transduction pathways Akt and Erk in ovarian follicles: in vitro and in vivo studies in cattle and sheep. J Ovarian Res. 2008;1(1):2.
110. Noma N, Kawashima I, Fan H-Y, Fujita Y, Kawai T, et al. LH-induced neuregulin 1 (NRG1) type III transcripts control granulosa cell differentiation and oocyte maturation. Mol Endocrinol. 2011;25(1):104–16.
111. Sekiguchi T, Mizutani T, Yamada K, Kajitani T, Yazawa T, et al. Expression of epiregulin and amphiregulin in the rat ovary. J Mol Endocrinol. 2004;33(1):281–91.

112. Choi J-H, Chen C-L, Poon SL, Wang H-S, Leung PCK. Gonadotropin-stimulated epidermal growth factor receptor expression in human ovarian surface epithelial cells: involvement of cyclic AMP-dependent exchange protein activated by cAMP pathway. Endocr Relat Cancer. 2009;16(1):179–88.
113. Yamashita Y, Hishinuma M, Shimada M. Activation of PKA, p38 MAPK and ERK1/2 by gonadotropins in cumulus cells is critical for induction of EGF-like factor and TACE/ADAM17 gene expression during in vitro maturation of porcine COCs. J Ovarian Res. 2009;2:20.
114. Ulloa-Aguirre A, Crépieux P, Poupon A, Maurel M-C, Reiter E. Novel pathways in gonadotropin receptor signalling and biased agonism. Rev Endocr Metab Disord. 2011;12(4):259–74.
115. Schiffer Z, Keren-Tal I, Deutsch M, Dantes A, Aharoni D, et al. Fourier analysis of differential light scattering for the quantitation of FSH response associated with structural changes in immortalized granulosa cells. Mol Cell Endocrinol. 1996;118(1–2):145–53.
116. Keren-Tal I, Suh BS, Dantes A, Lindner S, Oren M, et al. Involvement of p53 expression in cAMP-mediated apoptosis in immortalized granulosa cells. Exp Cell Res. 1995;218(1):283–95.
117. Amsterdam A, Dantes A, Liscovitch M. Role of phospholipase-D and phosphatidic acid in mediating gonadotropin-releasing hormone-induced inhibition of preantral granulosa cell differentiation. Endocrinology. 1994;135(3):1205–11.
118. Aharoni D, Dantes A, Oren M, Amsterdam A. cAMP-mediated signals as determinants for apoptosis in primary granulosa cells. Exp Cell Res. 1995;218(1):271–82.
119. Sun Q-Y, Miao Y-L, Schatten H. Towards a new understanding on the regulation of mammalian oocyte meiosis resumption. Cell Cycle. 2009;8(17): 2741–7.
120. Johnson AL, Bridgham JT, Swenson JA. Activation of the Akt/protein kinase B signalling pathway is associated with granulosa cell survival. Biol Reprod. 2001;64(5):1566–74.
121. Peter AT, Dhanasekaran N. Apoptosis of granulosa cells: a review on the role of MAPK-signalling modules. Reprod Domest Anim. 2003;38(3):209–13.
122. Craig J, Orisaka M, Wang H, Orisaka S, Thompson W, et al. Gonadotropin and intra-ovarian signals regulating follicle development and atresia: the delicate balance between life and death. Front Biosci. 2007;12:3628–39.
123. Grøndahl ML, Borup R, Lee YB, Myrhøj V, Meinertz H, Sørensen S. Differences in gene expression of granulosa cells from women undergoing controlled ovarian hyperstimulation with either recombinant follicle-stimulating hormone or highly purified human menopausal gonadotropin. Fertil Steril. 2009;91(5):1820–30.
124. Menon KM, Munshi UM, Clouser CL, Nair AK. Regulation of luteinizing hormone/human chorionic gonadotropin receptor expression: a perspective. Biol Reprod. 2004;70(4):861–6.
125. Bosch E, Vidal C, Labarta E, Simon C, Remohi J, Pellicer A. Highly purified hMG versus recombinant FSH in ovarian hyperstimulation with GnRH antagonists-a randomized study. Hum Reprod. 2008; 23(10):2346–51.
126. Ruvolo G, Bosco L, Pane A, Morici G, Cittadini E, Roccheri MC. Lower apoptosis rate in human cumulus cells after administration of recombinant luteinizing hormone to women undergoing ovarian stimulation for in vitro fertilization procedures. Fertil Steril. 2007;87(3):542–6.
127. Carone D, Caropreso C, Vitti A, Chiappetta R. Efficacy of different gonadotropin combinations to support ovulation induction in WHO type I anovulation infertility: clinical evidences of human recombinant FSH/human recombinant LH in a 2:1 ratio and highly purified human menopausal gonadotropin stimulation protocols. J Endocrinol Invest. 2012;35(11):996–1002.
128. Pacchiarotti A, Sbracia M, Frega A, Selman H, Rinaldi L, Pacchiarotti A. Urinary hMG (Menopur) versus recombinant FSH plus recombinant LH (Pergoveris) in IVF: a multicenter, prospective, randomized controlled trial. Fertil Steril. 2010; 94(6):2467–9.
129. Bühler KF, Fischer R. Recombinant human LH supplementation versus supplementation with urinary hCG-based LH activity during controlled ovarian stimulation in the long GnRH-agonist protocol: a matched case-control study. Gynecol Endocrinol. 2012;28(5):345–50.
130. Fábregues F, Creus M, Casals G, Carmona F, Balasch J. Outcome from consecutive ICSI cycles in patients treated with recombinant human LH and those supplemented with urinary hCG-based LH activity during controlled ovarian stimulation in the long GnRH-agonist protocol. Gynecol Endocrinol. 2013; 29(5):430–5.
131. Bühler K, Naether OG, Bilger W. A large, multicentre, observational, post-marketing surveillance study of the 2:1 formulation of follitropin alfa and lutropin alfa in routine clinical practice for assisted reproductive technology. Reprod Biol Endocrinol. 2014;12:6.
132. Papaleo E, Alviggi C, Colombo GL, Pisanelli C, Ripellino C, Longobardi S, Canonico PL. Cost-effectiveness analysis on the use of rFSH + rLH for the treatment of anovulation in hypogonadotropic hypogonadal women. Ther Clin Risk Manag. 2014;10:479–84.