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Disentangling the current role of LH activity in assisted reproduction: from biology to patient personalization

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ABSTRACT

The importance of determining the precise role of gonadotropins in assisted reproductive technology (ART) is increasingly recognized, as optimizing ovarian stimulation (OS) protocols is crucial for improving outcomes. Recent advances in reproductive biology highlight the multifaceted functions of luteinizing hormone (LH), revealing a complex interplay between its endocrine actions and local ovarian mechanisms. Traditionally, LH was primarily associated with its role in triggering ovulation and supporting the corpus luteum. However, accumulating evidence suggests that LH also influences follicular steroidogenesis, oocyte maturation, and endometrial receptivity. Despite these insights, the role of LH in OS remains controversial, particularly outside of patients with hypogonadotropic hypogonadism, characterized by hypothalamic amenorrhea. Notably, recent molecular and clinical evidence supports the benefits of including LH activity in OS protocols for select patient groups. LH activity can be derived from human menopausal gonadotropin (hMG), which contains both FSH and LH activity, with LH primarily driven by human chorionic gonadotropin (hCG); recombinant LH (rLH), or recombinant FSH + LH (rFSH+rLH). This review clarifies the evolving role of LH activity in ART, bridging foundational biological insights with the emerging paradigm of patient-specific treatment strategies to optimize reproductive outcomes.

Keywords: Gonadotropins, Follicle-stimulating hormone, Luteinizing hormone, Human chorionic gonadotropin, Ovarian stimulation

INTRODUCTION

Assisted reproductive technology (ART) has revolutionized the field of infertility treatment, offering hope to countless couples worldwide. Central to many ART protocols is the intricate orchestration of hormonal signals that regulate follicular development, ovulation, and luteal function. Refining ovarian stimulation (OS) protocols is key to improving outcomes like egg retrieval, fertilization, and pregnancy (Roque and Sunkara, 2025).

Gonadotropins - follicle-stimulating hormone (FSH), luteinizing hormone (LH), and human chorionic gonadotropin (hCG) - are pivotal hormones in regulating OS protocols, and their precise orchestration is crucial for successful reproductive treatment. FSH and LH are secreted by the pituitary gland under the pulsatile stimulus of gonadotropin-releasing hormone (GnRH) (Casati, et al., 2023) and play a complementary role in follicle development and ovulation (Anderson, et al., 2018). FSH initiates follicular growth and drives the growth of multiple ovarian follicles, while LH supports steroid production, contributing to final follicle maturation, fertilization and embryo quality (Hall et al., 2019). On the other hand, hCG is synthesised by the trophoblastic cells of the placenta in pregnant women and promotes progesterone production by ovarian corpus luteal cells during the first 3-4 weeks after pregnancy implantation, reaching a peak at 10 weeks of gestation (Narayan, et al., 2019; Cole, 2010). During the first trimester of pregnancy, hCG replaces the luteotrophic role of LH in sustaining the increasing demand for progesterone. In assisted reproduction protocols, hCG is used to mimic the mid-cycle LH surge due to the high degree of homology between the two hormones (Cole, 2010).

Recent advances in reproductive biology have shed new light on the multifaceted functions of LH, revealing a complex interplay between its endocrine actions and local ovarian mechanisms. The classical view of LH focused on its capacity to stimulate the conversion of cholesterol to androgens and, consequently, promote steroidogenesis. However, accumulating evidence suggests that LH may also influence follicle selection and growth from the early stages, further promoting oocyte maturation and endometrial receptivity (Arroyo et al., 2020; Mao et al., 2024)

Nevertheless, the role of LH activity in OS remains controversial, especially outside of hypogonadotropic hypogonadism, characterized by hypothalamic amenorrhea patients. While some studies suggest that adding LH activity can enhance follicular development, oocyte maturation, and embryo quality, its benefits in patients without clear LH deficiency are not definitively established (Di Segni et al., 2022). Variability in ovarian reserve, receptor sensitivity, and individual endocrine conditions contributes to inconsistent results. Consequently, aside from cases where there is an organic or functional deficiency of FSH and LH, the routine use of LH in OS protocols remains debatable, and further research is needed to identify the specific patient populations that might truly benefit.

This article seeks to disentangle the current role of LH activity in ART by bridging foundational biological insights with the emerging paradigm of patient personalization.

GONADOTROPIN'S STRUCTURE AND GLYCOSYLATION

Luteinizing hormone (LH) is a glycoprotein composed of a common α-subunit, which is shared among the gonadotropins, and a unique β-subunit of 121 amino acids that confers its specific biological activity. Both subunits undergo glycosylation, a post-

translational modification essential for proper folding, receptor interaction, and stability. In LH, glycosylation primarily occurs through N-linked oligosaccharides, though the overall carbohydrate content is less extensive than that found in hCG. The β-subunit of hCG, for instance, is larger at 145 amino acids and includes an additional 24-amino acid C-terminal peptide that carries extra glycosylation sites. This extra glycosylation in hCG results in a significantly prolonged half-life compared to LH. While FSH also shares the common α-subunit, its β-subunit is smaller (110 amino acids) and exhibits a distinct glycosylation pattern that affects its receptor binding and metabolic clearance differently. These structural and glycosylation differences among LH, FSH, and hCG are crucial in determining their specific physiological roles and durations of action in the body (Anderson*, et al.*, 2018; Cole, 2010; Choi et al., 2014; Narayan*, et al.*, 2019).

GONADOTROPINS RECEPTORS (LHCGR, FSHR)

Gonadotropins exert their activity through cell membrane receptors, members of the glycoprotein-hormone receptor subfamily of the rhodopsin-like class of G protein-coupled receptors (GPCRs), expressed in the gonads (Casarini and Simoni, 2021).

LH and hCG bind the same receptor (LH/hCG receptor, LHCGR), while FSH binds an exclusive receptor (FSH receptor, FSHR). Gonadotropin-specific intracellular signals and physiology rely on the structural diversity between the two receptors (Smits et al., 2003) Both receptors have a conserved molecular structure with an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular and transmembrane domains are connected through a hinge region, which is essential for receptor activation (Duan*, et al.*, 2021; Duan*, et al.*, 2023).

Gonadotrophin receptor sites with high specificity and affinity for LH and hCG have been identified in the interstitial cell fractions of rat testes. These binding sites have

been instrumental in radioligand receptor assays to study LH and hCG activity, as well as in exploring the structural features that determine how these hormones interact with their target tissues—ultimately confirming the central role of LH/hCG stimulation in initiating steroid hormone production and supporting reproductive function (Catt and Dufau, 1973).

The LH/hCG receptor exists in multiple isoforms—often referred to as Lhr, LHCGr type 1, and LHCGr type 2—which differ in structural features, ligand binding affinities, and intracellular signalling pathways (Troppmann et al., 2013). LH and hCG both target LHCGR and for many years, LH and hCG were considered interchangeable in clinical settings due to their shared receptor. However, it is now clear that they are not functionally equivalent as they activate distinct intracellular signalling pathways, leading to different physiological outcomes.

LH is secreted in pulses and has a short half-life (about 20–30 minutes), resulting in transient receptor activation that primarily triggers the ERK1/2 and AKT pathways, which drive rapid cell proliferation and survival—processes essential for follicular development and ovulation.

In contrast, hCG features an extra 24–amino acid C-terminal peptide that increases its glycosylation, conferring a much longer half-life (up to 24–36 hours) and sustained receptor stimulation. hCG induces cAMP production more effectively, thereby enhancing steroidogenesis (notably progesterone synthesis) and maintaining prolonged hormonal activity.

Some evidence suggests that the differences between hLH- and hCG-induced signalling are primarily determined by the L2-beta loop of the hormones and the hinge region of

the human LHCGR receptor, which contains a specific region capable of distinguishing between the two hormones (Grzesik et al., 2015).

LHCGR can qualitatively discriminate the LH- and hCG-dependent balance between proliferative/antiapoptotic and steroidogenic/proapoptotic signals occurring in granulosa cells in vitro (Casarini et al., 2012; 2018a; Riccetti et al., 2017). LH has a higher capacity to activate non-cAMP signalling pathways. In contrast, hCG demonstrates greater β-arrestin recruitment and cAMP generation, leading to prolonged receptor activation and a more sustained steroidogenic response.

An additional layer of complexity in LH/hCG signalling is provided by the concept of "spare receptors." Historical pharmacological studies in rodent Leydig cells indicated that maximal activation of LH-dependent steroidogenesis could occur at less than 1% receptor occupancy, suggesting an excess pool of LHCGR on theca cells. While this concept suggested that even low doses of LH or hCG could suffice, as there is an excess of receptors, recent findings——highlighting receptor heterodimerization (including FSHR–LHCGR complexes) and the distinct intracellular signalling of LH versus hCG—suggest that the traditional view of spare receptors might need to be revisited in human ovarian physiology and ART settings (Casarini et al., 2018b).

The FSHR is predominantly localized on granulosa cells of the developing ovarian follicle, while LHCGRs are found on three distinct cell types: the theca cells of the early antral follicle, the mural granulosa cells of the peri-ovulatory Graafian follicle induced by FSH/FSHR, and the luteal cells of the corpus luteum (Casarini et al., 2021). It has been shown that FSHR can exist as dimers, trimers, and recent findings show that FSHR can form heterodimers with the LHCGR. This complexity may explain why in mid-late follicular phase, when both LHCGRs and FSHRs are expressed on granulosa cells, LH is sufficient for follicular growth even in the presence of very low FSH levels,

while as LH levels rise, LHCGR/FSHR heteromers may then promote the LH-mediated pathways required for ovulation and luteinization (Jonas et al., 2018).

This receptor synergy suggests that FSH binding may activate LH-like signals through LHCGR, modulating follicular maturation even though FSH has about 100 times lower potency than LH in inducing cAMP production (50 nM for FSH vs. 500 pM for LH) (Simoni et al., 2020). Moreover, pharmacogenetic studies has shown that there are specific genetic variants of LHβ and LHCGR that could benefit from LH or FSH dose adjustment during ART (Conforti et al. 2025). The interplay between these hormones is critical for optimizing OS protocols. In ART, exogenous FSH and hCG do not induce granulosa cell apoptosis because high levels of oestrogens, such as oestradiol (E2), exert protective, antiapoptotic effects. By contrast, FSH enhances the potency of LH-dependent proliferative and antiapoptotic signals, resulting in higher cell viability compared with FSH and hCG co-treatment (Casarini et al., 2017).

Together, these intricate receptor interactions and signalling dynamics—ranging from receptor dimerization and heterodimerization to differential ligand potency and downstream signalling cascades—form the basis for fine-tuning OS protocols. A deep understanding of these mechanisms allows clinicians to maximize follicular recruitment and maturation while minimizing adverse outcomes like premature luteinization or ovarian hyperstimulation, thereby optimizing reproductive outcomes in ART.

SYNERGISTIC EFFECT OF LH AND FSH IN FOLLICULOGENESIS

Nowadays is accepted that both FSH and LH are indispensable for proper follicular growth and maturation, but they play distinct, stage-specific roles throughout folliculogenesis. The new paradigm in folliculogenesis reflects a move away from a simplistic, linear model toward one that emphasizes the dynamic, cooperative roles of

FSH and LH throughout the follicle's life cycle. Successful follicular development is a product of both hormones working in concert—each setting the stage for the other to ensure proper maturation and eventual ovulation (La Marca et al., 2023). Both gonadotropins contribute to maintain the autocrine–paracrine system governing dominant follicle's growth and LH/FSH coordination is also crucial in sustaining activity at different stages of folliculogenesis. This synergistic role of LH with FSH in follicular development is critical, particularly when FSH alone falls short in achieving optimal E2 production and follicle maturation (Shoham et al., 2002; Lisi et al., 2003; Bosch et al., 2021)

Early Follicular Growth and the Role of FSH

FSH is essential for the early phases of follicle growth. It primarily drives the proliferation and expansion of granulosa cells, which are critical for building the follicle's structure. However, in vitro studies have demonstrated that when small follicles—typically ranging between 85 and 110 μm—are cultured with FSH alone, they do not progress beyond the preantral stage (Wu et al., 2000). This finding indicates that while FSH stimulates initial follicular growth, its action by itself is insufficient to drive complete maturation.

Early Availability of LH Signalling

Even in these early stages, LH signalling is not absent. Studies have demonstrated that functional LHCGR are expressed on both granulosa and theca cells, albeit at very low levels, even in smaller follicles during a phase traditionally considered gonadotropin-independent (Jeppesen et al., 2012; Takao et al., 1997).

This finding indicates that LH may contribute to accelerating the progression of non-growing and primary follicles toward the preantral and antral stages (La Marca et al.,

2023). Moreover, this early expression means that LH can contribute to folliculogenesis right from the beginning, even though its role becomes more critical later. FSH primarily drives the growth of follicles, whereas LH complements this action by stimulating theca cells to produce androgens that are then aromatized into E2 by granulosa cells. In cases where FSH monotherapy does not yield sufficient E2 levels, LH activity supplementation ensures that follicles progress adequately toward maturation.

Transition to Later Stages and the Increasing Role of LH

As the follicle grows, the need for LH becomes more pronounced. The transition from the preantral to the antral stage—and ultimately to a fully mature, ovulatory follicle—requires LH signalling. In animal models, for instance, LHR knockout mice exhibit follicular arrest at the antral stage, clearly demonstrating that without functional LH receptors, follicles cannot progress further (Pakarainen et al., 2015). There is also evidence that LH activity alone is sufficient to support terminal follicle growth. Filicori et al. and Sullivan et al. have both demonstrated that follicles measuring >14 mm will continue to grow when only LH activity is provided (Filicori et al., 2002; Sullivan et al., 1999). It supports theca cells in producing androgens, which are converted to oestrogens in granulosa cells under FSH. This 2-cell, 2-gonadotropin model highlights the synergism between FSH and LH for oocyte maturation, follicle selection, ovulation, and luteinization (Sullivan et al., 1999).

Furthermore, administering low-dose LH activity after follicle selection aids in the continued development of the dominant follicle during the later stages of OS. This approach helps maintain the growth of selected follicles while minimizing the recruitment of new ones, potentially lowering the risk of ovarian hyperstimulation syndrome (OHSS) (Filicori et al., 2002; Sacchi et al. 2018). The LH surge triggers

meiosis resumption in oocytes, ensuring proper oocyte release and corpus luteum formation, vital for P4 production and maintaining early pregnancy.

Recent research shows that this strategy stabilizes the hormonal milieu, promoting a more controlled and efficient maturation process. A meta-analysis has shown that while FSH alone may result in a higher number of oocytes, supplementing with LH activity improves the quality of both oocytes and embryos, highlighting the advantages of a tailored gonadotropin supplementation approach (Santi et al., 2017).

Further studies have emphasized the role of LH in optimizing follicular fluid composition and increasing the expression of key enzymes, such as aromatase, in granulosa cells. This enhances oestrogen (E2) synthesis, which directly benefits oocyte quality and embryo development (Revelli et al., 2009).

This strategy, using LH activity supplementation alongside FSH, not only boosts oocyte competence but also helps synchronize follicular waves and is able to modulate several factors that play a role during embryo implantation, which leads to improved clinical outcomes, such as higher implantation and pregnancy rates (Rahman et al., 2017; Al-Inany *et al*., 2008; 2009; Coomarasamy *et al*., 2008; Van Wely et al., 2011). Endometrial maturation is disturbed in women with low endogenous LH but can be rescued by mid-cycle stimulation of LH receptor with exogenous LH activity supplementation (Tesarik et al., 2003). This is especially beneficial for specific groups of patients, such as those with diminished ovarian reserve or receptor mutations (Baerwald et al., 2020; Bosch et al., 2011).

LH ACTIVITY MODULATES FOLLICULAR DEVELOPMENT IN A CONTROLLED WAY

Advances in molecular biology have deepened our understanding of how LH affects follicular development at the genetic level. A study analysing the role of LH/hCG receptor-specific mRNA binding proteins in regulating LH receptor expression in ovarian granulosa cells found that LH receptor mRNA undergoes transient downregulation after the LH surge, mediated by mRNA binding proteins. This regulation influences receptor expression and oocyte maturation (Nair et al., 2006). Additionally, LH receptor expression decreases after hCG administration due to increased binding activity of Luteinizing Hormone Receptor Binding Protein (LRBP), which promotes mRNA degradation (Menon and Menon, 2014). This mechanism prevents overstimulation and premature luteinization of follicles. This study highlights a complex regulatory network in which LH not only activates immediate signalling pathways but also modulates receptor expression at the mRNA level through specific binding proteins. This dual role of LH ensures that follicular development proceeds in a controlled manner, balancing rapid cell proliferation and survival with the need to prevent overstimulation, thus optimizing conditions for successful oocyte maturation and subsequent fertility (Nair et al., 2006).

ROLE OF LUTEINIZING HORMONE ACTIVITY IN OVARIAN STIMULATION

Although FSH initiates follicular growth, the absence of LH activity leads to suboptimal follicular development. LH plays a crucial role in ovarian function by: (i) enhancing follicular recruitment through increased FSH receptor expression in granulosa cells; (ii) promoting follicular maturation via local growth factor recruitment; (iii) facilitating meiosis completion and first polar body extrusion; and (iv) inducing decidualization of endometrial stromal cells, essential for embryo implantation.

LH activity contributes to the prevention of premature luteinization, final oocyte maturation, and improved endometrial receptivity. In OS protocols where hCG has traditionally been used to trigger ovulation, the use of LH activity can offer benefits in situations where precise control over the timing and nature of follicle maturation is needed. For instance, in patients with low ovarian reserve or in those with specific receptor mutations, adding LH activity can help ensure that the follicles reach full maturity, and that ovulation occurs in a more physiologically synchronized manner (Filicori et al., 2002).

GONADOTROPIN ANALOGUES IN INFERTILITY TREATMENT

Gonadotropin analogues

In the late 1920s it was observed that the urine and blood of pregnant women contained a gonad-stimulating substance (hCG) that was able to induce follicular maturation. This gave rise to the first commercially available gonadotropin (Practice Committee of American Society for Reproductive Medicine*, et al.*, 2008).

Commercial gonadotropins have evolved from the first hCG extract marketed in 1931. Years later, the use of animal gonadotropins allowed the development of the "two-step protocol" for OS in which extracts containing FSH and LH were used to stimulate follicular growth followed by hCG administration to promote follicular maturation (Mazer and Ravetz, 1941). However, given its origin, these extracts were not able to induce a sustained response in women. This led to obtaining the human menopausal gonadotropin (hMG), containing FSH and LH, from the urine of post-menopausal women. Despite its effectiveness in inducing OS, these extracts presented some limitations such as the presence of impurities or their limited availability, although

advances in the manufacturing process have allowed for a new generation of highly purified gonadotropins, with higher quality and batch-to-batch consistency (Lunenfeld*, et al.*, 2019; Lunenfeld, 2012).

The emergence of recombinant DNA technologies revolutionised the treatment of infertility by providing recombinant forms of FSH (rFSH) and LH (rLH), allowing for their mass production and obtaining products with higher purity and batch-to-batch consistency and thus, arguably more effective, compared with the traditional urinary gonadotropins (Lunenfeld*, et al.*, 2019; Racca*, et al.*, 2020). Recombinant gonadotropins are produced using cell lines such as Chinese hamster ovary (CHO) cell line and human foetal retinal cells transfected with vectors encoding the human sequence (Lunenfeld*, et al.*, 2019).

Currently, rFSH and rLH as well as urinary forms of FSH and LH, hCG, and highly purified hMG (HP-hMG) are widely used for OS (**Table 1**) (Racca*, et al.*, 2020).

When comparing rFSH and hMG for OS, several studies have examined their effects on implantation, pregnancy, aneuploidy, and miscarriage rates. Although both agents are effective in promoting follicular development, four large systematic reviews have demonstrated a small but consistent increase in clinical pregnancy and live birth rates with hMG compared to rFSH (Al-Inany et al., 2008; 2009; Coomarasamy et al., 2008; Van Wely et al., 2011). Notably, more than two-thirds of participants in the hMG arms of these studies underwent stimulation with HP-hMG, suggesting that the improved in vitro fertilization (IVF) outcomes associated with hMG may be attributed to its intrinsic LH bioactivity.

Furthermore, there is some evidence suggesting that the LH activity in hMG may positively influence oocyte and embryo quality, potentially reducing aneuploidy rates

(Huddlestone et al., 2009; Weghofer et al., 2008). A study involving oocyte donors found that the euploidy rate was significantly associated with the proportion of gonadotropins derived from hMG (McCulloh et al., 2019). More recently, Cozzolino et al. (2024) investigated the impact of OS regimens on aneuploidy and blastocyst development in IVF cycles. Their study showed that the total gonadotropin dosage does not significantly affect aneuploidy rates, IVF outcomes, or cumulative live birth rates. However, women who received a higher proportion of hMG during OS had a significantly lower aneuploidy rate (P = 0.02). When results were stratified by age, younger women receiving higher hMG doses showed a marked reduction in aneuploidy rates (P < 0.001), whereas no significant differences were observed among older women, regardless of hMG dosage (Cozzolino et al., 2024). They concluded that the use of hMG should be evaluated on a case-by-case basis, according to the individual's characteristics and infertility type.

Differences in miscarriage rates between FSH- and hMG-based treatments are generally minimal and not consistently significant across studies. Overall, while hMG may offer certain advantages in selected patient populations, the choice of gonadotropin should be individualized based on patient characteristics and clinical context.

GONADOTROPIN PREPARATIONS CONTAINING LH ACTIVITY

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.

hMG is used for multifollicular development in women undergoing controlled ovarian hyperstimulation. It is usually composed of equal proportions of FSH and LH from the urine of post-menopausal women. During the purification process the FSH:LH proportions can be altered thus, requiring the addition of hCG to re-establish the LH activity (Lunenfeld*, et al.*, 2019).

The use of hCG for OS can mimic the LH activity, but there are differences between these two gonadotropins (**Table 2**). hCG has a longer half-life, higher binding affinity for the LHCGR and higher biopotency, providing more sustained LH stimulation (Ezcurra and Humaidan, 2014).

Additionally, the clinical safety and efficacy of gonadotropin preparations can vary depending on whether the hCG component is placental- or pituitary-derived, with placental hCG being more commonly used due to its longer half-life and established effectiveness. However, clinicians and patients are not always adequately informed about the specific origin of hCG in prescribed medications, which may impact informed decision-making in treatment selection.

In a comprehensive meta-analysis, Santi et al. investigated how different types of LH activity—specifically hMG, rLH, and hCG—affect various reproductive outcomes, including the total egg count, number of mature M2 eggs, implantation rates, and pregnancy rates. The analysis incorporated seventy randomized controlled trials (RCT) and adhered to the PRISMA guidelines for systematic reviews, while excluding women with PCOS. The results indicated that although FSH-only stimulation produced a higher number of eggs, it did not increase the proportion of mature eggs. In contrast, protocols that included LH activity led to the development of more embryos, as well as improved implantation and pregnancy rates. The study emphasized that the specific type of LH activity significantly influenced the outcomes, and the researchers suggest that

administering LH activity via hMG or rLH may be more advantageous than using hCG, as these options provide better pregnancy rates. Embryo number and implantation rate were higher when hMG was used instead of FSH alone (Santi et al., 2017). In intrauterine insemination cycles in women ≥35 years, stimulation with rFSH+rLH or HP-hMG offers similar results in terms of ovarian response, demonstrating that bioactivity is comparable between the two treatments when administered in the same ratio. The additional advantage of HP-hMG is the lower proportion of medium-sized follicles, which could be responsible for an increased risk of OHSS and multiple gestations (Moro et al., 2015). This finding has also been observed in IVF, where rFSH + rLH proved to be comparable to HP-hMG in terms of main IVF outcomes (pregnancy rate, implantation rate, oocyte and embryo quality) (Orvieto, 2019), although it may be associated with a higher risk of ovarian hyperstimulation in the rFSH + rLH group (Pacchiarotti et al., 2010). Administration of HP-hMG can be a valid and safe option for the induction of ovulation in intrauterine insemination (IUI) and IVF.

Evidence from experimental and clinical studies indicates that LH activity driven by hCG and rLH is not equivalent at either the molecular or functional level. While rLH offers the advantages of precise dosing and high purity, hMG, which contains both FSH and LH, provides benefits such as cost-effectiveness, long-term clinical validation, and simplified administration, making it a valuable option in many OS protocols. Ultimately, the choice between hMG and rLH (often used alongside rFSH) should be tailored to the individual patient's clinical profile and treatment goals (Shahrokh et al., 2017).

Despite 20 years of data on exogenous LH supplementation in OS for ART, the clinical role of LH remains unclear. This may be related to the fact that previous meta-analyses included different types of patients in the same group, making it difficult to determine in

which women LH supplementation could be recommended. However, recent publications that offer insights into the patient profiles most likely to benefit from this supplementation.

CLINICAL EVIDENCE ON THE USE OF LH ACTIVITY SUPPLEMENTATION IN SPECIFIC PATIENT PROFILES

Exogenous LH activity supplementation can increase the efficacy of ARTs (Nedresky and Singh, 2025). At the time of publication of the latest version of the ESHRE guidelines, the evidence on the use of LH in combination with FSH for OS suggested that this treatment was required for women with hypogonadotropic hypogonadism (Balasch*, et al.*, 1995; Loumaye*, et al.*, 1997) and resulted beneficial in patients showing a hyperresponsiveness to stimulation with rFSH alone (De Placido*, et al.*, 2001; Ferraretti*, et al.*, 2004). In contrast, it did not show a benefit in women of <35 years with a normal response (Griesinger*, et al.*, 2006; Bosch*, et al.*, 2011) and it might be beneficial for those of 36-39 years (Bosch*, et al.*, 2011; Hill*, et al.*, 2012). In poor responders, LH supplementation remains a subject of ongoing debate. While some clinicians advocate for routine LH activity supplementation in poor responders fulfilling the Bologna criteria, current evidence does not support a one-size-fits-all approach (Humaidan*, et al.*, 2017).

In addition, the evidenced obtained in a metanalysis of randomised controlled trials comparing rLH combined with rFSH versus rFSH alone in IVF/ISCI cycles did not report sufficient evidence to support the use of LH supplementation in order to improve rates of live births (Mochtar*, et al.*, 2017).

Similarly, initial meta-analysis comparing treatments with hMG versus rFSH in patients undergoing ART have reported no differences between treatments in terms of pregnancy

and live birth rates (Westergaard et al. 2003). There was no evidence of a difference between hMG and rFSH in ongoing pregnancy/live birth per woman (OR 1.27; 95% CI 0.98 to 1.64). The 2019 ESHRE guidelines, equally recommend the use of rFSH and hMG for OS. In addition, the use of highly purified FSH and hMG for OS in GnRH agonist protocols is equally recommended (ESHRE Reproductive Endocrinology Guideline Group, 2019).

However, subsequent meta-analyses suggested a potential benefit of hMG/rLH combined with rFSH administration over rFSH alone. A Cochrane analysis of rFSH vs hMG, published in 2011 concluded that there was no difference in live birth rate, but that there were significantly fewer live births after r-FSH than hMG (N=3197, OR 0.84, 95% CI 0.72 to 0.99), when combined in studies with a long GnRH agonist protocol (van Wely et al., 2011). This finding was confirmed in other meta-analyses which showed that hMG is superior to rFSH regarding clinical efficiency in ART cycles and concluded that the clinical superiority of hMG could be attributed to the LH activity it contains (Al-Inany et al., 2009; Coomarasamy et al, 2008; Santi et al., 2017; Van Melo et al., 2022).

Considering the heterogeneity of the evidence, the 2019 EHSRE guidelines working group did not reach a consensus regarding the use of LH supplementation and no recommendations were made. Since then, additional studies have been published, offering new evidence that may support the supplementation of LH activity during OS, as outlined below for the various groups of patients.

Hypogonadotropic/Hypogonadism

For patients with WHO type I anovulation (characterized by hypothalamic amenorrhea), experts agree that adding LH preparations is crucial. Women with hypothalamic

hypogonadism (including Kallman´s syndrome) struggle grow follicles in response to FSH stimulation and cannot produce normal levels of E2, unless LH activity is also provided (Balasch et al., 1995). Moreover, in these women when only FSH is given, the eggs don't perform as they should, which indicates that LH is crucial. LH is needed to support the production of steroid hormones in the follicle and to ensure that the oocyte develops with optimal quality (Shoham et al., 1991; 1993).

Medical societies recommend various treatment options—including hMG, a combination of FSH and rLH, or even FSH with hCG—but some important details must be considered. Studies have shown that a 2:1 ratio of rFSH to rLH is optimal for inducing a single, dominant follicle—a key goal in ovulation induction. In fact, a regimen using 150 IU of rFSH alongside 75 IU of rLH has been found to be the most physiological approach based on comparative research (The European Recombinant Human LH Study Group, 1998).

Deeply Suppressed LH Levels

Women with an extremely low LH nadir (<0.5 mIU/mL) during stimulation produced more eggs but had embryos with lower implantation rates, resulting in reduced clinical pregnancy and live birth rates (Propst et al., 2011). This indicates that very low LH levels may negatively affect oocyte quality. Similarly, previous 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 IU/L) after downregulation with GnRH agonists and OS with FSH monotherapy (Westergaard et al., 2000; Humaidan et al., 2002; Laml et al. 1999; Nakagawa et al. 2008). Nevertheless, while some studies have reported poorer outcomes associated with lower serum LH levels (Humaidan et al., 2002), others have not found such associations (Kolibianakis et al., 2004; Merviel et al., 2004). These discrepancies may be explained

by LH's short half-life and pulsatile secretion. Lahout et al. (2006) noted that although low mid-follicular LH levels significantly affect ovarian response, they do not necessarily impact live birth rates. However, a decline in LH levels of more than 50% from the early- to mid-follicular phase was associated with a reduced live birth rate. In line with this, Esposito et al. (2001) suggested that, when r-hFSH is used exclusively in ART cycles, it may be clinically beneficial to supplement LH in the late follicular phase or to further reduce the dose of GnRH agonist.

In cases where low follicular LH tone poses a risk, clinicians may either measure mid-follicular LH (or hCG) levels and supplement accordingly or proactively include LH activity in the stimulation protocol. The latter strategy appears to be safe—given the absence of a clearly defined ceiling effect—and may offer significant benefits for select patient populations.

Regarding the influence of the GnRH analogue type, some studies have reported improved pregnancy outcomes with LH activity supplementation in women undergoing GnRH agonist down-regulation (Platteau et al., 2008; Pezzuto et al., 2010). In GnRH antagonist cycles, improved implantation rates have been observed with LH supplementation in women with a history of implantation failure (Rahman et al., 2017). Nevertheless, these findings should be interpreted with caution, as the sample size in that study was very small, and the definition of "recurrent implantation failure" pertains to a relatively rare clinical scenario. Additionally, while the benefit of rLH supplementation in GnRH antagonist protocols remains debated, several studies suggest that LH activity may be associated with enhanced embryo development and higher live birth rates in these cycles (He et al., 2018; Mochtar et al., 2017; Paterson et al., 2015; Setti et al., 2021; Wang et al., 2022).

Researchers suggest that GnRH agonist protocols lead to more profound LH suppression, making the addition of LH activity to stimulation particularly beneficial (Hill et al., 2012). ln an antagonist protocol, the degree of LH recovery 24 h post first GnRH antagonist injection can identify those patients who may benefit from added LH. In addition, rising LH during the first 5 days of stimulation may predispose patients to a sharp LH drop following the first GnRH antagonist dose, and the need for added LH (Kol, 2020). The beneficial effect of LH activity on implantation has been associated not only with the development of higher-quality embryos and enhanced embryonic potential, but also with the optimization of decidualization and uterine receptivity (Wang et al., 2022).

Women with poor ovarian response/reserve

In 2014, Lehert et al., in a meta-analysis including over 6,000 IVF cycles, observed that supplementation with rLH improved the pregnancy rate by 9% in the general population. However, in "poor responder" patients, this beneficial effect was even greater, with a relative risk (RR) of 1.3 (95% CI: 1.01–1.67) (Lehert et al., 2014).

In the largest multi center RCT carried out to explore the use of LH activity in “poor responders” aligned with the Bologna criteria [presence of at least two of the following characteristics: (i) advanced maternal age (> 40 years) or any other poor ovarian response risk factor; or (ii) a previous episode of poor ovarian response (POR; defined as ≤ 3 oocytes retrieved after a conventional stimulation dose), a low ovarian reserve test in terms of anti-Müllerian hormone (AMH), and a low antral follicle count (AFC)], a total of 939 patients were included. The study compared OS using 300 IU of rFSH alone versus 300 IU of rFSH plus 150 IU of rLH. The study's results did not show

significant differences in the pregnancy rate. However, a post-hoc analysis of the same study—which stratified patients into mild, moderate, and severe "poor responders"—found that the live birth rate was significantly higher in the moderate and severe subgroups when treated with rLH (Humaidan et al., 2017). This stratification used the so-called Baseline Severity Score (BSC), which considers three criteria: (i) age ≥ 40 years; (ii) reduced ovarian reserve (AMH < 0.5 ng/mL); and (iii) retrieval of fewer than 2 oocytes during the most recent ART cycle. Based on this scoring system, patients were classified as mild (BSC = 0), moderate (BSC = 1), or severe (BSC = 2) “poor responders”. The BSC score was later renamed the PROsPeR (Poor Responder Outcome Prediction) score and demonstrated good predictive value for live birth rates in women meeting the Bologna criteria. In a large real-world study of 9,787 IVF cycles, Arvis et al. (2021) confirmed that patients with moderate or severe PROsPeR scores had significantly higher cumulative live birth rates when treated with a combination of r-hFSH and r-hLH, compared to those who received r-hFSH monotherapy. This effect may be attributed to improved oocyte quality, as suggested by a higher embryo-to-oocyte ratio observed in women receiving LH activity supplementation (Arvis et al., 2021).

Due to the lack of randomized studies analyzing the efficacy of LH supplementation across the different POSEIDON groups—a classification that stratifies patients with a low prognosis in ART based on age, ovarian reserve (AFC/AMH), and response to previous stimulation—some studies have retrospectively examined this issue. The POSEIDON criteria divide patients into four groups: Groups 1 and 2 include women with normal ovarian reserve but an unexpected poor or suboptimal response, with Group 1 being younger than 35 years and Group 2 aged 35 or older. Groups 3 and 4

include women with reduced ovarian reserve, with Group 3 being under 35 and Group 4 aged 35 or older.

For POSEIDON Groups 1 and 2, a low starting dose of gonadotropins and gonadotropin receptor polymorphisms have been suggested as possible causes of hypo response. In these patients, increasing the initial gonadotropin dose, adding rLH, or implementing dual stimulation have been proposed as potential strategies to improve outcomes (Chinta et al., 2021). Nevertheless, no high-quality RCTs have yet demonstrated a clear benefit of these interventions in this subgroup of patients.

In patients with a very low ovarian response (1–3 oocytes retrieved)—many of whom would fall into POSEIDON Groups 2 and 4—a retrospective study by Levi-Setti et al. (2019) found that the administration of rLH equalized clinical outcomes (in terms of pregnancy and live birth rates) to those achieved with FSH monotherapy, despite the LH-supplemented group having poorer baseline clinical characteristics.

Another relevant study evaluating LH activity supplementation in POSEIDON-defined patients demonstrated that, in Groups 3 and 4 (i.e., patients with reduced ovarian reserve), the addition of hMG from the start of stimulation significantly improved live birth rates and reduced cycle cancellations due to fertilization failure (Berker et al., 2021).

Other studies have also reported a beneficial effect of LH activity supplementation alongside FSH in women with poor ovarian response, showing an improvement in ovarian steroid production (17-hydroxy-progesterone, androstenedione, E2, and estrone), which could predict fertilization success (Marchiani et al., 2020).

Women with a previous inadequate response to FSH-only stimulation

The impact of LH-containing regimens on IVF outcomes in women with a previous inadequate response to FSH-only stimulation warrants careful consideration. LH activity supplementation has been shown to be more effective than increasing the dose of rFSH in terms of ovarian outcome in patients with an initial inadequate ovarian response to rFSH alone (De Placido et al., 2005). In these hypo-responsive patients, ovarian resistance can clinically manifest as an "initial slow response" or "stagnation" in follicle growth during OS with FSH monotherapy. A recent meta-analysis performed in women with a hypo-response profile concluded that the addition of r-hLH during OS lead to a significant increase in oocyte number, implantation rate, and clinical pregnancy rate (Conforti et al., 2019). Only one RCT reported data about live birth rate per cycle, suggesting an increased live birth rate in hypo-responders undergoing stimulation with r-hFSH and r-hLH co-treatment compared with those who received r-hFSH alone during their OS (Ferrareti et al., 2004).

The need to add LH activity in hypo-responders is not fully understood, but it's hypothesized that they have a relative LH deficiency (Alviggi et al. 2025). This may result from insufficient endogenous LH during pituitary suppression and could be linked to genetic variants affecting the LH system. In fact, a specific genetic variant of the LH β chain showed a typical hypo-response profile during OS with exogenous FSH (Alviggi et al., 2013). Similarly, hypo-responder women who express a common variant of the LHCGR receptor also seem to benefit from exogenous LH activity during OS (Ramaraju et al., 2021). In addition, LH activity supplementation in women with hypo-response could modify the follicular fluid steroid composition, changing it to a more physiologic composition in terms of estrogens and progestins (Marchiani et al., 2020).

Women with normal reserve/response

Despite the use of LH activity in women with a normal response might not be required, some studies suggest that the administration of hMG versus rFSH alone produced more top-quality embryos, led to the development of more embryos, as well as improved implantation and pregnancy rates (Santi et al., 2017; Smitz et al., 2007; Ziebe et al., 2007). Additionally, it has been suggested that the addition of both hMG and rLH to FSH may improve the chance of infertile women with normal ovarian responses to have more success in having live birth babies, specifically in those over 32 years of age or with overweight/obese patients who typically face challenges in conceiving and sustaining a pregnancy (Liang*, et al.*, 2024;). The authors suggest that the benefit of adding both hMG and rLH to FSH could be associated to the improvement in endometrial thickness, which is a major factor affecting IVF/ICSI success in these women (Liang*, et al.*, 2024;).

In women, the use of LH activity to prevent OHHS has also been investigated. Caserta et al. investigated 999 infertile women randomized to receive rFSH alone or rFSH + rLH (rLH supplementation at a dose of 75 IU/d from stimulation day 7 onward). No differences in age or mean number of embryos transferred were observed between groups. Although the number of eggs retrieved was lower in the supplemented group than in the rFSH–alone group (6.1 ± 3 vs. 6.6 ± 3.8; $P<.05$), the clinical pregnancy rate was higher in the supplemented group than in the rFSH–alone group (16.8% vs. 11.9%; $P<.05$). The proportion of cancelled cycles owing to OHSS risk (8.3% vs. 2.4%; $P<.000001$) and the proportion of patients who developed clinical OHSS (1.6% vs. 0.2%; $P<.05$) were significantly higher in the rFSH–alone group than in the rFSH + rLH group (Caserta et al., 2011).

Women with high ovarian response

The use of LH during OS has also been studied in women with predicted high response. A trial conducted in the US in these women comparing the effect of HP-hMG vs rFSH on ongoing pregnancy rate showed that the treatment with HP-hMG resulted in higher ongoing pregnancy rate as well as live birth rate and lower pregnancy loss rates after both fresh and frozen embryo transfers. In addition, the incidence of treatment-related adverse events, including ovarian hyperstimulation syndrome, was lower in women treated with HP-hMG (Witz*, et al.*, 2020). More recently, a cost-comparison analysis from the US private payer perspective comparing HP-hMG vs rFSH in predicted high-responders was published. The results of the analysis predicted that women treated with HP-hMG required fewer embryo transfers to achieve similar live birth rates and presented fewer cases of OHHS when compared with those treated with rFSH, which in turn, resulted in lower total costs per live birth (Khair*, et al.*, 2023).

Women of advanced maternal age

A systematic review and metanalysis of 12 RCT investigating the use of rLH+rFSH in women of advanced maternal age (35-40 years) undergoing OS for IVF found that, although more oocytes were retrieved in women with rFSH alone, the combination of rLH+rFSH was associated with higher implantation and clinical pregnancy rates (Conforti*, et al.*, 2021). The main limitation of this meta-analysis is that very few studies have specifically evaluated the clinical effects of r-hFSH/r-hLH co-treatment in well-defined age ranges, and only two studies reported live birth outcomes (Matorras et al., 2009; Vuong et al., 2015), despite live birth being considered the most relevant endpoint in reproductive medicine. Notably, one RCT (Matorras et al., 2009), which included only women aged 35–39 years, reported a significant benefit in live births per cycle (19.0% vs. 7.4%, $p = 0.047$). However, the majority of RCTs included in the meta-analysis focused on clinical pregnancy rates, and the available data suggest

that women between 35 and 40 years of age may benefit from r-hLH supplementation in terms of improved outcomes. The authors point out that, due to the age-dependent increase in miscarriage rates in this patient population, a substantial drop-out before delivery is commonly observed in similar trials. As a result, the sample size required to detect statistically significant differences in live birth rate becomes economically unfeasible. This also helps explain why even the largest RCTs published to date have been underpowered to detect differences in live birth outcomes between treatment arms in advanced maternal age groups.

Furthermore, in most trials, power calculations were based on clinical pregnancy rates or the number of oocytes retrieved, not on live birth rate. Consequently, when live birth rate—a secondary outcome in the original studies—is elevated to a primary outcome in meta-analyses, there is an increased risk of Type I error. In addition, live birth rate is more susceptible to bias than clinical pregnancy rate and is not directly influenced by the type of gonadotropin used for OS in ART. For these reasons, the authors argue that clinical pregnancy rate is a more appropriate endpoint for evaluating the effectiveness of different gonadotropin regimens for OS in this age group.

Similarly, a recent study using real-world data from a German registry showed that the combined treatment of rLH+rFSH (2:1 ratio) vs rFSH alone resulted in higher pregnancy and live birth rates in women of 35-40 years with normal ovarian reserve. No difference in the incidence of OHHS between treatment groups was observed (Bielfeld*, et al.*, 2024).

Overall, data from these studies indicate that the combination of rLH+rFSH in women of advance maternal age could compensate for the increase of fully glycosylated FSH variants (with lower affinity for the FSHR), decreased LH activity and reduced

androgen production observed in these women and therefore, increase pregnancy outcomes (Conforti*, et al.*, 2021; Bielfeld*, et al.*, 2024).

Different results were reported by König et al. in a RCT involving 253 couples undergoing IVF/ICSI. In their study, women aged 35 years or older underwent OS using a GnRH antagonist protocol with either rFSH at 225 IU/day or a combination of rFSH and rLH at 150 IU/day, starting on stimulation day 6. The intention-to-treat analysis revealed no significant differences in implantation rates (18.8% vs. 20.7%; mean difference −1.9%, 95% confidence interval [CI] −8.0 to 11.7) or clinical pregnancy rates (28.0% vs. 29.7%; mean difference −1.5%, 95% CI −9.4 to 12.7) (König et al., 2013). Similar findings were reported in the study by Vuong et al., in which the LH treatment protocol was also initiated on day 6 (Vuong et al., 2015).

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 (Bosch et al., 2014). Nevertheless, given the heterogeneity and limited quality of the published data, additional large RCTs examining the impact of rLH supplementation are needed to draw a conclusive recommendation about the routine incorporation of rLH in older women undergoing IVF/ICSI.

Fresh embryo transfer

In women undergoing OS with GnRH antagonists, LH activity could protect from elevated serum progesterone levels at the end of the follicular phase during OS thus, improving pregnancy and live birth rates in fresh embryo transfers (Werner et al. 2014). A recent retrospective study evaluated the effects of the addition of hMG on the change in serum progesterone levels and determined an optimal hMG:FSH ratio that reduced the risk of elevated peak serum during fresh embryo transfer (hMG:FSH ratio of >0.6).

Additionally, hMG:FSH ratios of 0.3-0.4 and 0.3-0.6 were associated with increased pregnancy rates and live birth rates, respectively (Wesevich*, et al.*, 2023). Similarly, the results of a clinical trial assessing the use of HP-hMG vs rFSH in donors with normal ovarian response showed that stimulation with hMG + rFSH led to significantly lower serum progesterone levels on stimulation days 6 and 8 and on day of trigger and lower pregnenolone:progesterone ratio on stimulation day 8 and on day of trigger compared with rFSH alone. In contrast, women in the hMG group presented higher serum androstenedione levels on stimulation day 8 and on day of trigger and higher pregnenolone:androstenedione ratio on stimulation days 6 and 8 and on day of trigger compared with those in the rFSH group (Bosch*, et al.*, 2024). hMG enhances the Δ5 steroidogenic pathway, facilitating the conversion of pregnenolone to androstenedione, which ultimately results in lower serum progesterone levels at the end of the cycle. In contrast, rFSH favours the conversion of pregnenolone to progesterone, leading to higher circulating progesterone levels (Bosch*, et al.*, 2024).

A recent meta-analysis including 68 RCTs and 34 different COS protocols has demonstrated that in women with predicted normal response undergoing long GnRH agonist cycles for pituitary suppression, the use of rFSH for OS may result in decreased fresh-cycle live birth rates compared to hMG (RR 0.80, 95% CI 0.68 to 0.95; 7 studies; 1575 women; I2 = 1%; low-certainty evidence) (Melo et al., 2022).

Therefore, LH activity would be recommended when considering a fresh embryo transfer, as it offers several advantages, (higher likelihood of maintaining adequate serum progesterone levels after OS, reducing FSH requirements, etc). This would reduce the possibility of having to switch to a delayed frozen embryo transfer, which would lead to a delayed process, causing higher economic costs, or to perform a fresh embryo transfer with lower success ratio.

COST-EFFECTIVENESS OF THE USE OF GONADOTROPINS

Another important aspect to consider is the cost-effectiveness of gonadotropin medications. Comparable clinical outcomes can be achieved at a lower cost when using HP-hMG versus rFSH in OS protocols, particularly in cohorts of predicted high-responders (Khair et al., 2023). An analysis of data from the MERIT and MEGASET clinical trials, along with real-world data from French ART practice, showed that HP-hMG was associated with lower overall costs for IVF/ICSI management compared with rFSH. In ART, when comparing different gonadotropins, several Incremental Cost-Effectiveness Ratio (ICER) drivers should be taken into account, including differences in live birth or aneuploidy rates, the number of stimulation cycles required, the incidence and management costs of OHSS, and the direct cost of the medications themselves. Deterministic sensitivity analyses identified the live birth rate as the most influential ICER driver, followed by the total gonadotropin dose used (Barrière et al., 2018).

The price of gonadotropins is largely influenced by their FSH content. Some authors suggest that hMG could offer a cost advantage among available options, as it provides equivalent stimulation to large follicles compared to 150 IU of rFSH at roughly half the cost, with the added benefit of including the LH component at no extra charge (Toner and Pirtea, 2025). However, it is important to note that no head-to-head pharmacoeconomic studies have definitively demonstrated cost-effectiveness differences between these two gonadotropin preparations.

CONCLUSIONS

The contemporary increase in the incorporation of LH activity in OS protocols is supported by a growing body of molecular and clinical evidence. This trend stems from

an evolving understanding of LH's fundamental role in follicular maturation, ovulation, and luteinization, distinguishing it from the action of hCG. Its inclusion in ART treatments has been shown to improve outcomes in specific patient groups, reinforcing its importance in modern OS protocols.

From a molecular perspective, the new paradigm in folliculogenesis reflects a move away from a simplistic, linear model toward one that emphasizes the dynamic, cooperative roles of FSH and LH throughout the follicle's life cycle. Successful follicular development is a product of both hormones working in concert—each setting the stage for the other to ensure proper maturation and eventual ovulation. LH ensures that follicular development proceeds in a controlled manner, balancing rapid cell proliferation and survival with the need to prevent overstimulation, thus optimizing conditions for successful oocyte maturation and subsequent fertility.

On a clinical level, various studies have demonstrated the effectiveness of LH activity supplementation in women with hypogonadotropic hypogonadism (HH) or hypothalamic amenorrhea (HA), where there is an endogenous LH deficiency. LH activity supplementation improves follicular growth and supports ovulation. Likewise, in women with diminished ovarian reserve (DOR), low endogenous LH production may impair ovarian response, and LH supplementation has been shown to optimize E2 production and follicular development, improving implantation and pregnancy rates.

Protocols containing some form of LH activity can also mitigate the risk of LH-deficiency due to protocol choices (i.e. patients who are over suppressed because of GnRH analogues or progestin protocols). Moreover, including LH in OS protocols may reduce the risk of OHSS by sustaining the growth of dominant follicles without recruiting new ones. This is particularly useful for patients with polycystic ovary

syndrome (PCOS) or high antral follicle counts, who are at greater risk of developing OHSS when stimulated with FSH alone.

Evidence suggests that LH activity supplementation could also be beneficial in certain profiles such as in women with unexpected poor/suboptimal responses (moderate and severe "poor responders") and in those of advanced maternal age (35-40 years). In these patients, it is important to determine the most appropriate timing for administration, and some studies have suggested that LH activity supplementation should be initiated on the first day of stimulation to fully maximize its effects on both theca and granulosa cells. In cycles with an increase in the P4 level above 1.5 ng/mL before trigger, the pregnancy rates are reduced. Based on recent studies with GnRH antagonist protocols, sufficient LH activity also reduces the chance for a premature P4 elevation and increase the opportunity for a fresh transfer.

LH activity supplementation with doses commonly used in clinical practice does not present higher adverse effects compared with the use of FSH (Wang*, et al.*, 2022; Bielfeld*, et al.*, 2024; Khair*, et al.*, 2023). The lack of a clear ceiling effect for LH, in contrast to FSH, suggests that its inclusion in OS protocols can be beneficial without significantly increasing the risk of overstimulation.

In conclusion, considering the evidence supporting the benefits of LH activity supplementation for almost all the patients we encounter in our clinical practice (older women, women with low ovarian reserve, poor responders, and women with PCOS, as well as its potential benefits in fresh transfers) (**Table 3**), its good safety profile, and the potential cost-effectiveness, it would be reasonable to consider the combination of LH and FSH as a standard practice for OS.

Ultimately, the choice among the different commercially available gonadotropin preparations containing LH activity should be tailored to the individual patient's clinical profile and treatment goals (Shahrokh et al., 2017). While rLH offers the advantages of precise dosing and high purity, hMG provides benefits such as cost-effectiveness, long-term clinical validation, and simplified administration, making it a valuable option in many OS protocols. As research into LH in OS continues to evolve, future studies should prioritize the identification of predictive biomarkers, particularly those that indicate which patients will benefit most from LH supplementation and how LH affects oocyte quality. Additionally, refining OS protocols by optimizing the timing and dosage of LH could enhance clinical outcomes in ART. Integrating molecular biology with reproductive medicine will pave the way for more personalized and effective fertility treatments, ensuring that each patient receives the most precise and efficient OS protocol based on her unique biological profile.

As a narrative review, the study lacks the methodological rigor of a meta-analysis, which may introduce potential biases in the selection of studies. However, it offers an exhaustive review of the main studies published on the subject, supported by the underlying biological mechanisms that validate them. Future studies are encouraged to explore recent developments in ART, such as pharmacogenomics—including FSH and LH receptor polymorphisms (FSHR, LHCGR)—to enable the customization of OS protocols based on individual genetic profiles. In parallel, the integration of artificial intelligence (AI) into clinical decision-making holds promise for further personalizing treatment regimens and improving the selection of the most appropriate gonadotropin for each patient.

Authors' roles

All authors have contributed to the conception and design, analysis and interpretation of

data, drafting the article and revising it critically for important intellectual content, and to the final approval of the version to be published.

Conflict of interest

The authors declare that there were no conflicts of interest.

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Table 1. Available gonadotropin analogues for ovarian stimulation

Gonadotropin	Name	Source	Origin
FSH	hFSH-HP	Urinary	Urine from post-menopausal women
	rFSH Alpha	Recombinant	Chinese hamster ovary cell line
	rFSH beta	Recombinant	Chinese hamster ovary cell line
	rFSH delta	Recombinant	Human foetal retinal cells
	Corifollitropin alfa	Recombinant	Chinese hamster ovary cell line
LH	rLH	Recombinant	Chinese hamster ovary cell line
hCG	u-hCG	Urinary	Urine from pregnant women
	r-hCG	Recombinant	Chinese hamster ovary cell line
hMG	hFSH-HP + placental hCG	Urinary	hMG extracted from urine from post-menopausal women. hCG extracted from urine from pregnant women
	hFSH-HP + pituitary hCG	Urinary	hMG and hCG extracted from urine from post-menopausal women

	rFSH alpha + rLH	Recombinant	Chinese hamster ovary cell line

HP: highly purified

Table 2. Main differential characteristics of LH activity from LH and hCG

	LH	Placental hCG	Pituitary hCG
Composition	rLH	Free hCG hCG	Sulphated hCG
Isoforms and glycoforms	One main molecule and ~39 isoforms	≥9 main molecules and an unknown number of isoforms	
Half-life	25 minutes	15-462 hours	2.32 days
Sialic acid content		hCGα: 2.4 mol/mol hCGβ: 9.4 mol/mol	hCGα: 1.7 mol/mol hCGβ: 4.6 mol/mol
Sulphate content		hCGα: 0.4 mol/mol hCGβ: 0.4 mol/mol	hCGα: 0.8 mol/mol hCGβ: 2.7 mol/mol
hCG content		10.5 ± 0.5 ng/ml (per 5.000 mIU/batch)	26.1 ± 0.7 ng/ml (per 5.000 mIU/batch)
LH content		No detectable	1.2 ± 0.02 ng/ml (per 5.000 mIU/batch)
EC_{50} required for cAMP activation	530.0 ± 51.21 ng/ml	107.1 ± 14.3 ng/ml 2.8 ± 1.1 ng/ml (Representation for hCG content)	11.0 ± 12.0 ng/ml (Representation for hCG content)
Maximum cAMP accumulation time	10 minutes	60 minutes	

Table 3. Summary of Clinical Indications for LH Activity supplementation in Assisted Reproductive Technology (ART)

Patient profile	Indication for LH activity Supplemmentation	Preferred LH source/Protocol
Hypogonadotropic Hypogonadism (WHO I)	Essential; LH required for follicular growth and steroidogenesis	rFSH + rLH (2:1 ratio) or hMG
Deeply Suppressed LH Levels (<0.5 mIU/mL)	Recommended; improves oocyte quality and implantation rates	Monitor LH; add rLH/hMG if low during stimulation
Poor Ovarian Response / Reserve **Bologna criteria and Severe PRosPeR score** **POSEIDON [groups 3 and 4 and very low response (1–3 oocytes)]**	Recommended in "poor responders" (Bologne criterio) with moderate/ severe PROsPeR scores; improves live birth rate Potential benefit in poor responders (POSEIDON); hMG commenced on early follicular period may increase live birth rates and reduce cycle cancelations due to fertilization failure in POSEIDON Group 3 and 4 poor responders	Consider early rLH or hMG rLH with rFSH from start; hMG also beneficial
Hypo-responsive to FSH (specific genetic variants of the LH β, etc.)	Recommended; better oocytes retrieval, implantation rate, clinical pregnancy rate. May increase live birth rate	rLH with rFSH from start
Normal Responders (≥32 years, overweight/obese)	May improve embryo quality, endometrial receptivity, and pregnancy rates	hMG or rLH with rFSH; particularly beneficial in selected subgroups
High Responders	May improve ongoing pregnancy rate and live birth rate, reduces OHSS risk	hMG preferred; HP-hMG favored over rFSH for cost and safety
Advanced Maternal Age (35–40 years)	Mixed evidence; early supplementation (day 1) may enhance outcomes	rFSH + rLH (2:1) from stimulation start; avoid late addition
Fresh Embryo Transfer Planned	Recommended; reduces premature progesterone rise, supports endometrial environment	HP-hMG or hMG with rFSH; optimal hMG:FSH ratio 0.3–0.6

hMG: human menopausal gonadotropin; HP-hMG: highly purified human menopausal gonadotropin; OHSS: PRosPeR: Poor Responder Outcome Prediction; rLH: recombinant luteinizing hormone; rFSH: recombinant follicle-stimulating hormone.

Key message

Recent molecular and clinical evidence supports the benefits of LH supplementation in contemporary ovarian stimulation protocols. Protocols that include some form of LH activity are recommended for certain patient profiles, ensuring that gonadotropins act synergistically to optimize reproductive outcomes.

SHORT BIOGRAPHY

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