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The Use of rLH, HMG and hCG in Controlled Ovarian Stimulation for Assisted Reproductive Technologies

Micah J. Hill and Anthony M. Propst

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1. Introduction

The physiologic roles of both follicle stimulating hormone and luteinizing hormone are well established in the natural menstrual cycle. Research by Ryan and colleagues in the 1960s established the concept of two different cells in the ovarian follicle, the thecal and granulosa cells, functioning in different manners to produce products of the steroid pathway, the “two cell hypothesis” (1, 2). Further work over the next two decades established the “two-cell two-gonadotropin” theory, demonstrating the action of FSH on granulosa cells and LH on thecal cells (3). Thecal cells alone were shown to express CYP17, the gene encoding for the critical enzyme in the conversion of progesterone and pregnenolone to androgens (3). Conversely, granulosa cells were demonstrated to be the cell expressing aromatase, allowing for the conversion of the androgens derived from the thecal cells to be converted to estrogens. The cooperation of both cells under the influence of both gonadotropins is essential for normal folliculogenesis and steroidogenesis in the ovary.

LH has several physiologic roles within the ovary in addition to its role in androgen production (Figure 1). LH receptor activation leads to increases in adenylate cyclase and cAMP, resulting in increased mitochondrial transport of cholesterol necessary for steroidogenesis through upregulation of StAR (4, 5). LH activity also induces the expression of EGF-like growth factors amphiregulin and epiregulin from luteinized granulosa cells (6). These factors protect these cells from apoptosis, induce pro-survival signaling cascades, and are critical in peri-ovulatory events (6, 7). The mid-cycle LH surge causes a cascade of events leading to ovulation of the oocyte from the ovarian follicle and take the oocyte out of meiotic arrest (8). Finally, LH receptors have been demonstrated in the endometrium during the implantation window, raising a possible role for LH in peri-implantation endometrial events

(9, 10). The specific importance of LH activity can be demonstrated in patients with LH β or LH receptor gene mutations. Case reports of these male and female patients have demonstrated hypogonadism, infertility, pseudohermaphroditism, and amenorrhea (11-13).

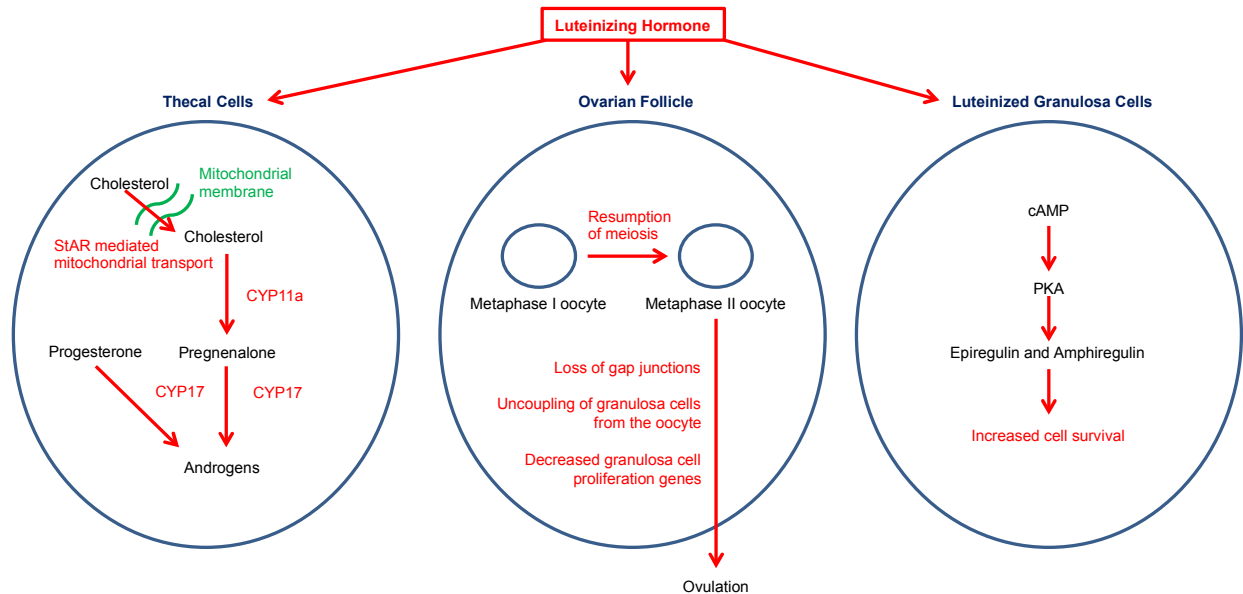


Figure 1. Key actions of LH within the ovary on the thecal cells, oocyte, and granulosa cells. Actions mediated by LH are indicated in red.

In assisted reproduction technologies (ART), the importance of LH is demonstrated clearly in hypogonadotropic hypogonadic patients. Patients with a profound lack of endogenous LH fail to undergo complete follicular maturation in the absence of exogenous LH (14, 15). Such patients require the exogenous administration of both LH and FSH to optimize reproductive outcomes (4, 16, 17). Urinary human menopausal gonadotropins were initially utilized in assisted reproductive technologies. These preparations were isolated and purified from large pools of human urine. One of the early urinary hMG products was Pergonal 75. One ampule of Pergonal 75 contained 75 international units (IU) of FSH and 75 IU of LH, which became an industry standard for ampules (18). These urinary hMG preparations contained both FSH and LH, as well as some hCG, and therefore patients were stimulated with both gonadotropins. Later advancements in monoclonal antibody technology enabled the production of urinary purified FSH and a more purified hMG, which is still used today (19, 20). Recombinant DNA technology using a mammalian cell culture system (Chinese hamster ovary cells) was used to produce recombinant human FSH, which was first licensed in 1995, and quickly replaced urinary FSH products. Recombinant human LH was later produced (18).

Despite the clear biologic importance of LH outlined in the preceding paragraphs, numerous studies have demonstrated successful ART outcomes with the use of exogenous FSH only (21, 22). A likely explanation is that LH is a very potent hormone, activating the LH receptor for adequate ovarian steroidogenesis when only 1% of LH receptors are bound (23). Even after GnRH agonist or antagonist down-regulation, a majority of patients will

have LH levels > 1 IU/L, a level presumably capable of driving adequate steroidogenesis (21). While the majority of patients have adequate endogenous LH levels to have successful ART cycles without exogenous LH, the value of additional exogenous LH administration has been a matter of debate. This chapter will review the scientific evidence surrounding the administration of exogenous LH in various forms (rLH, hMG, hCG) and its affect on ART outcomes.

2. Potential mechanisms of exogenous LH benefit in ART

There are theoretical benefits of the use of exogenous LH for the oocyte and the endometrium. The putative purpose of controlled ovarian stimulation in ART is to maximize the number of oocytes retrieved. However, the evidence is clear that the addition of LH is not associated with an increase in the number of oocytes or the number of mature metaphase II oocytes (MII) retrieved. Indeed, the use of hMG has been shown to decrease the number of follicles, oocytes, and metaphase II oocytes (MII) as compared to rFSH alone (21, 22, 24-28), presumably due to the action of LH contained in hMG. This is confirmed by similar data comparing rLH plus rFSH versus rFSH alone which has shown a decrease in developing follicles and oocytes retrieved with rLH (29, 30). In the majority of these trials, the decrease was in oocytes from small to intermediate follicles, and the number of oocytes retrieved from large follicles and the number of MIIs retrieved were not different. This suggests the possibility that the use of exogenous LH activity is associated with a decreased in the development of small follicles which may have been unlikely to yield a fertilized 2PN. There appears to be no negative effect on the development of larger follicles.

In a series of *in vivo* studies evaluating the effect of LH activity on follicle growth, Filicori and colleges confirmed the findings that LH activity can decrease the growth of small follicles without impacting the continued growth and maturity of larger follicles. First, they demonstrated that the number of follicles under 10mm in size during ART stimulation positively correlated with FSH dose ($r=0.193$, $p<0.05$) but negatively correlated to LH dose ($r=0.648$, $p<0.0001$) (31). In another study, it was demonstrated that incrementally decreasing the dose of FSH from day 7 of stimulation and increasing the dose of LH resulted in a decrease in the number of follicles <10 mm in size, without affecting follicles over 14mm in size (32). To evaluate if this effect was due to the decreasing FSH dose or the increasing LH dose, they performed a similar experiment where FSH was held steady at 150IU per day and patients were placed into groups of incrementally increasing LH doses. In this experiment, increasing doses of LH (in the presence of a constant dose of FSH) was again associated with a decrease in number of small follicles while not affecting the larger follicles (33). When the experiments were repeated utilizing hMG, hMG was also associated with a decrease in small follicles (34). These experiments and the results of many randomized controlled trials demonstrate that any beneficial effect of LH activity is not the result of an increase in oocyte yield.

While the number of total oocytes, especially from small follicles, appears to be diminished in ART cycles utilizing LH, the quality of those oocytes may be increased. While direct

measures of oocyte quality are difficult to assess clinically, some studies have noted an increased fertilization rate in oocytes obtained from cycles stimulated with LH (24, 30). Numerous trials have also demonstrated that the addition of LH activity results in an increase in serum estradiol on the day of hCG (Figure 2), which may represent a higher quality cohort of developing follicles (22, 26, 30, 35-43). LH supplementation was demonstrated to result in lower levels of apoptosis in cumulous cells as compared to FSH stimulation only (44). Cumulous cell apoptosis has been used a marker of oocyte quality and the decrease in apoptosis with the addition of LH is consistent with its post-receptor effects through increased epiregulin and amphiregulin.

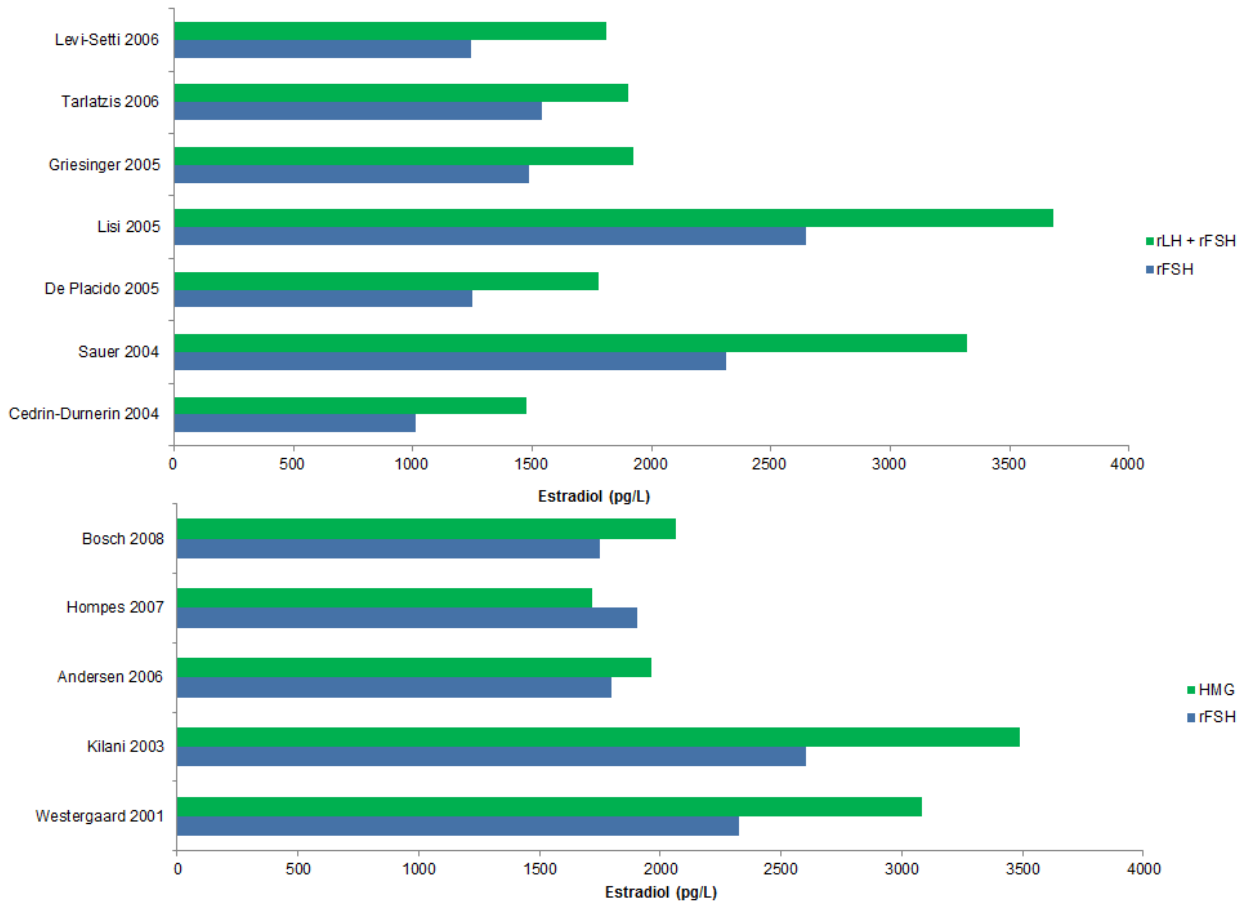


Figure 2. Randomized controlled trials demonstrating an increased estradiol level on the day of hCG with rLH (top) or hMG (bottom) as compared to rFSH alone (adapted from Hill *et al.*, 2012 (21)).

Another possible effect of LH is on the endometrium and embryo implantation. LH receptors are present in the endometrium during the window of implantation (9, 10), but whether these receptors play a direct role in embryo implantation needs further investigation. An indirect effect on the endometrium has been proposed via decreased premature progesterone secretion (24, 45). There is a growing body of evidence to suggest that prematurely elevated progesterone levels on the day of hCG have a negative impact on embryo implantation without affecting embryo quality (46-51). The evidence that this is an endometrial effect is supported by studies in oocyte donor cycles, where elevation of

progesterone in the donor is not associated with decreased implantation in the recipient (52). Progesterone is necessary for endometrial development and embryo implantation. However, premature rises in progesterone can advance the development of the endometrium and lead to asynchrony with the embryo development (46, 51, 53). FSH drives the conversion of cholesterol to progesterone but lacks CYP17 to further convert progesterone to androgens (54, 55). LH stimulates CYP17 in thecal cells to further convert the progesterone to androgens, which are subsequently aromatized in the granulosa cell (3). Under the two-cell two gonadotropin model, LH is protective of premature progesterone elevations prior to luteinization (24, 45) (Figure 3). Further investigation is needed to determine if exogenous LH administration is protective for the endometrium.

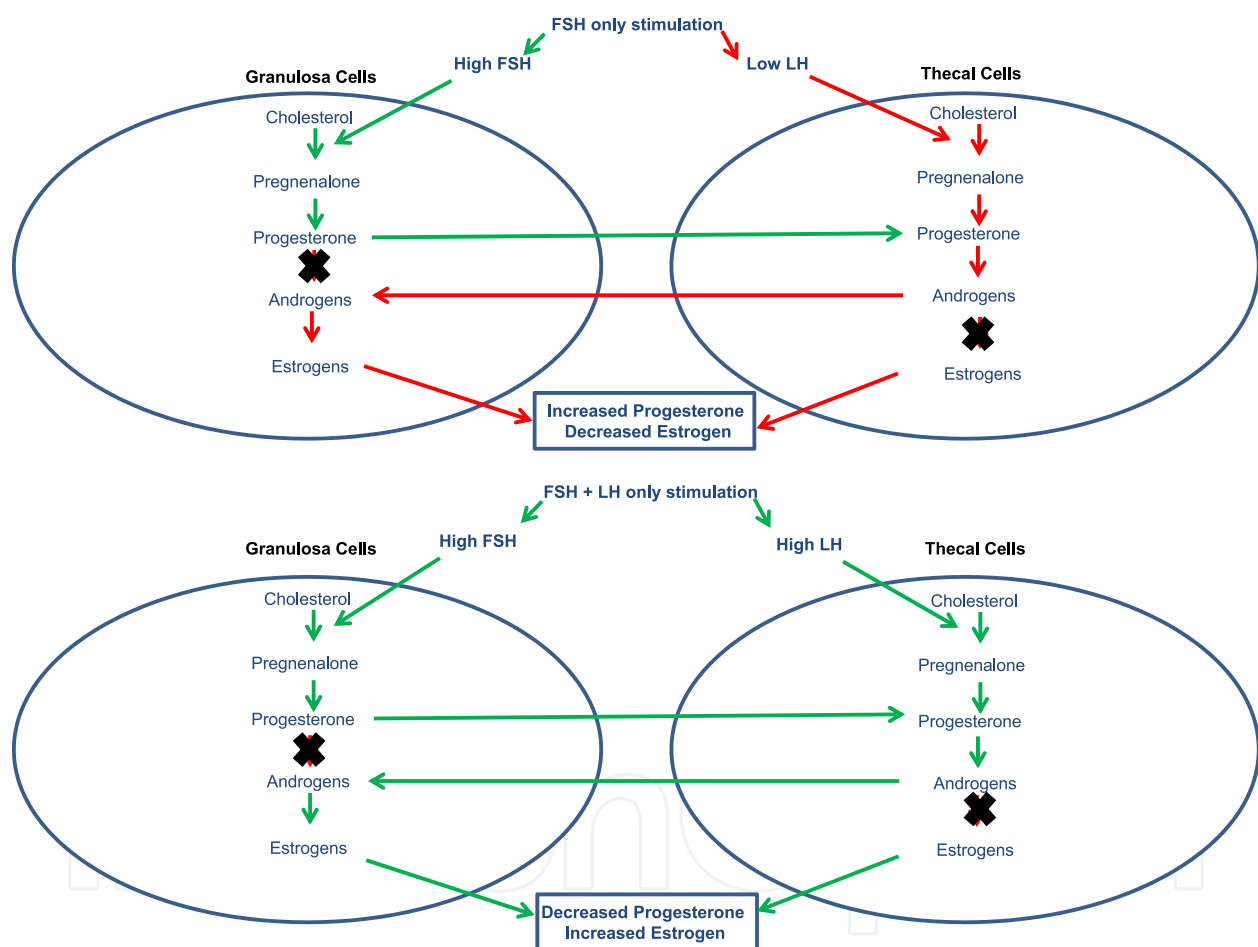


Figure 3. Model demonstrating a possible mechanism by which the administration of exogenous LH decreases premature rises in serum progesterone. FSH stimulates granulosa cells to convert cholesterol to progesterone. Lacking CYP17, the granulosa cells cannot convert progesterone to androgens and thus progesterone is secreted from the cells. In the absence of adequate LH levels, this progesterone is secreted into the circulation where it can advance the endometrium prematurely. In the presence of adequate LH levels, the progesterone is converted into androgens by CYP17 in the thecal cells. The androgens are then taken up by the granulosa cells and converted to estrogens. In this model exogenous LH protects the endometrium from exposure to premature progesterone rises. Green arrows represent increased action. Red arrows represent decreased action.

There is evidence to suggest that suppressed LH levels in women during ART stimulation can have negative effects (Figure 4). Depending on the study, adverse outcomes have been demonstrated with LH below 0.5-1.2 IU/L. LH levels < 1.2 IU/L have been reported to be associated with decreased serum estradiol, poor follicular development, decreased oocyte yield, decreased high quality embryos, and lower pregnancy rates (14, 15). Below LH levels of 1IU/L, other researchers demonstrated slower follicular growth and decreased estradiol (56). Finally, LH levels < 0.5 IU/L have been associated with increased pregnancy loss, lower implantation rates, and lower live birth rates (57, 58).

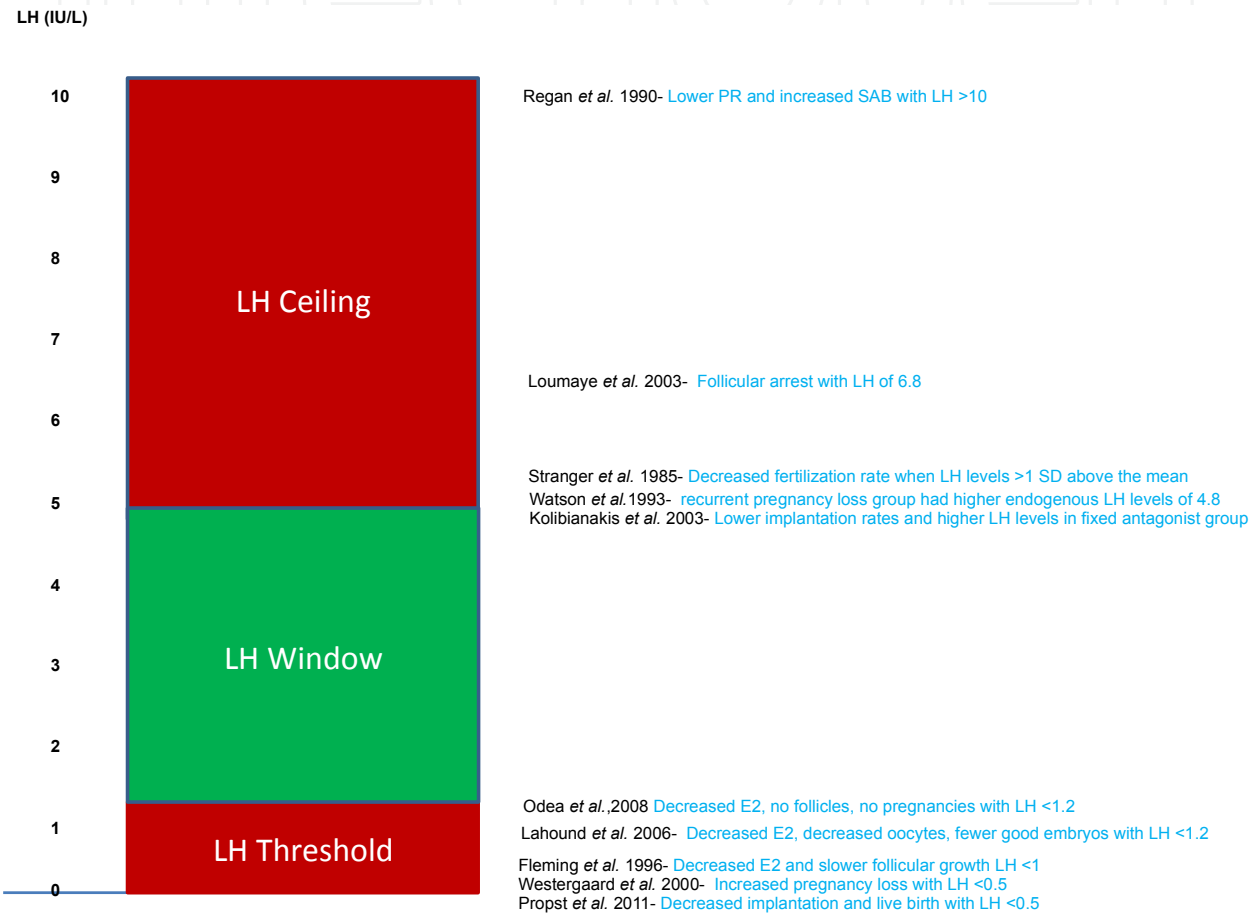


Figure 4. Demonstrates the concept of an LH window. Low LH levels have been associated with decreased poor pregnancy outcomes with levels below 0.5-1.2 IU/L, demonstrating a threshold below which low LH causes poor outcomes. High LH levels have also been associated with poor pregnancy outcomes with levels over 6.8-10 IU/L. This gives rise to the concept of a therapeutic LH window (in green) to maximize ART outcomes.

It has also been demonstrated that elevated LH levels are associated with negative ART cycle outcomes. Decreased pregnancy rates and increased spontaneous abortion were reported with LH levels above 10 IU/L (59). Increased follicular arrest, decreased fertilization, higher recurrent pregnancy loss, and lower implantation rates have all been reported in patients with higher LH levels that controls, although ceiling values were not established in these studies (56, 60-63). This evidence that too much or too little LH activity can have negative outcomes has led to the concept of an LH window (4, 61, 64). In reality,

with GnRH analogues, most patients are not in danger of having elevated endogenous LH levels. Indeed, by day 6 of GnRH antagonist administration, endogenous LH levels are depressed to a mean level of 1.6 IU/L, a value much closer to the LH threshold than the ceiling (65). Similarly, long agonist protocols also suppress endogenous LH levels to a mean near 1 IU/L (66). The evidence would suggest the clinician should be more concerned with replacing an adequate LH level in patients under pituitary down-regulation and the threat of high LH levels is less prevalent.

2.1. Summary points

1. LH activity causes atresia of small follicles during ART stimulation
2. Indirect evidence suggests increased oocyte quality with LH
3. LH activity decreases oocyte yield due to a loss of these small follicle
4. LH activity increases estradiol production from follicles
5. LH activity may protect the patient from premature progesterone elevations

3. Human menopausal gonadotropin

hMG is a urinary gonadotropin preparation consisting equal activity of both LH and FSH and some hCG. It is available in highly purified forms, minimizing earlier preparation disadvantages of protein contamination leading to the risk of allergic reactions. Evaluating studies with hMG have the advantage of homogeneity. Due to the nature of hMG containing equal FSH and LH activity, all patients in the hMG group receive equal amounts of LH and FSH activity and start LH activity on the same day as the FSH activity is started. As hMG has been available for longer than rFSH, there are more studies and total data available for analysis.

When looking at intermediate outcomes and surrogate markers for ART, hMG has not been demonstrably different than rFSH for ovarian stimulation. The results are similar in the proportion of MII oocytes, the number of high quality embryos, zona pellucida morphology, and polar body evaluation (42, 67-69). Studies have also shown no benefit in the number of oocytes retrieved with hMG and indeed numerous studies have shown a small decrease in the number of oocytes retrieved (typically around 1 oocyte less per retrieval) (22, 25-28, 41, 67). In the majority of studies, the decrease in oocytes did not translate into a decrease in the number of MIIs retrieved per cycle, indicating the loss was in smaller, immature oocytes. hMG administration has been associated with higher serum and follicular fluids androgens and estrogens and lower serum progesterone levels on the day of hCG (22, 25, 41-43, 56, 70-72). It has been proposed that this more favorable endocrine milieu reflects a healthier cohort of developing follicles in hMG cycles. One study also demonstrated increasing implantation rates with increasing doses of LH supplementation (73). This dose dependent benefit of LH could be due to an increase in the quality of the oocytes retrieved or due to an endometrial effect on implantation. However, this study was small and we are not aware that the findings have been confirmed.

There is a large body of randomized controlled trials available for analysis comparing hMG to rFSH only. These trials are relatively homogenous, with similar dosing strategies and primarily GnRH agonist pituitary downregulation. These RCT have been systematically evaluated in several meta-analyses shown in Table 1 (74-78). The number of patients required to show a benefit in hMG had been calculated at over 2100 (76). This is demonstrated in a 2005 meta-analysis by Al-Inany *et al.* where 8 RCTs including 2031 ART cycles failed to show a statistically significant improvement in live birth (OR 1.18, 95%CI 0.93-1.50) although a trend to benefit may have been seen (76). When the same authors repeated a meta-analysis in 2008, there were 11 RCTs including over 2900 patients available for analysis (75). This time a significant improvement in live birth (OR 1.20, 95%CI 1.01-1.42) was demonstrated with the use of hMG versus rFSH alone (75). This data was confirmed in a separate meta-analysis by Coomarasamy *et al.* showing an improvement in live birth with hMG (OR 1.18, 95%CI 1.02-1.38) (77). Two more recent meta-analyses in 2010 each failed to show a significant improvement in live birth with the use of hMG (74, 78). However, the p values for these studies were borderline significant (0.051-0.06) and the odds ratio of pregnancy was similar to the other trials. Indeed, the last four meta-analyses had all demonstrated between a 3-4% absolute increase in pregnancy and a 10-21% relative increase in pregnancy with the use of hMG as compared to rFSH alone. These numbers translate to a NNT of approximately 32 patients with hMG to achieve one additional live birth. The clinical relevance of this number has been a matter of debate, but there is a clear statistical benefit to utilizing hMG. The majority of these source RCTs for these meta-analysis were from cycles utilizing a GnRH agonist protocols.

Author, Year	RTCs	Number of Patients	Absolute Pregnancy Benefit	Relative Pregnancy Benefit	Pregnancy OR (95% CI)
Al-Inany, 2005	8	2031	-	-	1.18 (0.93-1.50)
Al-Inany, 2008	11	2937	+3%	+21%	1.20 (1.01-1.42)
Coomarasamy, 2008	7	2159	+4%	+18%	1.18 (1.02-1.38)
Jee, 2010	5	2299	+3%	+12%	1.14 (0.98-1.33)
Lehert, 2010	16	3952	+3%	+10%	1.10 (0.97-1.25)

Table 1. Recent meta-analysis comparing hMG versus rFSH for ovarian stimulation in ART cycles.

A recent RCT published in 2012 has provided similar evidence for the benefit of hMG in GnRH antagonist cycles. Devroey *et al.* randomized 749 patients to receive either hMG or rFSH (79). There were numerous strengths to this trial: rigorously described randomization, allocation and concealment, the use of 25 clinics in 7 countries, all patients were only allowed a single blastocyst transfer, and the follow-up included live births from the fresh cycle plus subsequent frozen cycles of embryos obtained during the study. Patients in the hMG arm had higher estradiol, LH, and FSH measured in the serum on the day of hCG. There was a significant reduction in the number of oocytes retrieved in the hMG group (-1.6 oocytes per retrieval). Importantly, an absolute difference of +3% in live birth with the use of

hMG in the pre-protocol analysis and +2% in the intent-to-treat analysis, although the findings did not achieve statistical significance (79). The cumulative live birth rate (fresh and frozen cycles) was 40% in the hMG group and 38% in the rFSH group. This study was in agreement with a prior publication evaluating 280 patients using a GnRH antagonist protocol with hMG or rFSH, also showing a non-significant 3% improvement in live birth rate (22). While there is not enough data to definitively conclude that hMG is beneficial in GnRH antagonist cycles, the available data shows a similar improvement to that seen in GnRH agonist cycles.

3.1. Summary points

1. hMG is a urinary derived gonadotropin formulation containing equal amounts of LH and FSH activity
2. hMG may decrease the number of oocyte retrieved by 1 oocyte per retrieval as compared to FSH
3. hMG increases live birth by 3-4% as compared to rFSH in GnRH agonist cycles
4. hMG may also increase live birth in GnRH antagonist cycles

4. Recombinant luteinizing hormone

The advent of recombinant DNA technology eventually led to the availability of recombinant LH in clinical practice (80). Urinary isolation of LH is an inefficient process, with 60-250 IU/mg of protein isolate (81). Conversely, recombinant LH contains 20,000 – 30,000 IU/mg of protein (81). The pharmacodynamics of recombinant and urinary derived LH preparations show similar clearance, half-life, and concentration curves (16, 81). The pharmacodynamics profiles of rLH are similar whether it is administered subcutaneously or intramuscular and it does not impact the pharmacodynamics of co-administered rFSH (82-84). In hypogonadotropic hypogonadal patients, a dose of 75IU of rLH has been demonstrated to promote adequate folliculogenesis when administered with FSH (85). rLH has potential advantages over the LH activity in hMG in that there is less risk of protein contamination and allergic reaction and it allows for the LH dose to be specifically adjusted without affecting the FSH dose.

There are numerous RCT evaluating rLH plus rFSH versus rFSH alone, but the data is complicated by significant heterogeneity between the trials (29, 30, 36-41, 86-95). The fact that the rLH dose can be administered at a separate starting time and doses from the rFSH dose has allowed researchers and clinicians to be more varied in the approach to rLH administration as compared to hMG. While this has allowed for the investigation of interesting protocols, it makes interpretation and meta-analysis of the data more complex. rLH has been investigated as a priming agent started up to 7 day prior to rFSH administration, as an early follicular phase agent beginning on days 1-3 of rFSH, and as a late follicular agent starting day 5-8. The dosing of rLH has also varied from 75IU to 300IU per day or as a fixed ratio to the FSH dose.

Three RCT have shown higher implantation or pregnancy rates in women receiving rLH supplementation (24, 38, 94). Patients with an inadequate response to rFSH alone have also been shown to benefit from the addition of 150IU of rLH as compared to increasing the FSH dose by 150IU (39). However, the vast majority of RCT evaluating rLH have failed to show an improvement in clinical pregnancy when compared to rFSH alone (29, 30, 36, 37, 40, 41, 86-89, 91, 92, 95). The majority of these trials were underpowered to detect for small differences in pregnancy outcomes between the study arms.

Four meta-analyses have been done to compare the outcomes of RCTs evaluating the use of rLH in ovarian stimulation (96-99). Kolibianakis *et al.* demonstrated no difference in live birth with the use of rLH, including in sub-analysis of early and mid-follicular administration or GnRH antagonist and agonist administration (97). Baruffi *et al.* did demonstrated a higher serum estradiol on the day of hCG (+514 pg/ml) and a higher number of MII oocytes retrieved (+0.88) with the use or rLH, but these differences did not translate into improve clinical pregnancy (96). In the largest meta-analysis, Mochtar *et al.* demonstrated a trend towards improved live birth with rLH, but the result did not reach statistical significance (OR 1.22, 95%CI 0.95-1.56) (98). However, pooled analysis did show an improvement in live birth for poor responders who were stimulated with rLH (OR 1.85, 95% CI1.10-3.11) (98). rLH was shown to have increased estradiol, fewer days of stimulation, and lower FSH administration in a fourth meta-analysis, although once again no improvement in pregnancy outcomes was demonstrated (99).

Author, Year	RTCs	Number of Patients	Pregnancy Odds Ratio (95% CI)
Baruffi, 2007	5	434	0.89 (0.57-1.36)
Kolibianakis, 2007	7	701	0.92 (0.65-1.31)
Mochtar, 2007	14	2612	1.22 (0.95-1.56)
Oliveira, 2007	5	1225	1.10 (0.85-1.42)

Table 2. Meta-analyses comparing rLH plus rFSH versus rFSH only.

The data from the RCT and meta-analyses evaluating rLH is similar to that of hMG in showing a reduction in the amount of FSH required for stimulation and an increase in serum estradiol. However, these data differ in that they do not show a convincing increase in pregnancy outcomes. It is possible that this is due to the smaller numbers in the rLH meta-analysis. Only the Mochtar *et al.* paper had a power similar to that of the hMG meta-analysis to detect for live birth as an outcome. The heterogeneity within the design and results of the rLH studies themselves also is associated with a decreased power to detect for pregnancy outcomes and a wide confidence interval. It is also possible that the differences seen in the meta-analyses between rLH and hMG is not only statistical, but also due to the differences in the pharmaceuticals themselves. Differences in the glycosylation of LH between urinary and recombinant preparations and the addition of hCG to urinary preparations may lead to fundamental differences in biologic action which affect clinical results.

4.1. Summary points

1. rLH increases serum estradiol
2. rLH decreases the amount of rFSH needed for ovarian stimulation
3. It is uncertain if the addition of rLH increases pregnancy outcomes in ART

5. Human chorionic gonadotropin:

hCG and LH have a significant degree of structural homology and both act on the LH receptor. hCG has a 6-8 fold affinity for the LH receptor as compared to LH only. Glycosylation additions to hCG also give it a longer half-life than LH. This has resulted in trials investigating the ability of hCG to replace LH for ovarian stimulation.

Two studies have reported the use of hCG in the early follicular phase of ovarian stimulation (100, 101). These trials and two case reports have used various dosing strategies to deliver the hCG, including 200 IU per day for four or seven days, 50 IU per day for 14 days, and 1,250 IU in a single dose on cycle day two (100-103).

In one trial, the addition of hCG resulted in significantly greater highly quality embryos (85% versus 47%) and pregnancy rates (46% versus 31%) (100). Overall, there is a lack of randomized controlled data evaluating the use of hCG from the early follicular phase, but what data is available is promising.

hCG has been evaluated as a mid-to-late cycle supplement to rFSH stimulation cycles in six trials. The dose of hCG utilized was 200IU per day in five trials and 250IU in another (104-109). All trials were initiated with rFSH only for stimulation with hCG added when the follicles were between 12-14mm in size. Five of the trials reported a significantly higher estradiol level on the day of hCG in patients randomized to receive hCG stimulation, with increases in estradiol ranging from 700-1500 pg/ml (104-107, 109). A study by Filicori *et al.* further demonstrated a significantly higher fertilization rate in patients receiving hCG versus rFSH only (74% versus 48%) (104). The remainder of the trials did not show any differences in outcomes with hCG with regards to fertilization, implantation, or pregnancy (105-107, 109). These RCT total 614 patients and demonstrate that the addition of hCG results in higher estradiol levels and at least comparable ART outcomes to rFSH stimulation only.

In a retrospective analysis, Van Horne *et al.* demonstrated that the addition of daily hCG (50-100 IU per day) to a rFSH only stimulation protocol resulted in a decrease in average FSH administration by 1000IU per patient and resulted in a cost savings of \$600 in a military healthcare facility (110). In a subsequent publication, this same group demonstrated that low dose hCG was effective at significantly improving implantation rates (54% vs. 19%) and live-birth rates (64% vs. 25%) in patients who had endogenous LH levels ≤ 0.5 IU/L, while it had no benefit in patients with LH levels >0.5 IU/L (58). A meta-analysis of over 1,000 patients has demonstrated that the addition of hCG to ovarian

stimulation results in a decreased requirement for rFSH, leading to a cost savings with comparable outcomes (108).

A recent meta-analysis summarized the evidence on the use of hCG in ovarian stimulation (111). The analysis included 11 RCT and 1,068 ART cycles. While the conclusions were limited due to heterogeneity with the source studies, significant conclusions were reached. It was demonstrated that the total dose of FSH was decreased by over 800 IU in patients who were supplemented with hCG. The use of hCG resulted in a small decrease in the number of MII oocytes retrieved (WMD -0.30, 95%CI -0.44 to -.66) (111). This data is consistent with the effect of LH on follicular growth discussed earlier in the chapter and a reduction of 0.3 oocytes per patient may be of small clinical impact. In analysis of 3 of trials reporting on early follicular phase hCG administration, there was no demonstrable benefit in clinical pregnancy. However, analysis of five of the trials reporting on late follicular phase hCG demonstrated a significant benefit in clinical pregnancy (RR 1.32, 95%CI 1.06-1.64).

5.1. Summary points

1. hCG can be used to provide LH action
2. 50-200 IU per day is an appropriate hCG dose
3. hCG supplementation decreases FSH requirement and ART cycle cost
4. hCG supplementation when the lead follicle is 12-14mm improves clinical pregnancy

6. Special patient groups

The use of rLH has been evaluated specifically in patients of advanced reproductive age, defined as 35 years of age and older in most studies. Eight RCT trials have compared rLH with rFSH versus rFSH stimulation only in this patient population (24, 29, 41, 88, 91, 93, 94). None of the trials reported a significant difference in oocytes retrieved with rLH. One trial reported a significant decrease in MII oocytes retrieved (5.5 versus 6.9) per patient with the use of rLH (29). The majority of the trials were small and no differences in outcomes were demonstrated with the use of rLH. The largest trial published by Bosch *et al.* enrolled 720 total patients (24). In patients 35 years old and younger, there was no benefit to rLH administration. However, in the advanced reproductive age group, there was a significantly increased fertilization rate (68% versus 61%) and implantation rate (26.7% versus 18.6%) with the use of rLH (24). There was a trend towards increased clinical pregnancy in the patients of advanced reproductive age who were supplemented with rLH (33.5 versus 25.3, $p=0.09$) (24).

A meta-analysis by Hill *et al.* evaluated seven of these trials (45). In that analysis, there was a significant increase in implantation (OR 1.36, 95%CI 1.05-1.78) and in clinical pregnancy (OR 1.37, 95%CI 1.03-1.83) with the use of rLH (45). While the smaller trials have been underpowered to detect important clinical outcomes such as implantation and clinical

pregnancy, both the largest trial and the meta-analysis suggest a clinical benefit to including rLH in the ovarian stimulation of patients with advanced reproductive age.

It has also been suggested that poor responders will benefit from the addition of LH. A common approach to increase LH in poor responders involves the use of the microdose flare protocol. This protocol avoids the profound suppression of endogenous LH and FSH in the early follicular phase normally achieved with long luteal downregulation protocols. Scott and Novat's initial investigation of the microdose flare found it to have higher peak estradiol, more mature follicles and more mature oocytes than a traditional agonist protocol (112). While this protocol represents a well-established approach to increasing endogenous LH and FSH, randomized controlled trials have been small and inconclusive on whether this protocol increases live birth rates (113-116). One RCT did not show any benefit to adding either rLH or low-dose rHCG to a microdose flare protocol for poor responders (117). A Cochrane review has suggested that poor responders may benefit from the addition of rLH (98). In this meta-analysis there was a marked increase in live birth with the use of rLH (OR 1.85, 95%CI 1.10-3.11) (98).

6.1. Summary points

1. rLH increases implantation and clinical pregnancy in patients 35 years and older
2. rLH increases live birth in poor responders

7. Conclusion

The action of LH is vital to both natural and assisted human reproduction. Normogonadotropic patients often have adequate endogenous LH levels, even after GnRH analogue pituitary downregulation, to have successful assisted reproduction with FSH stimulation alone. However, the addition of LH activity to ovarian stimulation has been demonstrated to improve the odds of achieving a live birth. We find the 3-4% improvement in live birth with the use of LH activity to be clinically relevant. The inclusion of LH in the stimulation of poor responders and women thirty-five and older has been shown to improve ART outcomes. Since there are currently no proven methods to determine which patients will benefit most from the addition of LH, we recommend clinicians consider some form of LH activity in the ovarian stimulation of all patients.

Author details

Micah J. Hill

Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA

Anthony M. Propst

Uniformed Services University of the Health Sciences, Department of Obstetrics and Gynecology, Bethesda, MD, USA

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