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ORIGINAL PAPER

Recombinant luteinizing hormone supplementation to recombinant follicle-stimulation hormone during induced ovarian stimulation in the GnRH-agonist protocol: A meta-analysis

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Abstract *Purpose*: to compare the efficacy of recombinant LH supplementation for controlled ovarian stimulation in recombinant FSH and GnRH-agonist protocol.

Methods: Search strategies included on-line surveys of databases. The fixed effects model was used for odds ratio and effect size (weighted mean difference). Four trials fulfilled the inclusion criteria.

Results: a fewer days of stimulation ($p < 0.0001$), a fewer total amount of r-FSH administered ($p < 0.0001$) and a higher serum estradiol levels on the day of hCG administration ($p < 0.0001$) were observed for the r-LH supplementation protocol. However, differences were not observed in number of oocyte retrieved, number of mature oocytes, clinical pregnancy per oocyte retrieval, implantation and miscarriage rates.

Conclusions: more randomized controlled trials are necessary before evidence-based recommendations regarding exogenous LH supplementation in ovarian stimulation protocols with FSH and GnRH-agonist for assisted reproduction treatment can be provided.

Keywords GnRH agonist · Recombinant FSH · Recombinant LH · Ovarian stimulation

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Introduction

The pharmacology of ovarian stimulation has been strongly influenced by the two-cell, two gonadotrophin theory, and follicular stimulation protocols historically have included both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in an attempt to mimic normal physiology [1]. During recent years, the effect of LH on follicular maturation and pregnancy outcome during the course of ovarian stimulation in relation to assisted reproduction has received increasing attention. This interest reflects the fact that modern stimulation protocols have resulted in LH concentrations substantially lower than those observed in the natural cycle and in previously used protocols. The introduction of gonadotrophin-releasing hormone agonists (GnRH-a) in the mid-1980s successfully circumvented the problems of a premature LH surge. During the same period of time, there has been a gradual shift from human gonadotrophin (HMG) with equal amounts of FSH and LH like activity over pure urinary derived FSH preparations to recombinant human FSH (r-FSH), without LH activity [2].

Some studies have suggested that the suppression of the endogenous LH secretion does not seem to affect the majority of IVF patients treated with supplementation in women undergoing assisted reproduction and stimulation

with r-FSH. Other studies have indicated that a group of normogonadotrophic women, down regulated and stimulated with pure FSH preparations may experience low LH concentrations that compromise the parameters of the IVF treatment [2].

According to current concepts in the folliculogenesis, LH plays an essential role in the final stages of follicular maturation [3, 4]. Once an appropriate stage of follicular development has been achieved in response to treatment with FSH, granulosa cells became receptive to LH stimulation and LH becomes capable of exerting its actions on both theca cells and granulosa cells. In fact, at non-saturating concentrations of FSH and LH, the response are additive. Moreover, it has been postulated that the maturing follicle reduces its dependence on FSH by acquiring LH receptors [3, 4]. Thus, LH may play an essential role in determining oocyte maturity and development potential in IVF cycles. However, exposure of the developing follicle to inappropriately high concentrations of LH may interfere with follicular and oocyte maturation and thus adversely affect the reproductive process [5, 6].

The objective of this meta-analysis is to compare the efficacy and safety of recombinant LH (r-LH) supplementation in women undergoing assisted reproduction and stimulation with r-FSH in the GnRH-a protocol of ovarian stimulation in IVF/ICSI cycles.

Materials and methods

Criteria for considering studies for this meta-analysis

All published and ongoing randomized controlled trials (RCT) comparing the effect of r-LH supplementation in women undergoing assisted reproduction and stimulation with r-FSH in the GnRH-a protocol were analyzed.

Types of outcome measures

The primary outcome measures used for this meta-analysis were the number of days of stimulation, the total amount of r-FSH administered, serum estradiol levels on the day of hCG administration, the number of retrieved and mature (MII) oocytes. The secondary outcomes were clinical pregnancy rate (CPR) per oocyte retrieval, implantation rate and miscarriage rate.

Identification of studies

Search strategies included on-line surveys of databases (MEDLINE, EMBASE, Science Citation Index, Cochrane Controlled Trials Register and OVID) from 1990 to 2006. There was no language restriction. The following Medical Subject Headings and text words were used: "ovarian stimulation," "recombinant FSH," "recombinant LH," "GnRH agonist" and "randomized controlled trial." The principal inclusion criterion was randomized controlled trial.

Validity assessment and data extraction

Each trial was assessed independently by two reviewers and ranked for its methodology rigor and its potential to introduce bias. Missing data were obtained from the authors when possible.

Statistical analysis

Data management and analysis were conducted using the StatsDirect statistical software (Cheshire, UK). Effectiveness was evaluated using the Mantel-Haenszel method. A confidence interval for the Mantel-Haenszel odds ratio in StatsDirect was calculated using the Robins, Breslow and Greenland variance formula. A chi-square test statistic was used with its associated probability that the pooled odds ratio (OR) was equal to one. The StatsDirect also gives the option to base effect size calculations on weighted mean difference (WMD) as described in the Cochrane Collaboration Handbook [7]. The measure of heterogeneity (non-combinability) was evaluated by Cochran's Q and the Breslow-Day test. A non-significant result (i.e. lack of heterogeneity) indicates that no trial has either an OR or a WMD that is statistically significantly worse or better than the overall common OR or WMD obtained by pooling the data. The fixed effects model was used for odds ratio (OR) and effect size (WMD). Since a fixed effects model has been employed it is important to acknowledge that inferences refer only to the particular studies included in the analysis. Meta-analysis used in this way is simply a device to pool the information from the various studies to provide a composite finding, but only for those studies. In the alternative random effect model, the individual studies are regarded as a random sample from the (infinite) population of studies. Global inferences would be permissible, but the random errors used would need to reflect inter-study variation. Since each of our analyses contained only four studies, we decided to derive our inferences from a fixed effects model.

Results

Search results

Four trials fulfilled the inclusion criteria [2, 8–10]. In 3 trials, the pituitary down-regulation with GnRH-a started in the mid-luteal phase of the preceding cycle and in 1 trial in the beginning of the menstrual cycle. All trials compared ovarian

stimulation with r-FSH alone (control group) versus ovarian stimulation with r-FSH + r-LH (study group).

Description of the studies included

Humaidan et al. [2] A total of 231 women undergoing IVF or ICSI treatment from November 2001 to October 2002 were included in this prospective randomized non-assessor-blind study. Patients fulfilling the following inclusion criteria were prospectively enrolled in a consecutive manner: (i) female age < 40 years; (ii) baseline FSH < 10 IU/L; (iii) menstrual cycles between 25 and 34 days; (iv) standard hormonal treatment as follows: pituitary down-regulation with GnRH-a (Suprefact; Hoechst, Horsholm, Denmark) 0.5 mg s.c. daily from the mid-luteal phase for 14 days after which the dose of GnRH-a was reduced to 0.2 mg s.c. and ovarian stimulation was initiated with r-FSH (Gonal F; Serono Nordic, Copenhagen, Denmark or Puregon; Organon, Skovclunde, Denmark) using individualized doses according to baseline FSH, BMI, age and ovarian volume. Patients <35 years of age were stimulated with 150IU of r-FSH and patients >35 years were stimulated with 225 IU of r-FSH. An additional 75IU of r-FSH was administered in patients with baseline FSH between 8 and 10IU/L, BMI above 30 and ovarian volume below 3 ml. The ovarian response was starting on day 8 of stimulation and the dose of FSH was adjusted if necessary. On this same day of stimulation (day 8), patients were randomized by the study nurse, using computer generated random numbers in sealed, unlabelled envelopes, each containing a unique study number, to receive either r-FSH plus supplementation of r-LH (Luveris; Serono Nordic, Copenhagen, Denmark) ($n = 116$) or r-FSH alone ($n = 115$). Doses of r-FSH and r-LH were given in a ratio of 2:1.

Lisi et al. [8] All patients ($n = 453$) began treatment during a set period (treatment run) in which patients were allocated r-FSH only or r-FSH and r-LH on the basis of randomization by allocation of treatment to every third patient. Exclusion criteria were a body mass index <18 or >35, an abnormal karyotype or any endocrinopathy/illness. Patients were included if there was evidence of tubal damage and endometriosis, but their frequency was not significantly different among all groups studied. Patients had no evidence of abnormal menstrual cycles and evaluation of basal hormone concentrations occurred on day 3 of the spontaneous cycle. The down-regulation was induced with triptoreline 0.1 mg (Ipsen, France) subcutaneously from the mid-luteal phase of previous cycle (day 21) for 3 weeks before start gonadotrophin stimulation. All patients commenced on a daily dose of 150IU r-FSH; group A patients ($n = 122$) were supplemented daily with 75IU r-LH (Luveris; Serono, Geneva, Switzerland) from day 7 of stimulation and group B patients ($n = 331$) were maintained on r-FSH only.

Marrs et al. [9] The study included ($n = 431$) normo-ovulatory women aged 18–40 with serum/plasma FSH ≤ 11.2 mIU/ml; presence of both ovaries; male partner with a diagnosis of male factor infertility; and a requirement for ICSI. Exclusion criteria included: clinically significant systemic disease; smoking more than 10 cigarettes per day; any contraindication to pregnancy; serum/plasma LH:FSH ratio >2; and more than two previous ICSI cycles in which gonadotrophin stimulation was used. This was a randomized, open-label, multi-centre study carried out in 44 centers in the USA. Patients were randomized 1:1 to undergo stimulation with r-FSH (Gonal-F; Serono Laboratories, Geneva, Switzerland) alone ($n = 219$) or r-FS + r-LH (Luveris; Serono Laboratories) ($n = 212$). Pituitary down-regulation was carried out using leuprolide acetate (Lupron; TAP Pharmaceuticals, Inc., IL, USA), 0.5 mg/day starting 7–8 days after estimated ovulation. Treatment with r-FSH (225 IU/day) was started when serum estradiol was <75pg/ml. After 5 days, the r-FSH could be increased about 75–150IU/day every 2–3 days if necessary. The combination group began treatment with r-LH (150 IU/day) on stimulation day 6. The dose of r-LH remained constant throughout the treatment period.

Tarlatzis et al. [10] The trial was a double-blind, randomized, placebo-controlled, prospective study performed at six centres in four countries. Women were eligible for inclusion in the study if they aged between 18 and 37 years, had a normal uterus and two ovaries, and were scheduled to undergo controlled ovarian stimulation prior to IVF with ICSI. All women had normal ovulatory cycles of 24–35 days, with maximum FSH and prolactin concentrations of 12 IU/l and 1040 IU/l, respectively, during the early follicular phase (days 2–6). No evidence of other gynaecological pathology (except tubal) was present. Women in whom a previous IVF cycle had been unsuccessful due to a poor response (≤2 oocytes recovered) were not eligible for the study. Patients underwent pituitary down-regulation with buserelin (Suprefact, Hoecht, Frankfurt, Germany), using a fixed daily dose of 200 mg s.c, according to the long agonist protocol, starting on day 2 of the normal menstrual cycle. Treatment with r-FSH (Gonal-F, Laboratories Serono S.A, Aubonne, Switzerland) was then started in women with serum E_2 concentrations <200pmol/l and no follicles >15 mm in diameter or ovarian cysts on ultrasonographic examination. The initial r-FSH dose was 150 IU s.c. daily for 5 days, after which the dose was adjusted to a maximum of 450 IU per day according to the ovarian response. Once the leading follicle had reached a diameter of 14 mm, patients were randomized to receive r-LH (Luveris, Laboratories Serono S.A), 75IU s.c. ($n = 55$), or placebo ($n = 57$) for a maximum of 10 days.

Table 1 Cycles with and without LH supplementation: Days of stimulation

Trail	rec FSH + rec LH		rec FSH		WMD	95%CI fixed
	n	mean ± sd	*n*	mean ± sd		
Tarlatzis 2006	55	9.7 ± 2.3	57	9.9 ± 3.2	−0.2	−1.24, 0.84
Humaidan 2004	116	11.1 ± 1.4	115	11.1 ± 1.4	0	−0.36, 0.36
Marrs 2004	212	9.3 ± 1.6	219	9.5 ± 2.0	−0.2	−0.54, 0.14
Lisi 2002	122	12.57 ± 0.15	331	12.77 ± 0.27	−0.2	−0.25, −0.15
Total	505		722			
Pooled effect size Fixed effects (Mulrow-Oxman)					−0.198	−0.24, −0.16

Note. Z (test WMD + differs from 0) = −9.96, $p < 0.0001$.
Non-combinability: Cohran $Q = 1.16$ (df = 3), $p = 0.76$.

Primary outcome

Days of stimulation (Table 1): All studies were included. The mean days of stimulation was significantly lower in the 505 women using r-LH than in the 722 women not using ($p < 0.0001$; WMD: −0.198 95% CI: −0.24, −0.16). There was no heterogeneity in this comparison (Cohran Q: 1.16, df: 3, $p = 0.76$).

Total amount of r-FSH administered (Table 2): All studies were included. A significantly fewer amount of r-FSH was administered for the 505 women using r-LH than in the 722 women not using ($p < 0.0001$; WMD: − 192, 95% CI: − 220, − 164). There was heterogeneity in this comparison (Cohran Q: 9.79, df:3, $p = 0.02$).

Serum estradiol levels on the day of hCG administration (Table 3): Three trials reported this data. Significant higher serum estradiol level was found in 389 women using r-LH than in 607 women not using ($p < 0.0001$; WMD: 49.4, 95% CI: 38.4, 60.4). There was no heterogeneity in this comparison (Cohran Q: 4.41, df:2, $p = 0.11$).

Number of oocytes retrieved (Table 4): All studies were included. The mean number of oocytes retrieved was not significantly different in the 505 women using r-LH than in 722 women not using ($p = 0.37$; WMD: 0.03, 95% CI: −0.03, 0.09). There was no heterogeneity in this comparison (Cohran Q: 1.84, df:3, $p = 0.61$)

The number of mature oocytes (MII) (Table 5): All studies were included. The mean number of mature oocytes retrieved was not significantly different in the 497 women using r-LH than in 717 women not using ($p = 0.94$; WMD: 0.016, 95% CI: −0.46, 0.50). There was no heterogeneity in this comparison (Cohran Q: 2.57, df:3 $p = 0.46$).

Secondary outcome

Clinical pregnancy rate oocyte retrieval (CPR) (Table 6): All studies were included. The CPR per oocyte retrieval

Table 2 Cycles with and without LH supplementation: Total amount of r-FSH administered

Trail	rec FSH + rec LH		rec FSH		WMD	95%CI fixed
	n	mean ± sd (UI)	*n*	mean ± sd(UI)		
Tarlatzis 2006	55	1837.5 ± 802.5	57	1755 ± 682.5	82.5	−193, 358
Humaidan 2004	116	2008 ± 776	115	1997 ± 749	11	−185.7, 207.7
Marrs 2004	212	2161.4 ± 716.55	219	2266.4 ± 826.21	−105	−251.2, 41.2
Lisi 2002	122	2998 ± 112	331	3201 ± 198	−203	−240.2, −165.8
Total	505		722			
Pooled effect size Fixed effects (Mulrow-Oxman)					−192	−220, −164

Note. Z (test WMD + differs from 0) = −13.4, $p < 0.0001$.
Non-combinability: Cohran $Q = 9.79$ (df = 3), $p = 0.02$.

Table 3 Cycles with and without LH supplementation: Serum estradiol levels on the day of hCG administration

Trail	rec FSH + rec LH		rec FSH		WMD	95%CI fixed
	n	mean ± sd (pmol/l)	*n*	mean ± sd (pmol/l)		
Tarlatzis 2006	55	1901 ± 1073	57	1539 ± 723	362	24.2, 670
Marrs 2004	212	9004 ± 5162	219	8435 ± 4853	569	−376, 1514
Lisi 2002	122	1221 ± 58	331	1072 ± 36	49	40.1, 57.9
Total	389		607			
Pooled effect size Fixed effects (Mulrow-Oxman)					49.4	38.4, 60.4

Note. Z (test WMD + differs from 0) = 8.8, $p < 0.0001$.

Non-combinability: Cohran $Q = 4.41$ (df = 2), $p = 0.11$.

procedure was not significantly different in cycles using r-LH (171/497, 34.4%) than in cycles not using (202/705, 28.6%) ($p = 0.52$; OR:1.1, 95% CI: 0.85, 1.42). There was no heterogeneity in this comparison (Breslow-Day: 3.42, df:3, $p = 0.33$; Cochran Q:3.39, df:3, $p = 0.34$)

Implantation rate (Table 7): Two studies were included. The implantation rate was not significantly different in the cycles using r-LH (97/464, 20.9%) than in cycles not using (127/940, 13.5%) ($p = 0.06$; OR:1.35 95% CI: 0.99, 1.83). There was no heterogeneity in this comparison (Breslow-Day:0.68, df:1, $p = 0.41$; Cochran Q:0.68, df:1, $p = 0.41$)

Miscarriage rate (Table 8): Two trials were included. The miscarriage rates were not significantly different in the pregnancies after the use of r-LH (10/51, 19.6%) than in those in which it was not used (16/49, 32.6%) ($p = 0.23$; OR:0.52, 95% CI: 0.21, 1.3). There was no heterogeneity in this comparison (Breslow-Day:1.24, df:1, $p = 0.26$; Cochran Q:1.22, df:1, $p = 0.27$)

Discussion

The validity of the two-cell, two-gonadotrophin hypothesis, which suggests that both LH and FSH are required for ovarian steroidogenesis in a gonadotrophin-deficient population (World Health Organization I classification), is clear. However, there is still a considerable controversy on the need of additional LH supplementation in cycles of assisted reproduction techniques using a GnRH-a [10]. It has been proposed that "resting" concentrations of LH are sufficient to maintain steroidogenesis and normal folliculogenesis [11].

It has been widely demonstrated that, during ovarian stimulation with FSH and concomitant administration of a GnRH-a, endogenous levels of LH decrease reaching lowest values during the late stimulation phase. Thus, it would seem logical that if LH supplementation is to have any benefit, then the late follicular phase would be appropriate time for its administration especially if, as has been reported, ± 50% of agonist/FSH treated women are LH deficient (plasma LH concentration <0.5IU/l) [12]. The proportion of positive

Table 4 Cycles with and without LH supplementation: Number of retrieved oocytes

Trail	rec FSH + rec LH		rec FSH		WMD	95%CI fixed
	n	mean ± sd	*n*	mean ± sd		
Tarlatzis 2006	55	10.1 ± 5.4	57	9.8 ± 7.0	0.3	−2.02, 2.62
Humaidan 2004	116	9.3 ± 5.4	115	10.0 ± 4.7	−0.7	−2.00, 0.60
Marrs 2004	212	13.6 ± 6.8	219	14.1 ± 7.5	−0.5	−1.85, 0.85
Lisi 2002	122	7.0 ± 0.32	331	6.97 ± 0.20	0.03	−0.02, 0.08
Total	505		722			
Pooled effect size Fixed effects (Mulrow-Oxman)					0.03	−0.03, 0.09

Note. Z (test WMD + differs from 0) = 0.89, $p = 0.37$.

Non-combinability: Cohran $Q = 1.84$ (df = 3), $p = 0.61$.

Table 5 Cycles with and without LH supplementation: Number of mature oocytes (MII)

Trail	rec FSH + rec LH		rec FSH		WMD	95%CI fixed
	n	mean ± sd	*n*	mean ± sd		
Tarlatzis 2006	47	6.9 ± 4.9	52	6.2 ± 4.8	0.7	−1.21, 2.61
Humaidan 2004	116	8.3 ± 4.9	115	9.1 ± 4.5	−0.8	−2.01, 0.41
Marrs 2004	212	10.3 ± 5.9	219	10.4 ± 6.3	−0.1	−1.25, 1.05
Lisi 2002	122	5.71 ± 2.96	331	5.52 ± 3.04	0.19	−0.44, 0.82
Total	497		717			
Pooled effect size Fixed effects (Mulrow-Oxman)					0.016	−0.46, 0.50

Note. Z (test WMD + differs from 0) = 0.64, *p*: 0.95.

Non-combinability: Cohran $Q = 2.57$ (df = 3), $p = 0.46$.

pregnancy tests was similar in the two groups (30% versus 34% per started cycle), but the final clinical treatment outcome was significantly different, with a five-fold higher risk of early pregnancy loss (45% versus 9%; $p < 0.05$) in the low LH group and consequently a significant poorer chance of delivery than in the normal LH group [12].

On the other hand, the rationale for using a dose 150IU/day was on the concentrations of LH measured in women down regulated with GnRH-a. This dose of r-LH achieved a C-max of 1.2IU/L, a concentration found to be important in hypogonadotropic women stimulated with r-FSH alone [13]. Concentrations of LH < 1.2IU/L were associated with insufficient estradiol concentrations and a failure to become pregnant. It seems to be a therapeutic "window" of LH concentrations, because high concentrations of LH have been associated with atresia of developing follicles in women with hypogonadotropic hypogonadism or polycystic ovary disease [13].

It has recently been suggested that, in a subset of patients, a suboptimal ovarian response to the GnRH-a protocol associated with the use FSH-only gonadotrophin preparations may be due to low LH activity caused by low serum concentrations of LH and/or LH bioactivity [14]. The following facts support this possibility. The ovarian response to stimulation with gonadotrophins is often reduced in patients receiving long-term down-regulation [15] or in those who required prolonged GnRH-a treatment to achieve down-regulation, with a subsequent profound suppression of endogenous gonadotrophins [16].

When this meta-analysis was carried out advantages for the LH supplementation protocol with respect to a higher serum estradiol levels on the day of hCG administration

Table 6 Cycles with and without LH supplementation: clinical pregnancy rate per oocyte retrieval

Trail	rec FSH + rec LH	rec FSH	% Weights	Odds ratio	95%CI fixed
Tarlatziz 2006	9/55	14/57	10.4	0.60	0.21, 1.68
Humaidan 2004	42/116	35/115	20.3	1.3	0.72, 2.33
Marrs 2004	90/204	91/202	46.4	0.96	0.64, 1.45
Lisi 2004	30/122	62/331	58.9	1.41	0.83, 2.38
Total	171/497	202/705			
Pooled odds ratio Fixed effects (Mantel-Haenszel, Robins-Breslow-Greenland)				1.1	0.85, 1.42

Note. Chi^2 (test odds ratio differs from 1) = 0.41, $p = 0.52$.

Non-combinability of studies.

Breslow-Day = 3.42 (df = 3), $p = 0.33$.

Cochran $Q = 3.39$ (df = 3), $p = 0.34$.

Table 7 Cycles and without LH supplementation: Implantation rate

Trail	rec FSH + rec LH	rec FSH	% Weights	Odds ratio	95%CI fixed
Humaidan 2004	56/176	53/187	49.9	1.2	0.73, 1.9
Lisi 2002	41/288	74/753	50.1	1.52	0.98, 2.3
Total	97/464	127/940			
Pooled odds ratio Fixed effects (Mantel-Haenszel, Robins-Breslow-Greenland)				1.35	0.99, 1.83

Note. Chi^2 (test odds ratio differs from 1) = 3.54, $p = 0.06$.

Non-combinability of studies.

Breslow-Day = 0.68 (df = 1), $p = 0.41$.

Cochran $Q = 0.68$ (df = 1), $p = 0.41$.

($p < 0.0001$; WMD: 49.4 95% CI 38.4, 60.2) were observed. How estradiol might influence or reflect human oocyte health has not been fully elucidated, although preliminary research has suggested a potential role as a growth factor. Diminished serum estradiol concentrations most probably represent impaired ovarian steroidogenesis after GnRH-a down-regulation, and assessment of estradiol response patterns have been considered important markers for IVF cycle success [17]. Multiple novel roles of LH have been recently proposed and it has been postulated that LH may affect IVF results both determining oocyte quality and by influencing uterine receptivity via ovarian estradiol secretion or through direct effects on endometrium, myometrium, and uterine artery and vein [18–20]. In essence, LH should no longer be viewed only as a gonadal regulating hormone. LH has a number of other target organs in the body, and what it does in them could be important for better understanding of biology as well as a benefit to patients. As we learn more about the non-classical LH action our thinking may yet again have to be revised [18].

In addition, this meta-analysis also showed advantages for the LH supplementation protocol with respect to a fewer total amount of r-FSH administered ($p < 0.0001$; WMD: −192 95% CI −220, −164) and a fewer days of stimulation ($p < 0.0001$; WMD: −0.20 95% CI −0.24, −0.16). The reduction of total amount of the r-FSH in the group with r-LH supplementation confirms that FSH and LH act synergistically in the last part of the cycle [2]. However, the variable total amount of r-FSH had a significant heterogeneity (Cohran Q: 9.79, df:3 $p = 0.02$). De Placido et al. [21] observed that the administration of a daily r-LH resulted in a significant decrease in that cumulative r-FSH amount and in a trend toward a reduction of the stimulation length. Balasch et al. [22] reported that with a fixed regimen of 150IU r-FSH or HMG during the first 14 days of treatment, the duration of ovarian stimulation and the per cycle gonadotrophin amount dose were lower in group HMG.

On the other hand, in this meta-analysis no differences were observed in the number of oocytes retrieval, mature

Table 8 Cycles with and without LH supplementation: Miscarriage rate

Trail	rec FSH + rec LH	rec FSH	% Weights	Odds ratio	95%CI fixed
Tarlatziz 2006	3/9	4/14	16.1	1.25	0.13, 10.5
Humaidan 2004	7/42	12/35	83.9	0.38	0.11, 1.26
Total	10/51	16/49			
Pooled odds ratio Fixed effects (Mantel-Haenszel, Robins-Breslow-Greenland)				0.52	0.21, 1.3

Note. Chi^2 (test odds ratio differs from 1) = 1.41, $p = 0.23$.

Non-combinability of studies.

Breslow-Day = 1.24 (df = 1), $p = 0.26$.

Cochran $Q = 1.22$ (df = 1), $p = 0.27$.

oocytes, CPR per oocyte retrieval, implantation rate and miscarriage rate.

Some authors think that the mid-follicular LH levels have a significant impact on ovarian response and pregnancy outcome. Concerning the group of patients with high LH levels after down-regulation, we have to await the results of future trials to draw firm conclusions. The dynamic changes of the endogenous LH levels during down-regulation outcome as well as the bioactivity of the LH molecule in individual patients, however, should receive more attention, as they seem to be of importance for the reproductive outcome of the down-regulated cycle [23].

Kolibianakis et al. [24] in a systematic review concluded that, the available evidence suggests, among women with normal ovulation or WHO II oligo-anovulation, low endogenous LH levels during ovarian stimulation for IVF using GnRH analogues are not associated with a decreased probability of ongoing pregnancy beyond 12 weeks. On the contrary, there is evidence to suggest that the opposite may be true. Unless further prospective studies modify the direction of the current evidence, LH supplementation in ovarian stimulation for IVF using GnRH analogues cannot be based on the rationale that low endogenous LH levels have an adverse effect on the probability of ongoing pregnancy beyond 12 weeks of gestation.

On the contrary, Balasch and Fabregues [25] concluded that the LH plays an essential physiological role in follicle steroidogenesis and development and oocyte maturation. Thus, exogenous LH is an essential tool in ovulation induction. Current concepts of gonadotrophic control of ovarian function have established that both a 'threshold' and a 'ceiling' for LH concentrations exist during the follicular phase of menstrual and induced cycles. Therefore, concentrations of LH should be neither too high nor too low during ovulation induction, in order to not compromise reproductive performance.

Although there may be specific subgroups of endocrinologically healthy assisted reproduction treatment patients needing LH supplementation, it is well accepted that the best and only true model to investigate any LH hypothesis correctly is the hypogonadotrophic woman who may be totally LH deficient. It seems clear that normally ovulating women with pituitary down-regulation are not comparable to WHO group I anovulatory patients, as in most cases an absolute deficiency does not really exist, as demonstrated by a very different steroidogenic response to FSH alone. In addition, the use of GnRH analogues represents a major effect modifier. Therefore, to establish the threshold value of LH that should be used to discriminate between LH concentrations considered sufficient and those considered too low or too high in assisted reproduction treatment patients is not an easy task. Thus, more data (randomized controlled trials) are necessary before evidence-based recommendations regarding exogenous r-LH supplementation in ovarian stimulation protocols with r-FSH and GnRH-a for assisted reproduction treatment can be provided.

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