Article

Recombinant LH supplementation to recombinant FSH during induced ovarian stimulation in the GnRH-antagonist protocol: a meta-analysis

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Abstract

This study aims to compare the efficacy of recombinant LH (r-LH) supplementation for ovarian stimulation in gonadotrophin-releasing hormone-antagonist protocol for IVF/intracytoplasmic sperm injection cycles. Search strategies included online surveys of databases. The fixed effects model was used for odds ratio (OR) and effect size (weighted mean difference, WMD). Five trials fulfilled the inclusion criteria. When the meta-analysis was carried out, advantages were observed for the LH supplementation protocol with respect to higher serum oestradiol concentrations on the day of human chorionic gonadotrophin administration ($P < 0.0001$; WMD: 514, 95% CI 368, 660) and higher number of mature oocytes ($P = 0.0098$; WMD: 0.88, 95% CI 0.21, 1.54). However, these differences were not observed in the total amount of recombinant FSH (r-FSH) administered, days of stimulation, number of oocytes retrieved, the clinical pregnancy rate per oocyte retrieval, the implantation rate and miscarriage rate. This result demonstrates that the association of r-LH with r-FSH may prevent any decrease in oestradiol after antagonist administration and that a significantly higher number of mature oocytes was available for laboratory work. Nevertheless, it failed to show any statistically significant difference in clinically significant end-points in IVF (implantation and pregnancy rates). Additional randomized controlled trials are needed to confirm these results further.

Keywords: *GnRH antagonist, ovarian stimulation, recombinant FSH, recombinant LH*

Introduction

The pharmacology of ovarian stimulation has been strongly influenced by the two-cell, two-gonadotrophins theory. In this way, follicular stimulation protocols historically have included both FSH and LH in an attempt to mimic normal physiology (Sills *et al.*, 1999). 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 (GnRH) agonists 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 menopausal 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 (Humaidan *et al.*, 2004). In addition, the recent introduction of GnRH antagonists offers the opportunity to control the endogenous LH surge in a rapid and more convenient way. Indeed, GnRH antagonists act on gonadotrophin secretion through an immediate competitive blockade of GnRH receptors, and have induced a marked decrease in serum LH concentrations and a less pronounced decreased in FSH secretion (Albano *et al.*, 1997).

However, evidence has shown that the serum LH concentration can interfere with the results in assisted reproduction. The Ganirelix Dose-Finding Study Group (1998) found low endogenous LH concentrations and impaired oestrogen synthesis associated with decreased implantation, with increasing doses of antagonist, despite similar number of oocytes and embryos. Lindheim and Morales (2003), analysing donor cycles, concluded that GnRH antagonists has an unpredictable effect on oestradiol production during follicular recruitment that appears adversely to affect pregnancy outcome if a decline in serum oestradiol occurs. In addition, Tesarik *et al.* (2003) concluded that endometrial maturation is disturbed in women with low endogenous LH, and suggested that a direct action of LH on uterine LH receptors is needed to support both endometrial growth and uterine receptivity in the implantation window. Lahoud *et al.* (2006) demonstrated that a fall in LH concentration of ≥50% from the early-to mid-follicular phase resulted in a lower live birth rate. On the other hand, there is clinical evidence that excessive stimulation of the ovaries by LH adversely affects normal pre-ovulatory development (Balasch and Fábregues, 2006). In their reviews, Borini and Dal Prato (2005) and Balasch and Fábregues (2006) related that a therapeutic 'LH concentrations window' has been described, below which oestradiol production is not adequate and above which LH may be detrimental to follicular development.

Some studies have suggested that in GnRH agonist protocols, the suppression of the endogenous LH secretion does not seem to affect the majority of women undergoing assisted reproduction and stimulation with recombinant FSH (Balasch *et al.*, 2001; Lisi *et al.*, 2002; Humaidan *et al.*, 2004; Marrs *et al.*, 2004; Tarlatzis *et al.*, 2006), although some benefits appears in special situations: women who required >3000 IU to reach follicular maturity in previous cycles (Lisi *et al.*, 2001); patients in whom LH concentration was <1.0 IU/l at down-regulation or required excessive FSH stimulation, (Lisi *et al.*, 2002), advanced reproductive age (patients aged ≥35 years: Humaidan *et al.*, 2004; Marrs *et al.*, 2004; patients aged ≥38 years: Gomez-Palomares *et al.*, 2005); hypo-responders to r-FSH (De Placido *et al.*, 2004, 2005; Ferraretti *et al.*, 2004). Humaidan *et al.* (2004) have indicated that a group of normogonadotrophic women down-regulated and stimulated with pure FSH preparations may experience such low LH concentrations that parameters of the IVF treatment are compromised. Lower LH concentrations have also been associated with increased miscarriage rates (Westergaard *et al.*, 2000).

Trials in GnRH antagonist protocols failed to show a benefit of LH supplementation (Cédrin-Durnerin *et al.*, 2004; Sauer *et al.*, 2004; Griesinger *et al.*, 2005; Levi-Setti *et al.* 2006). Moreover, in a prospective study of patients stimulated with r-FSH only and GnRH antagonists, it was shown that the lower the LH concentrations on day 8 of stimulation 2 days after antagonist initiation, the higher the pregnancy rates achieved (Kolibianakis *et al.*, 2004). However, other studies showed better clinical results with the use of LH supplementation: oocyte donation cycles (Acevedo *et al.*, 2004), patients with risk of ovarian hyperstimulation syndrome (Kol and Muchtar, 2005), patients showing basal FSH >9 IU/l and/or age >37 years (De Placido *et al.* 2006) In addition, Huirne *et al.* (2005) indicate that both excessive and insufficient suppression of LH during GnRH antagonist administration seems to be associated with impaired clinical pregnancy rates.

Based on the above considerations, this meta-analysis aimed to compare the efficacy of recombinant LH supplementation in women undergoing assisted reproduction and stimulation with recombinant FSH for protocols of ovarian stimulation with antagonists in IVF/intracytoplasmic sperm injection (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 recombinant LH supplementation in women undergoing assisted reproduction and stimulation with recombinant FSH were analysed. Only trials performed in normogonadotrophic or 'good prognosis' women were considered.

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 oestradiol concentrations on the day of human chorionic gonadotrophin (HCG) administration, and 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 antagonist', '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 rigour 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-squared test statistic was used with its associated probability that the pooled odds ratio (OR) was equal to 1. The StatsDirect also gives the option to base effect size calculations on weighted mean difference (WMD) as described in the Cochrane Collaboration Handbook (Mulrow and Oxman, 1996). The measure of heterogeneity 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 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 OR and WMD. Since a fixed effects model has been employed here, 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 then be permissible, but the random errors used would then need to reflect inter-study variation. Since each of the analyses contained only five studies, it was decided to derive the inferences from a fixed effects model. Fixed effects model assumed that the true treatment difference for all study is identical and only reason to estimates varies between trials is the variance (or standard error square) within of each trial (within-study variance). Therefore, under the fixed-effect model, smaller studies (greater standard error) provide less precise estimates of the common effect than the larger ones (smaller standard error). Confidence intervals are calculated from variance that is companion of all estimates. In the fixed-effects model only within-study variances are taken into account in the assessment of the confidence intervals that are more influenced by the larger trials.

Results

Search results

Five trials fulfilled the inclusion criteria (**Figure 1**). A multiple low-dose regimen (cetrorelix, 0.25 mg) was applied in three and a fixed dose (cetrorelix, 3.0 mg) regimen was applied in two.

Description of studies included

Acevedo et al. (2004)

Forty-two young volunteer donors were randomly assigned with a computer-generated list to the GnRH antagonist alone protocol (n = 20) or the GnRH antagonist + r-LH (n = 22). The donors were healthy, age ranged from 18 to 35, and with normal menstrual cycle. Donors with polycystic ovarian disease (PCO), endometriosis, hydrosalpinges and severe male factor infertility were excluded from this study. Twenty donors were subjected to a step-down protocol in which LH activity was suppressed with a GnRH antagonist. On day 3 or 4 of their menstrual period, a fixed dose of 225 IU/day of r-FSH (Gonal-F®; Serono, Madrid, Spain) was given for 5 days. At day 6 of ovarian stimulation, 0.25 mg/day of GnRH antagonist (Cetrotide®; Serono, Spain) was subcutaneously injected until HCG was given. Twenty-two donors were subjected to the same GnRH antagonist + r-FSH step-down protocol, except when the GnRH antagonist was initiated, 75 IU/day r-LH (Luveris®; Serono, Spain) was added and maintained until GnRH antagonist was discontinued.

Cédrin-Durnerin et al. (2004)

A total of 200 patients who were submitted to oocyte retrieval from three centres were enrolled in this study. Ovulatory women were included with the following criteria: age 19–38 years; body mass index (BMI) <30; normal ovulatory function assessed by hormonal determinations at day 3 of a spontaneous cycle. All patients with history of low (<5 oocytes) or high (>15 oocytes) ovarian response in a previous IVF/ICSI attempt were excluded from the study. Pre-treatment with an oral contraceptive pill (levonorgestrel 0.15 mg + ethynylestradiol 30 μg) was given during the cycle prior to the IVF/ICSI procedure. Three days after pill discontinuation, stimulation was started by daily injection of 150–300 IU of r-FSH (Gonal F; Serono SA). The starting dose was chosen according to patient's age (150 IU/day, age ≤35 years; 225 IU/day, age >35 years), BMI (starting dose increased by 75 IU/day if BMI was 27–30) and ovarian responsiveness in previous cycles. This dose was maintained constant for 5 days. From day 6 of the stimulation, r-FSH doses were individually adjusted according to hormonal determinations and ultrasound data. When the follicle reached 14–16 mm diameter, patients received a single injection of cetrorelix 3.0 mg (Cetrotide; Serono SA,) and received (n = 107) or not (n = 94), according to randomization, a daily injection of 75 IU r-LH (Luveris; Serono SA) from the time of cetrorelix injection up to HCG administration. In both groups, r-FSH dose adjustment was not allowed at the time of cetrorelix administration. If criteria for triggering ovulation were not met within 4 days after cetrorelix administration, additional injections of cetrorelix 0.25 mg per day were performed until HCG administration. Randomization for r-LH supplementation was performed in centre 1 on day 1 of the stimulation by means of serially numbered, opaque, sealed envelopes. The allocation sequence was generated by a random permutation table and was concealed from clinicians enrolling participants. In centres 2 and 3, patients were randomized when they received their prescription according to the even on uneven year of the woman's birth. This latter quasi-randomization procedure was applied for clinician's convenience and explains the unequal number of patients in each group for these centres. Neither patients nor clinicians were blinded to r-LH administration. Patients were included in the study for only one cycle.

Sauer et al. (2004)

This was an open label, randomized, multi-centre study. In the present study, three groups of patients were compared: group A, GnRH agonist + r-FSH; group B, GnRH antagonist + r-FSH; and group C, GnRH antagonist + r-FSH + r-LH. Since the association of r-LH is relevant for this meta-analysis, only (data) comparison between group B and C were considered. A total of 42 infertile women (group B, n = 21 and group C,

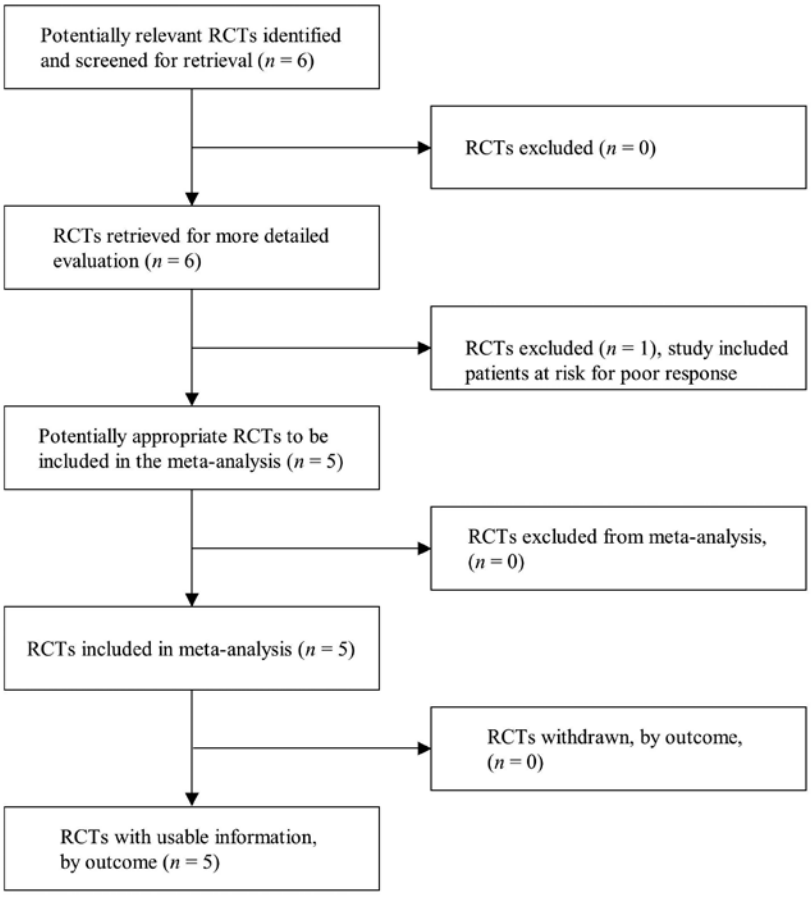

Figure 1. QUOROM statement flow diagram. RCT = randomised controlled trials.

n = 21) (aged 18–39 years), who were planning to undergo ICSI were recruited and were eligible for inclusion if all of the following criteria were satisfied within three menstrual cycles prior to randomization: regular menstrual cycles, BMI <35, both ovaries present, no clinical signs of pelvic or uterine abnormalities, normal cervical cytology, wash-out period completed for any previous IVF drug protocols and FSH concentration in the normal range. The principal exclusion criteria included clinically significant systemic disease, infection with human immunodeficiency virus, hepatitis C or B viruses, the presence of endometriosis or medical conditions likely to interfere with the study. Women were also excluded if previous assisted reproduction cycle had failed through insufficient response to gonadotrophin stimulation or absence of motile spermatozoa, or if they had undergone three or more consecutive assisted reproduction cycles without a clinical pregnancy, or had a history of extrauterine pregnancy or abnormal gynaecological bleeding. Patients were randomized using a computer-generated allocation. All patients took an oral contraceptive (Orthocept 21®; Ortho-McNeill, Raritan, NJ, USA) from day 1 of menses for 14–28 days. Women in group B and C received an injection of cetrorelix (Cetrotide; Serono Inc., USA), 3 mg s.c., on day 7 of the FSH stimulation cycle. If the patient did not achieve follicle maturation by day 11, an injection of cetrorelix, 0.25 mg s.c., was administered on day 11 and on each proceeding day up to, but not including, the day of r-HCG administration. The patients received r-FSH (Gonal-F; Serono) 225IU sc 5 days after the last oral contraceptive. From day 6, the dose was individualized according to patient response, with doses in the range 75–450 IU/daily. Women in group C received r-LH (Luveris; Serono), 150 IU s.c., on days 7–10 following the cetrorelix injection and at the same time as the r-FSH injection.

Griesinger et al. (2005)

This study was a prospective, randomized, open, single-centre, group-comparative clinical trial assessing a starting dose of 150 IU r-FSH versus 150 IU r-FSH plus 75 IU r-LH (2:1) for ovarian stimulation in the GnRH-antagonist multiple-dose protocol. The randomization process was conducted by drawing sealed envelopes and patients were free to start ovarian stimulation within the next three spontaneous menstrual cycles after randomization. Main inclusion criteria were: indication for treatment with IVF or ICSI; age between 20 and 39 years; BMI between 18 and 35; regular menstrual cycle ranging from 24 to 35 days, intra-individual cycle variability of ≤3 days. Main exclusion criteria were: >3 previous unsuccessful assisted reproduction techniques attempts; poor response to gonadotrophin stimulation defined as <3 pre-ovulatory follicles; history of ovarian hyperstimulation syndrome grade II–III; polycystic ovarian syndrome; any other endocrine disorder; no natural luteal phase prior to treatment cycle; abnormal uterine cavity; presence of a clinically significant systemic disease.

Ovarian stimulation started on day 2 of the natural cycle with r-FSH (Gonal-F; Serono) in the control group ($n = 54$), and 150IU r-FSH (Gonal-F) plus 75 IU r-LH (Luveris; Serono) in the study group ($n = 54$). After 5 days of gonadotrophin treatment, GnRH antagonist cetrorelix 0.25 mg (Cetrotide; Serono) administration was started. Gonadotrophin and antagonist treatment was continued up to and including the day of HCG administration. From day 6 onwards, the r-FSH dosage could be increased to 225 or 300 IU according to the ovarian response. In cases of a dose increment to 300 IU r-FSH in the study group, the r-LH was concomitantly adjusted to 150 IU.

Levi-Setti et al. (2006)

Forty patients undergoing ovarian stimulation for ICSI were included in this investigation. Indication for treatment was male-factor fertility, including only patients with ejaculated spermatozoa and excluding patients with frozen or testicular spermatozoa. All patients were normo-ovulatory, with regular menstrual cycles ranging from 25–35 days, aged ≤37 years, had a BMI <25, and had basal FSH measurements <12 IU/ml, measured no more than three cycles before starting the induction therapy. Patients with previous surgery or endometriomas at transvaginal ultrasound were excluded from the study. In all patients, a pre-treatment with an oral contraceptive (Minulet®; Wyeth, Aprilia-Latinia, Italy) was used. On day 2 of the cycle starting the administration of 225IU r-FSH (Gonal–F; Serono, Rome, Italy). When follicles reached the mean diameter of 14 and 15mm, the administration of cetrorelix (Cetrotide; Serono) was initiated at a daily dose of 0.25 mg s.c. At this time, the patients were randomly allocated by a computer-generated list to one of two groups: in group I ($n = 20$), ovarian stimulation was performed with the combination of 225 IU of r-FSH alone was continued, and in group II ($n = 20$) ovarian stimulation was performed with the combination of 150 IU of r-FSH and 75 IU of r-LH (Luveris; Serono). In group II, the dose of r-FSH was reduced to give to the two groups of patients the same amount of gonadotrophins.

Primary outcome

*Days of stimulation (**Table 1**)*

All studies were included. The mean days of stimulation was not significantly different in the 225 women using r-LH than in the 209 women not using it ($P = 0.65$; WMD: 0.07 95% CI –0.24, 0.37). There was no heterogeneity in this comparison (Cochran Q: 4.49, $df = 4$, $P = 0.34$).

*Total of r-FSH administered (**Table 2**)*

Four studies were included (Acevedo *et al.*, 2004; Cédrin-Durnerin *et al.*, 2004; Sauer *et al.*, 2004; Griesinger *et al.*, 2005). The mean amount of r-FSH administered was not significantly different in the 205 women using r-LH than in the 189 women not using it ($P = 0.87$; WMD: 8.51, 95% CI-98.2, 115.2). There was no heterogeneity in this comparison (Cochran Q: 3.18, $df = 4$, $P = 0.36$).

*Serum oestradiol concentrations on day of HCG administration (**Table 3**)*

All studies were included. Significantly higher serum oestradiol concentration was found in 221 women using r-LH than in 205 women not using it ($P < 0.0001$; WMD: 514, 95% CI 368, 660). There was no heterogeneity in this comparison (Cochran Q = 1.85, $df = 4$, $P = 0.76$).

*Number of oocytes retrieved (**Table 4**)*

Four trials reported this data (Acevedo *et al.*, 2004; Cédrin-Durnerin *et al.*, 2004; Griesinger *et al.*, 2005; Levi-Setti *et al.*, 2006). The mean number of oocytes retrieved was not significantly different in the 201 women using r-LH than in 185 women not using it ($P = 0.34$; WMD = 0.41, 95% CI –0.44, 1.3). There was no heterogeneity in this comparison (Cochran Q = 0.75, $df = 3$, $P = 0.85$).

*Number of mature oocytes (MII) (**Table 5**)*

All studies were included. A significantly higher number of mature oocytes was retrieved in 214 women using r-LH than in 193 women not using it ($P = 0.0098$; WMD = 0.88, 95% CI 0.21, 1.54). There was no heterogeneity in this comparison (Cochran Q = 6.19 $df = 4$, $P = 0.18$).

Secondary outcome

*Clinical pregnancy rate per oocyte retrieval (**Table 6**)*

Four studies were included (Cédrin-Durnerin *et al.*, 2004; Sauer *et al.*, 2004; Griesinger *et al.*, 2005; Levi-Setti *et al.*, 2006). The CPR per oocyte retrieval procedure was not significantly different in cycles using r-LH (59/200, 29.5%) compared with cycles not using it (59/186 31.7%) ($P = 0.69$; OR = 0.89, 95% CI 0.57, 1.39). There was no heterogeneity in this comparison (Breslow-Day = 0.95, $df = 3$, P = 0.81; Cochran Q = 0.94, $df = 3$, $P = 0.81$).

*Implantation rate (**Table 7**)*

Three studies were included (Cédrin-Durnerin *et al.*, 2004; Griesinger *et al.*, 2005; Levi-Setti *et al.*, 2006). The implantation rate was not significantly different in the cycles using r-LH (58/357, 16.2%) than in cycles not using it (54/336, 16.1%) ($P = 0.96$; OR = 0.99, 95% CI –0.66, 1.48). There was no heterogeneity in this comparison (Breslow-Day = 2.16, $df = 2$, $P = 0.34$; Cochran Q = 2.13, $df = 2$, $P = 0.34$).

*Miscarriage rate (**Table 8**)*

Two trials were included (Cédrin-Durnerin *et al.*, 2004; Levi-Setti *et al.*, 2006). The miscarriage rates were not significantly different in the pregnancies in which r-LH was used (6/41, 14.6%) from those that did not use r-LH (5/36, 13.9%) ($P = 0.82$; OR = 1.06, 95% CI –0.29, 3.8). There was no heterogeneity in this comparison (Breslow-Day = 0.03, $df = 1$, $P = 0.86$; Cochran Q = 0.03, $df = 1$, $P = 0.86$).

Table 1. Cycles with and without LH supplementation: days of stimulation.

Trial	*r-FSH + r-LH*		*r-FSH*		*WMD*	*95% CI fixed*
	n	*Mean ± SD*	n	*Mean ± SD*		
Levi-Setti *et al.*, 2006	20	9.8 ± 0.95	20	10.3 ± 1.25	–0.5	–1.19, 0.19
Griesinger *et al.*, 2005	55	12.0 ± 2.4	54	11.4 ± 2.1	0.6	–0.25, 1.45
Sauer *et al.*, 2004	21	9.4 ± 1.7	21	9.3 ± 1.1	0.1	–0.77, 0.97
Acevedo *et al.*, 2004	22	9.7 ± 2.1	20	9.3 ± 1.6	0.4	–0.74, 1.54
Cédrin-Durnerin *et al.*, 2004	107	11.8 ± 1.7	94	11.7 ± 1.5	0.1	–0.34, 0.54
Total	225		209			
Pooled effect size: fixed effects (Mulrow–Oxman)					0.07	–0.24, 0.37

Z (test WMD + differs from 0) = 0.44, *P* = 0.65.
Non-combinability of studies, Cochran Q = 4.496929 (*df* = 4), *P* = 0.34.
CI = confidence interval; r = recombinant; WMD = weighted mean difference.

Table 2. Cycles with and without LH supplementation: Amount of r-FSH administered.

Trial	*r-FSH + r-LH*		*r-FSH*		*WMD*	*95% CI fixed*
	n	*Mean ± SD (IU)*	n	*Mean ± SD (IU)*		
Griesinger *et al.*, 2005	55	2082.8 ± 695.7	54	1875.4 ± 646.4	207.4	–44.8, 459.6
Sauer *et al.*, 2004	21	2214.2 ± 612	21	2228.6 ± 359.8	–14.4	–318.0, 289.2
Acevedo *et al.*, 2004	22	1738 ± 407.2	20	1807.1 ± 97.05	–69.1	–252.3, 114.1
Cédrin-Durnerin *et al.*, 2004	107	2235 ± 729	94	2239 ± 620	–4	–192.5, 184.5
Total	205		189			
Pooled effect size: fixed effects (Mulrow-Oxman)					8.51	–98.2, 115.2

Z (test WMD + differs from 0) = 0.156, *P* = 0.87.
Non–combinability of studies, Cochran Q = 3.183282 (*df* = 4), *P* = 0.3642.
CI = confidence interval; r = recombinant; WMD = weighted mean difference.

Table 3. Cycles with and without LH supplementation: Serum oestradiol concentrations on day of human chorionic gonadotrophin administration.

Trial	*r–FSH + r–LH*		*r–FSH*		*WMD*	*95% CI fixed*
	n	*Mean ± SD (pg/ml)*	n	*Mean ± SD (pg/ml)*		
Levi–Setti *et al.*, 2006	18	1825 ± 545	20	1253 ± 490	572	243, 901
Griesinger *et al.*, 2005	54	1924.7 ± 1256.4	54	1488 ± 824	436.4	35.6, 837
Sauer *et al.*, 2004	20	2440.5 ± 1181.7	17	1540 ± 951.2	900.5	201, 1600
Acevedo *et al.*, 2004	22	1596 ± 988	20	989.6 ± 597	606.4	106, 1106
Cédrin-Durnerin *et al.*, 2004	107	1476 ± 787	94	1012 ± 659	464	162, 666
Total	221		205			
Pooled effect size: fixed effects (Mulrow–Oxman)					514	368, 660

Z (test WMD + differs from 0) = 6.9, $P < 0.0001$.
Non–combinability of studies, Cochran Q = 1.853585 ($df = 4$), $P = 0.76$.
CI = confidence interval; r = recombinant; WMD = weighted mean difference.

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

Trial	*r–FSH + r-LH*		*r–FSH*		*WMD*	*95% CI fixed*
	n	*Mean ± SD*	n	*Mean ± SD*		
Levi–Setti *et al.*, 2006	18	9.9 ± 2.6	18	9.2 ± 2.9	0.7	–1.2, 2.5
Griesinger *et al.*, 2005	54	7.9 ± 5.3	54	7.7 ± 5.1	0.2	–1.8, 2.2
Acevedo *et al.*, 2004	22	9.3 ± 3.6	20	8.2 ± 3.5	1.1	–1.1, 3,2
Cédrin-Durnerin *et al.*, 2004	107	9.9 ± 4.7	93	9.8 ± 4.7	0.1	–1.2, 1.40
Total	201		185			
Pooled effect size: fixed effects (Mulrow–Oxman)					0.41	–0.44, 1.3

Z (test WMD + differs from 0) = 0.945, $P = 0.34$
Non–combinability of studies, –Cochran Q = 0.756465 ($df = 3$), $P = 0.85$.
CI = confidence interval; r = recombinant; WMD = weighted mean difference.

Table 5. Cycles with and without LH supplementation: number of mature oocytes (metaphase II).

Trial		*r–FSH + r–LH*		*r–FSH*		*WMD*	*95% CI fixed*
		n	*Mean ± SD*	n	*Mean ± SD*		
Levi–Setti *et al.*, 2006		18	6.9 ± 2.1	18	6.5 ± 2.4	0.4	–1.1, 1.9
Griesinger *et al.*, 2005		46	6.57 ± 4.17	41	6.49 ± 4.26	0.08	–1.7, 1.8
Sauer *et al.*, 2004		21	14.6 ± 9.5	21	13.7 ± 6.8	0.9	–4.1, 5.9
Acevedo *et al.*, 2004		22	7.4 ± 1.6	20	5.2 ± 2.4	2.2	0.98, 3.4
Cédrin–Durnerin *et al.*	2004	107	8.2 ± 4.1	93	7.8 ± 4.0	0.4	–0.73, 1.53
Total		214		193			
Pooled effect size: fixed effects (Mulrow–Oxman)						0.88	0.21, 1.54

Z (test WMD + differs from 0) = 2.58, $P = 0.0098$
Non–combinability of studies, Cochran Q = 6.196684 ($df = 4$), $P = 0.18$.
CI = confidence interval; r = recombinant; WMD = weighted mean difference.

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

Trial	*r–FSH + r–LH*	*r–FSH*	*% weights*	*Odds ratio*	*95% CI fixed*
Levi–Setti *et al.*, 2006	7/18	6/18	8.8	1.30	0.26, 6.2
Griesinger *et al.*, 2005	8/54	12/54	24.6	0.61	0.20, 1.8
Sauer *et al.*, 2004	10/21	11/21	13.9	0.83	0.21, 3.3
Cédrin–Durnerin *et al.*, 2004	34/107	30/93	52.7	0.98	0.52, 1.8
Total	59/200	59/186			
Pooled odds ratio: fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)				0.89	0.57, 1.39

Chi-squared test (odds ratio differs from 1) = 0.15, $P = 0.69$.
Non–combinability of studies: Breslow–Day = 0.95 ($df = 3$), $P = 0.81$; Cochran Q = 0.94 ($df = 3$), $P = 0.81$.
CI = confidence interval; r = recombinant.

Table 7. Cycles with and without LH supplementation: implantation rate.

Trial	*r–FSH + r–LH*	*r–FSH*	*% weights*	*Odds ratio*	*95% CI fixed*
Levi–Setti *et al.*, 2006	11/54	8/49	14.3	1.30	0.43, 4.2
Griesinger *et al.*, 2005	8/99	15/109	28.2	0.55	0.19, 1.5
Cédrin-Durnerin *et al.*, 2004	39/204	31/178	57.5	1.12	0.64, 2.0
Total	58/357	54/336			
Pooled odds ratio: fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)				0.99	0.66, 1.48

Chi-squared test (odds ratio differs from 1) = 0.002, $P = 0.96$.
Non–combinability of studies: Breslow–Day = 2.16 ($df = 2$), $P = 0.34$; Cochran Q = 2.13 ($df = 2$), $P = 0.34$.
CI = confidence interval; r = recombinant.

Table 8. Cycles with and without LH supplementation: miscarriage rate.

Trial	*r–FSH + r–LH*	*r–FSH*	*% weights*	*Odds ratio*	*95% CI fixed*
Levi-Setti *et al.*, 2006	1/7	1/6	20.3	0.83	0.01, 78.3
Cédrin-Durnerin *et al.*, 2004	5/34	4/30	79.7	1.12	0.21, 6.3
Total	6/41	5/36			
Pooled odds ratio: fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)				1.06	0.29, 3.8

Chi-squared test (odds ratio differs from 1) = 0.05, $P = 0.82$.
Non–combinability of studies: Breslow–Day = 0.03 ($df = 1$), $P = 0.86$; Cochran Q = 0.03 ($df = 1$), $P = 0.86$.
CI = confidence interval; r = recombinant.

Discussion

Most data available in the medical literature suggest that recombinant gonadotrophins are more efficient than urinary gonadotrophins in inducing multiple follicular growth and maturation (Daya, 2002). The advantages of r-FSH are its excellent consistency from batch to batch and the fact it is free from contaminating human proteins. Although the role of LH in ovulation induction remains controversial, and it is well established that FSH alone can induce follicular growth, small amounts of LH are necessary to promote adequate oestradiol secretion and also to allow the follicle to luteinize when exposed to HCG (Couzinet *et al.*, 1988). The action of LH on follicular development is probably not limited to providing androgen substrate for aromatization, but it also exerts a direct effect on the stimulation and modulation of folliculogenesis (Filicori *et al.*, 2001).

On the other hand, GnRH antagonists are GnRH molecules with amino-acid modifications at positions 1, 2, 3, 6 and 10 and they immediately block the GnRH receptor in a competitive fashion and hence reduce LH and FSH secretion within a period of 8 h. The inhibition of LH secretion is more pronounced than that of FSH, this being most likely due to the different forms of gonadotrophin regulation, the prolonged FSH half-life or the immunoactive and bioactive forms of FSH (Matikainen, 1992). GnRH antagonist administration induces a fast and profound pituitary suppression, with a clear advantage in terms of premature LH avoidance. Nevertheless, LH activity is quickly and dramatically reduced in the phase in which this hormone activity is crucial: follicles that have been recruited in a physiological FSH and LH environment are dramatically deprived of their LH sustenance (Alviggi *et al.*, 2006).

Garcia-Velasco *et al.* (2001) observed that GnRH antagonist therapy in women undergoing ovarian stimulation had a significant effect on ovarian follicular steroidogenesis. The mean oestradiol concentration in follicular fluid was significantly lower in patients treated with a GnRH antagonist than in those treated with GnRH agonist. Lindheim and Morales (2003) in 37 donor cycles, starting GnRH antagonist administration on day 6 of stimulation, reported that 35% of cycles had a decrease in

serum oestradiol prior to HCG administration and 93% of them showed a decrease in serum oestradiol at >3 days after GnRH antagonist administration. In addition, clinical pregnancy rate per initiated cycle [14 (2/14) versus 54% (14/26)], ongoing pregnancy rate per initiated cycle [7 (1/14) versus 46% (12/26)] and implantation rate (4 versus 24%) were all significantly less ($P < 0.05$) following a decrease in serum oestradiol after initiation of GnRH antagonist. They concluded that the use of GnRH antagonists has an unpredictable effect on oestradiol production during follicular recruitment, which appears to adversely affect pregnancy outcome if a decline in oestradiol occurs. The interaction of the antagonist (at clinical working doses) with its receptors and the initiation of any intra-cellular signalling cascade that could affect gene transcription need further demonstration and clarification at the molecular level. Some of the indirect (oestradiol-mediated) action through which the GnRH antagonist could impact cell function, and ultimately implantation rates, can be substantiated and evaluated in a clinical setting. For example, it is well established that the production of some growth factors and peptides involved differentiation and mitosis of the oocyte, the embryo, the endometrium, and the granulosa cells, are oestrogen dependent. Because the hypo-oestrogenic environment that develops may compromise cell function, maintaining the oestradiol synthesis could lessen some of the indirect (oestradiol-mediated) GnRH antagonist action at the ovarian or endometrial concentrations (Acevedo *et al.*, 2004)

In this meta-analysis, a good effect of r-LH supplementation in ovarian stimulation was observed in serum oestradiol concentrations on the day of HCG administration. Significantly higher serum oestradiol concentrations were found in 221 women using r-LH compared with 205 women not using it ($P < 0.0001$; WMD = 514, 95% CI 368, 660). This result demonstrates that the association of r-LH with r-FSH may prevent a decrease in oestradiol after antagonist administration. In addition, significantly higher numbers of mature oocytes were retrieved in 214 women using r-LH compared with 193 women not using it ($P = 0.0098$; WMD = 0.88, 95% CI –0.21, 1.54). In another RCT, De Placido *et al.* (2006) found the same results. Although the trial was performed in women at risk for low ovarian response (age >37 years and/or basal FSH >9), different from this population, results are clearly consistent with those of the meta-analysis (i.e. increased number of mature oocytes in the r-LH group) and support the hypothesis that r-LH supplementation at the daily dose of 150 IU during GnRH antagonist treatment may be useful. Interestingly, in this study a progressive increase in the GnRH antagonist dose is also suggested.

On the other hand, these good results were not observed in total amount of r-FSH administered ($P = 0.87$; WMD = 8.51, 95% CI –98.2, 115.2), days of stimulation ($P = 0.65$; WMD = 0.07 95% CI –0.24,0.37), number of oocytes retrieved ($P = 0.34$; WMD = 0.41, 95% CI –0.44,1.3), the CPR per oocyte retrieval ($P = 0.69$; OR = 0.89, 95% CI –0.57,1.39), the implantation rate ($P = 0.96$; OR = 0.99 95% CI –0.66,1.48) and miscarriage rate ($P = 0.82$; OR = 1.06, 95% CI –0.29,3.8).

The need for reviews of medical literature is growing, stimulated by the increasing volume of information. Meta-analysis differs from the narrative review by the rigorous and complete quantitative and qualitative methodological approaches. Meta-analyses address a well-defined clinical question, use an explicit strategy to locate relevant evidence, evaluate the retrieved studies using prospectively defined methodological criteria, and formally synthesize the results (Cook *et al.*, 1997; Shojania and Bero, 2001; Borenstein, 2005; Delaney *et al.*, 2005). It is an analytical approach where different and independent studies are integrated and the results combined in a unique common result. When it is compared with narrative review, meta-analysis has the great advantage of being less influenced by the personal opinion of the reviewer and providing impartial conclusions. Moreover, all of the results of each study examined are reported, and the reader easily can recalculate the data and compare with the authors' conclusions (Leandro, 2005). The meta-analysis, even if not producing definite conclusions about the utility of a treatment, can support the necessity for new randomized trials on the subject. Besides, previously meta-analysis allows identification of the most important questions to be analysed in new researches.

Meta-analysis also presents problems, such as the quality of the primary studies or the form in which data are reported and the dependence on a great enough number of eligible studies to justify the statistical analysis (Delaney *et al.*, 2005; Heijnen *et al.*, 2006). Methodological problems caused by clinical heterogeneity and insufficient power (low sample size) make it difficult to draw inferences about the meta-analysis. In this study no heterogeneity was found for either outcome. This reflects the relative agreement between trials regarding the studied parameters and has particular importance when statistically significant outcomes were found. Nevertheless, it should be stressed that tests of heterogeneity among studies have low power. Therefore, where there is a non-significant test for heterogeneity among studies, it may be that a relationship is present but undetected.

On the other hand, the overall sample size is not large, with three of trials including fewer than 50 patients (Acevedo *et al.*, 2004; Sauer *et al.*, 2004; Levi-Setti *et al.*, 2006). In addition, the sample size of one study (Cédrin-Durnerin *et al.*, 2004), depending on the outcome analysed, varies from a minimum of 46% (days of stimulation) up to a maximum of 83% (miscarriage rate) of the general sample size. In particular in the fixed effects model, the common result tends to follow larger studies. In this meta-analysis, however, the individual results and the absence of heterogeneity denote similarity of the results. In addition, it was found that a statistically significant outcome resulted when the study with highest sample size it did not itself show a difference (number of mature oocytes). However, although smaller studies conducted on diverse populations may better reflect the natural heterogeneity of treatment effectiveness found in daily practice (Chalmers *et al.*, 1987), large studies may produce a more precise answer (Yusuf *et al.*, 1984). Thus, additional large sample-size RCT will be helpful for corroboration of the results.

As in many studies on the strategy of stimulation, this meta-analysis drew attention to variables more directly related to the stimulation (primary outcomes). However, some authors suggest that the meta-analysis should be patient orientated, i.e. primary outcomes should be clinical results (e.g. implantation rate); all other outcomes should be listed as secondary outcomes. Number of mature oocytes and oestradiol concentrations are intermediate end-points, which do not necessarily predict a better outcome and/or a more advantageous cost/effectiveness ratio. This meta-analysis failed to show any statistically significant difference in the most relevant and clinically significant end-points in IVF: the pregnancy rate, implantation rate and miscarriage rate. This observation can be related to a small cumulative sample size.

Based on the pregnancy rate per oocyte retrieval obtained in the group without LH (59/186, 31.7%), to detect a difference of 5% with a power of 80%, around 2800 patients would be necessary for a definitive conclusion, i.e. above the total number included here. However, it is very near to the numbers indicated by Daya and Gunby (1999) and Daya (2002) in meta-analyses comparing pregnancy rates in cycles with r-FSH and with urinary-FSH. On the other hand, neither did all studies supply data about pregnancy, implantation or miscarriage rates, nor was it possible to include the data from all trials in the statistical analysis. Additionally, as stated by Griesinger and Diedrich (2006), one must be aware of the fact that a number of other significant predictors of the chance of pregnancy achievement exist in an individual patient, besides the endocrine situation in the follicular phase, including cause of infertility, chromosomal status of the transferred embryo, quality and origin of spermatozoa. Moreover, an increased number of mature oocytes may exert possible good effects on cumulative pregnancy rates following the transfer of frozen embryos; an evaluation of this type was not possible to carry out in this study. Thus, for a more consistent conclusion, this meta-analysis again guides researchers to wait for the results of new randomized controlled trials that have more information about clinical parameters.

From other trial data and these meta-analysis results, other points should be stressed. First, it is interesting to observe that the only study performed in oocyte donation cycles (Acevedo *et al*., 2004), a model in which the endometrial variable is not conditioned by the stimulation regimen, demonstrated a more relevant impact of r-LH supplementation on implantation rate of recipients. Second, data from De Placido *et al*. (2004) showed that in normogonadotrophic women with suboptimal response to r-FSH, a daily dose of r-LH of 150 IU was more effective than 75 IU in improving ovarian response. Furthermore, data from the same trial indicates similar benefits of r-LH supplementation, at least for subgroups (Lisi *et al*., 2001, 2002; De Placido *et al*., 2004, 2006; Ferraretti *et al*., 2004; Humaidan *et al*., 2004; Marrs *et al*., 2004; De Placido *et al*., 2005; Gomez-Palomares *et al*., 2005; Kol and Muchtar, 2005). Accordingly, such findings should be considered hypotheses and can provide a basis for recommendations for future RCT on r-LH supplementation.

In conclusion, considering all the outcomes, there is probably no reason to be concerned that the use of r-LH supplementation imposes a harmful effect. Moreover, the results indicated that the association of r-LH with r-FSH may prevent any decrease in oestradiol after antagonist administration, and that a significantly higher number of mature oocytes were available for laboratory work. Nevertheless, it failed to show any statistical difference in clinically significant end-points in IVF (implantation and pregnancy rates). Additional randomized controlled trials with better planning are needed to further confirm these results.

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Received 26 May 2006; refereed 24 August 2006; accepted 6 October 2006.