Article

GnRH agonist versus GnRH antagonist in poor ovarian responders: a meta-analysis

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Abstract

The aim of this meta-analysis was to compare the efficacy of gonadotrophin antagonist (GnRH-ant) versus GnRH agonist (GnRHa) as coadjuvant therapy for ovarian stimulation in poor ovarian responders in IVF/intracytoplasmic sperm injection cycles. Search strategies included on-line surveys of databases such as MEDLINE, EMBASE and others. A fixed effects model was used for odds ratio (OR) and effect size (weighted mean difference, WMD). Six trials fulfilled the inclusion criteria (randomized controlled trials). There was no difference between GnRH-ant and GnRHa (long and flare-up protocols) with respect to cycle cancellation rate, number of mature oocytes and clinical pregnancy rate per cycle initiated, per oocyte retrieval and per embryo transfer. When the meta-analysis was applied to the two trials that had used GnRH-ant versus long protocols of GnRHa, a significantly higher number of retrieved oocytes was observed in the GnRH-ant protocols [$P = 0.018$; WMD: 1.12 (0.18, 2.05)]. However, when the meta-analysis was applied to the four trials that had used GnRH-ant versus flare-up protocols, a significantly higher number of retrieved oocytes ($P = 0.032$; WMD: –0.51, 95% CI –0.99, –0.04) was observed in the GnRHa protocols. Nevertheless, additional randomized controlled trials with better planning are needed to confirm these results.

Keywords: *GnRH agonist, GnRH antagonist, ovarian stimulation, poor responder*

Introduction

Recruitment and development of multiple follicles in response to ovarian stimulation are the key factors leading to successful treatment by IVF/intracytoplasmic sperm injection (ICSI) and embryo transfer. Garcia *et al*. (1983) first described as a poor responder the patient with peak oestradiol concentrations <300 pg/ml and a decreased follicular response, expressed as fewer retrieved and fertilized oocytes and also fewer transferred embryos.

Poor ovarian response, on the other hand, is usually associated with poor pregnancy rates, and many of these cycles are cancelled without proceeding to egg collection (Keay *et al*., 1997). Several strategies have been suggested to prevent cycle cancellation, such as decreasing the dosage and timing of gonadotrophin-releasing hormone agonists (GnRHa) (Scott and Navot, 1994) or the use of GnRHa flare-up regimens (Surrey *et al*., 1998). These procedures should theoretically eliminate excessive ovarian suppression while taking advantage of the additional gonadotrophin stimulus provided by the agonistic effect of GnRHa.

The introduction of GnRH antagonist (GnRH-ant) into clinical practice might be a new hope for poor responder patients (Craft *et al*., 1999). GnRH-ant prevents the LH surge occurring within a few hours, which is a common cause of cancellation in poor ovarian responder patients. The action of GnRH-ant does not result in early folliculogenesis inhibition, which is a critical point for patients with a limited cohort of follicles (Akman *et al*., 2000, 2001).

The objective of this meta-analysis was to compare the efficacy of GnRH-ant to that of a GnRHa as coadjuvant medicine for

protocols of ovarian stimulation in poor ovarian responder patients in IVF/ICSI cycles.

Materials and methods

Criteria for considering studies for this meta-analysis

All published randomized controlled trials (RCT) comparing GnRH-ant with GnRHa in ovarian stimulation protocols for poor responders were analysed. Ongoing RCT, where data were available via websites and theses, were also considered.

Types of outcome measures

The primary outcome measures used for this meta-analysis were the number of retrieved and mature oocytes. The secondary outcomes were cycle cancellation rate (CCR) due to poor response, clinical pregnancy rate (CPR) per cycle initiated, CPR per oocyte retrieval and CPR per transfer.

Identification of the 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 and grey literature (for example, Gateway, TrialsCentral, OMNI and others) was included. The following Medical Subject Headings and text words were used: 'poor responder', 'ovarian stimulation', '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 methodological 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 by 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 (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 an OR or WMD that is significantly worse or better than the overall common OR or WMD obtained by pooling the data. The fixed effect model was used for odds ratio (OR) and effect size (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 two (GnRH-ant versus GnRHa long protocol) and four (GnRH-ant versus GnRH flare-up) studies, it was decided to derive the inferences from a fixed effects model.

Search results

Six trials fulfilled the inclusion criteria (**Figure 1**). In all studies, the multiple low-dose (0.25 mg) antagonist regimen was applied (cetrorelix: 4, ganirelix: 2). In two trials, a long protocol with a GnRHa (leuprolide: 1, buserelin: 1) starting in the mid-luteal phase of the preceding cycle was used as a reference treatment. In the four remaining trials, a flare-up protocol with a GnRHa (triptorelin: 2, leuprolide: 2) was used as reference treatment. Three trials were excluded: D'Amato *et al.* (2004) due to a randomization problem (weekday is not a random assignment), Fasouliotis *et al.* (2003) was a retrospective study, and Palazón *et al.* (2005), due to no clear randomization description.

Results

Description of the studies included

Akman *et al.* (2001) conducted a prospective RCT (randomization by consecutive number method) including a total of 48 poor responder patients described from previous cycles (at least two failed IVF attempts) whose poor response was due to one of following reasons: baseline FSH concentrations >15 mIU/ml or oestradiol concentration on the day of human chorionic gonadotrophin (HCG) injection <500 pg/ml or fewer than four mature oocytes retrieved. The patients were divided into two groups: group I, 24 patients (mean age 38 years, range 28–46 years), in 24 cycles in which leuprolide acetate (Lucrin, Abbott, France) at the dose of 40 μg s.c. per day (GnRHa microdose flare-up protocol) was initiated on cycle day 2, followed by exogenous gonadotrophins [highly purified (HP)-FSH: Metrodin HP, Serono Laboratories, Switzerland] on cycle day 3; group II, 24 patients (mean age 38.5, range 28–44 years) in 24 cycles in which ovarian stimulation included a GnRH-ant (Cetrotide, Asta Medica, Germany), 0.25 mg daily, administered during the late follicular phase from the time when the leading follicle reached 14 mm in diameter until the day of HCG injection. In group I, the FSH on cycle day 3 (mIU/ml) was 9.03 (5.76–22.4) versus 10.3 (4.4–15.93) in group II (not significantly different). All patients in each group received 300 IU of HP-FSH together with 300 IU of human menopausal gonadotrophin (HMG) (Humegon: Organon Laboratories, USA) daily for 4 days. While the HMG dose remained constant until the injection of HCG, the HP-FSH dose was adjusted individually according to the response of the ovaries and the oestradiol concentrations. The number of oocytes retrieved was significantly higher ($P < 0.032$) in the GnRHa flare-up group (5.5 ± 2.2) when compared with GnRH-ant group (4.5 ± 1.8). However, the number of mature oocytes retrieved was not significantly different between GnRHa flare-up group (4.5 ± 1.9) versus GnRH-ant group (4.0 ± 1.8).

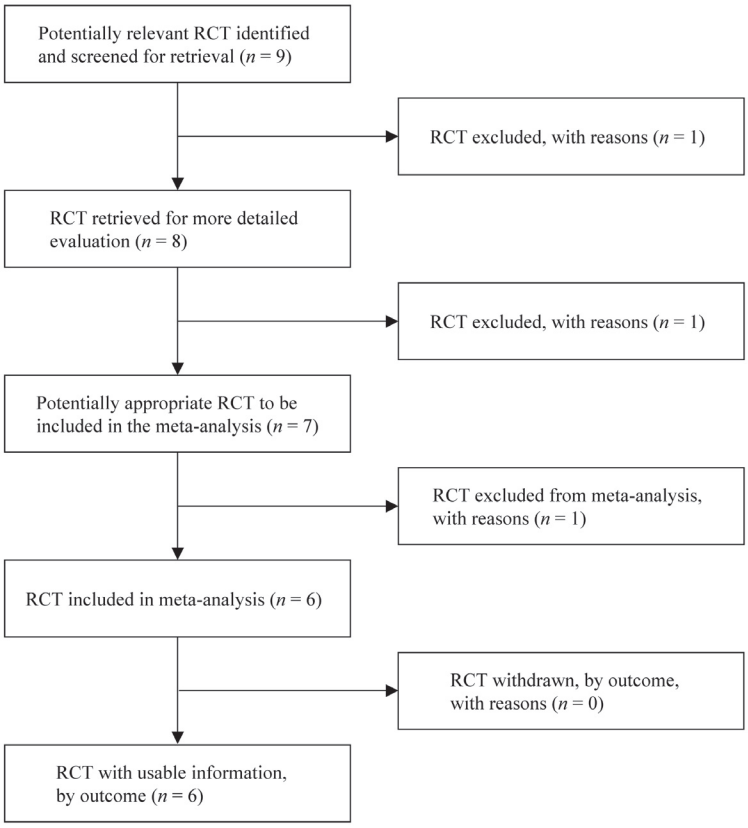

Figure 1. QUOROM statement flow diagram illustrating selection of trials included in the meta-analysis (http://www.consort-statement.org/Evidence/evidence.html). RCT = randomized controlled trials.

Malmusi *et al.* (2005) conducted a prospective RCT (randomization list). The poor responders (no ovarian response when ≥300 IU of FSH were administered for ≥15 days or a low number of oocytes ≤4) were randomized into two groups. The first group consisted of 30 patients (age 36.6 ± 0.8 years) in 30 cycles in which triptorelin, 0.1 mg (Decapeptyl: Ipsen, France) was initiated on day 1 of menstruation, followed by exogenous gonadotrophins (Gonal-F: Serono, Switzerland) administered from day 2 of menstruation. The second group consisted of 25 patients (age 36.2 ± 1.2 years), poor responders (25 cycles) in whom the exogenous gonadotrophins (Gonal-F: Serono, Switzerland) were started on day 1 of the menstrual cycle, followed by ganirelix (Orgalutran: Organon, Netherlands), 0.25 mg, administered daily when the leading follicle reached 14 mm in diameter until HCG injection. There was no difference in the basal FSH between the two groups (8 ± 3 and 7.5 ± 3.5). In each group, all patients initially received 450 IU of recombinant human FSH and the dose was adjusted individually according to the response of the ovaries. In the GnRHa flare-up group, the number of oocytes retrieved was significantly greater ($P < 0.05$) than in the GnRH-ant group (3.5 ± 1.4 versus 2.5 ± 1.2). In addition, the number of mature oocytes was significantly higher ($P < 0.05$) in the GnRHa flare-up group (3.2 ± 1.5) than in the GnRH-ant group (1.7 ± 1.2).

Marci *et al.* (2005) conducted a prospective RCT (randomized 1:1). The poor responders (oestradiol concentrations <600 pg/ml on the day of HCG administration and the number of retrieved oocytes <3 after a previous standard long protocol with GnRHa) were divided into two groups. Group A patients ($n = 30$; age = 39 ± 3.1) were stimulated with a standard long protocol using down-regulation with GnRHa, an injection of 3.75 mg leuprolide (Enantone: Takeda, Italy), and an s.c. injection of recombinant FSH (Gonal-F: Serono, Italy) at a dose of 375 IU daily from day 3 of the next cycle. In group B ($n = 30$; age = 38.8 ± 2.9), ovarian stimulation started at day 2 with recombinant FSH at a dose of 375 IU. The GnRH-ant cetrorelix (Cetrotide: Serono, Switzerland), 0.25 mg per day, was then administered when the two leading follicles had reached 14 mm in diameter, irrespective of the day of the cycle, and continued until the day of HCG injection. The number of oocytes retrieved was found to be significantly increased ($P = 0.022$) in the GnRH-ant group (5.6 ± 1.6) with respect to GnRHa long protocol group (4.3 ± 2.2). No information about basal FSH and the number of mature oocytes was reported.

Cheung *et al.* (2005) reported a prospective, RCT (computer-generated randomization concealed in opaque envelopes). A research nurse coordinated the randomization process and

distribution of medication throughout the treatment cycles. Doctors and embryologists involved in the study were blinded to the treatment allocation. Poor responders were classified as patients who had exhibited a poor ovarian response with <3 mature follicles on a long GnRHa protocol in their previous IVF cycles or those with repeated high basal concentrations of FSH >10 IU/l. The patients were randomly allocated to receive a GnRH-ant or an agonist. All patients received the oral contraceptive pill (Nordett: Wyeth, Australia; 30 µg of ethinyl oestradiol and 150 µg levonorgestrel), one tablet daily, for the rest of the cycle for a total of 21 days. The control group ($n = 32$; age = 36.3 ± 3.0) received a long GnRH-a protocol, buserelin acetate (Suprecur: Hoechst AG, Germany) nasal spray was given at a daily dose of 600 µg starting in the mid-luteal phase of the preceding cycle, and co-administered during the final week of oral contraceptive pre-treatment. Buserelin was continued until the day of HCG administration. The study group ($n = 31$; age = 36 ± 2.6) received a fixed multi-dose (0.25 mg daily) GnRH-ant protocol (Cetrotide; Serono, Switzerland) from day 6 of stimulation until the day of HCG. The ovarian stimulation was started with recombinant FSH (Gonal-F; Serono, Switzerland), 300 IU daily in both groups. The basal FSH on cycle day 3 was similar between the two groups (11.1 ± 3.3 and 11.8 ± 3.4 IU/l). There were no statistically significant differences between the control and study groups in the number of oocytes retrieved and number of mature oocytes.

Schmidt *et al*. (2005) reported a prospective, RCT. Computer-generated randomization was used for patient assignment to two treatment protocols at a 1:1 ratio. Sealed envelopes were used for protocol allocation. A poor responder was defined as a woman with serum peak oestradiol concentration ≤850 pg/ml and/or ≤4 preovulatory follicles ≥15 mm in average diameter present on the day of HCG administration during a previous cycle. A total of 48 patients were randomized to one of two groups: ganirelix acetate (group A; $n = 24$) or microdose leuprolide flare-up (group B; $n = 24$). Patients undergoing IVF treatment were between the ages of 25 and 43 years and were required to have a cycle day 3 serum FSH concentration <13 mIU/ml. On cycle day 2, patients randomized to group A received 300 IU of recombinant FSH every morning and 150 IU of human menopausal gonadotrophin (HMG) every evening for 5 days. A multidose regimen of ganirelix was used with daily morning injections (when oestradiol concentration ≥250 pg/ml and follicle ≥12 mm) up to and including the day of HCG administration. The patients randomized to group B received leuprolide acetate with a regimen of 40 µg s.c. every 12 h (GnRHa microdose flare-up protocol). The FSH and HMG dosing, monitoring, and individualized dosing adjustments were the same as for group A. There were no statistically significant differences between the GnRHa flare-up and GnRH-ant groups in the number of oocytes retrieved (9.0 ± 1.2 versus 8.9 ± 0.9) and number of mature oocytes (6.5 ± 1.1 versus 7.7 ± 0.9).

De Placido *et al*. (2006) reported a prospective randomized trial with a personalized flexible protocol that provided both a gradual increase in the GnRH-ant dose and the addition of recombinant LH (rLH), with the standard GnRHa short protocol in patients at risk for poor ovarian response when undergoing ICSI. The following inclusion criteria were used: age ≥37 years or day 2 serum FSH concentration ≥9 IU/l. Only couples undergoing ICSI were included because the mean number of mature oocytes was the primary end-point. All patients received a daily dose of 300 IU of recombinant FSH (Gonal-F; Serono, Italy) beginning on day 2 of their cycles. The daily dose of recombinant FSH was adjusted on the basis of the ovarian response beginning on day 5 of stimulation. The FSH mean concentrations measured on day 2 of the cycle were 7.64 ± 4.12 IU/l in the antagonist group and 8.45 ± 4.23 IU/l in the agonist group. Patients were randomized into two groups using a computer-generated list. In the antagonist group (patient mean age 37.16 ± 4.14 years), a dose of 0.125 mg/day of the GnRH-ant cetrorelix (Cetrotide: Serono, Italy) was administered for 2 days beginning when at least one follicle ≥14 mm was present. Thereafter, the full GnRH-ant dose of 0.25 mg/day was given until the day of exogenous HCG administration. Beginning on the same day of GnRH-ant administration, a daily dose of 150 IU of rLH (Luveris: Serono, Italy) was also added until the day of HCG. The agonist group (patient mean age 37.32 ± 3.72 years) received a daily dose of triptorelin (decapeptyl 0.1 mg: Ipsen, Italy) of 0.1 mg s.c., beginning on the same day as the first rFSH administration. In addition, in this group, a dose of 150 IU/day of rLH was added when at least one follicle reached 14 mm. The mean number of mature oocytes retrieved was significantly higher ($P < 0.05$) in the GnRH-ant group (6.09 ± 3.36 versus 5.02 ± 1.86). Conversely, no statistically significant difference in the total number of cumulus oocyte complexes retrieved (7.23 ± 3.60 versus 7.06 ± 2.55) was observed between the groups.

Primary outcome measures

When the meta-analysis was carried out on the two trials that had used GnRH-ant versus long protocols of GnRHa, a significantly higher number of retrieved oocytes was observed in the GnRH-ant protocols [$P = 0.018$; WMD: 1.12 (0.18, 2.05)] (**Table 1**). However, no difference was observed with respect to number of mature oocytes [WMD: 0.32 (–1.55, 2.19)] (**Table 2**). On the other hand, when the meta-analysis was performed with the four trials that had used GnRH-ant versus flare-up protocols, a significantly higher number of retrieved oocytes ($P = 0.032$; WMD: –0.51, 95% CI –0.99, –0.04) was observed in the GnRHa protocols (**Table 1**). In addition, no differences were observed regarding the number of mature oocytes (WMD: 0.07, 95% CI –0.38, 0.53) (**Table 2**).

Secondary outcome measures

In the two trials with a long protocol of GnRHa as a reference treatment versus GnRH-ant, no differences were observed in the CCR due to a poor ovarian response (OR 0.67, 95% CI 0.26, 1.68) (**Table 3**); CPR per cycle initiated (OR 2.23 (0.71–6.98) (**Table 4**) per oocyte retrieval (OR 2.30, 95% CI 0.71, 7.4) (**Table 5**) or CPR per transfer (OR 2.03, 95% CI 0.62, 6.55) (**Table 6**). On the other hand, when meta-analysis was performed only with the four trials that used the flare-up protocol of GnRHa as reference treatment, no other differences were observed in the GnRH-ant protocol with respect to secondary outcome measures: CCR due to a poor ovarian response (OR 0.95, 95% CI 0.48, 1.87) (**Table 3**); CPR per initiated cycle (OR 1.05, 95% CI 0.59, 1.87) (**Table 4**), CPR per oocyte retrieval (OR 1.02, 95% CI 0.56, 1.87) (**Table 5**); CPR per transfer (OR 1.09, 95% CI 0.59, 2.00) (**Table 6**). There was no significant heterogeneity of treatment effect (odds ratio and weighted mean difference) across the trials except for the GnRH-ant versus GnRHa flare-up protocols with respect to number of mature oocytes.

Table 1. Meta-analysis of gonadotrophin-releasing hormone antagonist (GnRH-ant) versus gonadotrophin-releasing hormone agonist (GnRHa) (long protocol and flare-up) treatments in poor responders for number of oocytes retrieved.

Trial	*No. oocytes retrieved (mean ± SD)*		*WMD (fixed)*	*95% CI*
	GnRH-ant	*GnRHa*		
Long protocol				
Cheung *et al.*, 2005	19 (5.89 ± 3.02)	21 (5.62 ± 4.17)	0.27	–2.0, 2.54
Marci *et al.*, 2005	29 (5.60 ± 1.6)	26 (4.30 ± 2.2)	1.3	0.29, 2.30
Fixed effects (Mulrow–Oxman): pooled effect size			1.12	0.18, 2.05
Z (test WMD + differs from 0) = 2.35, *P* = 0.018				
Non-combinability/Cochrane Q = 0.67 (*df* = 1), NS				
Flare-up				
Akman *et al.* 2001	20 (4.5 ± 1.8)	20 (5.5 ± 2.0)	–1	–2.18, 0.18
De Placido *et al.*, 2006	62 (7.23 ± 3.60)	62 (7.06 ± 2.55)	0.17	–0.92, 1.2
Malmusi *et al.*, 2005	18 (2.5 ± 1.2)	24 (3.5 ± 1.4)	–1	–1.80, –0.19
Schmidt *et al.*, 2005	13 (8.9 ± 0.9)	11 (9.0 ± 1.2)	–0.1	–0.94, 0.74
Fixed effects (Mulrow–Oxman) pooled effect size			–0.51	–0.99, –0.04
Z (test WMD + differs from 0) = –2.13, *P* = 0.032				
Non-combinability/Cochrane Q = 4.49 (*df* = 3), NS				

WMD = weighted mean difference; NS= not significant.

Table 2. Meta-analysis of gonadotrophin-releasing hormone-antagonist (GnRH-ant) versus gonadotrophin-releasing hormone agonist (GnRHa) (long protocol and flare-up) treatments in poor responders for number of mature oocytes.

Trial	*No. mature oocytes (mean ± SD)*		*WMD fixed*	*95% CI*
	GnRH-ant	*GnRHa*		
Long protocol				
Cheung *et al.*, 2005	19 (5.32 ± 2.73)	21 (5.0 ± 3.26)	0.32	–1.55, 2.19
Flare-up				
Akman *et al.*, 2001	20 (4.0 ± 1.8)	20 (4.5 ± 1.9)	–0.5	–1.65, 0.65
De Placido *et al.*, 2006	62 (6.09 ± 3.36)	62 (5.02 ± 1.86)	1.07	0.11, 2.02
Malmusi *et al.*, 2005	18 (1.7 ± 1.2)	24 (3.2 ± 1.5)	–1.5	–2.34, –0.65
Schmidt *et al.*, 2005	13 (7.7 ± 0.9)	11 (6.5 ± 1.1)	1.2	0.40, 1.99
Fixed effects (Mulrow–Oxman), pooled effect size			0.07	–0.38, 0.53
Z (test WMD + differs from 0) = –0.32, NS				
Non-combinability/Cochrane Q = 26.75 (*df* = 3), *P* < 0.0001				

WMD = weighted mean difference; NS= not significant.

Table 3. Meta-analysis of gonadotrophin-releasing hormone-antagonist (GnRH-ant) versus gonadotrophin-releasing hormone agonist (GnRHa) (long protocol and flare-up) treatments in poor responders for cycle cancellation rate due to poor response.

Trial	*Cycle cancellation rate*		*%*	*Odds*	*95% CI*
	GnRH-ant	*GnRHa*	*Weights*	*ratio*	
Long protocol					
Cheung *et al.*, 2005	10/31	11/32	65.5	0.90	0.27, 2.94
Marci *et al.*, 2005	1/30	4/30	34.5	0.22	0.004, 2.51
Pooled odds ratio				0.67	0.26, 1.68
Fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)					
χ^2 (test odds ratio differs from 1) = 0.37, NS					
Non-combinability of studies					
Breslow-Day = 1.31 (*df* = 1), NS					
Cochrane Q = 1.23 (*df* = 1), NS					
Flare-up					
Akman *et al.*, 2001	2/24	2/24	10.5	1.00	0.07, 14.94
De Placido *et al.*, 2006	4/66	5/67	26.8	0.80	0.15, 3.91
Malmusi *et al.*, 2005	7/25	6/30	22.5	1.55	0.37, 6.63
Schmidt *et al.*, 2005	10/24	12/24	40.2	0.71	0.19, 2.57
Pooled odds ratio				0.95	0.48, 1.87
Fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)					
χ^2 (test odds ratio differs from 1) = 0.018, NS					
Non-combinability of studies					
Breslow–Day = 0.90 (*df* = 3), NS					
Cochrane Q = 0.90 (*df* = 3), NS					

NS= not significant.

Table 4. Meta-analysis of gonadotrophin-releasing hormone-antagonist (GnRH-ant) versus gonadotrophin-releasing hormone agonist (GnRHa) (long protocol and flare-up) treatments in poor responders for clinical pregnancy rate per cycle initiated.

Trial	*Clinical pregnancy rate per cycle initiated*		*%*	*Odds*	*95% CI*
	GnRH-ant	*GnRHa*	*Weights*	*ratio*	
Long protocol					
Cheung *et al.*, 2005	5/31	3/32	59.8	1.85	0.32, 13
Marci *et al.*, 2005	5/30	2/30	40.2	2.80	0.40, 31.3
Pooled odds ratio				2.23	0.71, 6.98
Fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)					
χ^2 (test odds ratio differs from 1) = 1.27, NS					
Non-combinability of studies					
Breslow–Day = 0.12 (*df* = 1), NS					
Cochrane Q = 0.12 (*df* = 1), NS					
Flare-up					
Akman *et al.*, 2001	4/24	5/24	18.6	0.76	0.13, 4.16
De Placido *et al.*, 2006	17/67	14/66	45.9	1.30	0.54, 3.2
Malmusi *et al.*, 2005	3/25	6/30	21.4	0.54	0.07, 2.96
Schmidt *et al.*, 2005	5/24	4/24	14.1	1.31	0.24, 7.65
Pooled odds ratio				1.05	0.59, 1.87
Fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)					
χ^2 (test odds ratio differs from 1) = 0.00006, NS					
Non-combinability of studies					
Breslow–Day = 1.32 (*df* = 3), NS					
Cochrane Q = 1.31 (*df* = 3), NS					

NS = not significant.

Table 5. Meta-analysis of gonadotrophin-releasing hormone-antagonist (GnRH-ant) versus gonadotrophin-releasing hormone agonist (GnRHa) (long protocol and flare-up) treatments in poor responders for clinical pregnancy rate per oocyte retrieval.

Trial	*Clinical pregnancy rate per oocyte retrieval*		*% Weights*	*Odds ratio*	*95% CI*
	GnRH-ant	*GnRHa*			
Long protocol					
Cheung *et al.*, 2005	5/19	3/21	54.6	2.1	0.34, 15.9
Marci *et al.*, 2005	5/29	2/26	44.5	2.5	0.36, 28.3
Pooled odds ratio				2.3	0.71, 7.4
Fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)					
χ^2 (test odds ratio differs from 1) = 1.27, NS					
Non-combinability of studies					
Breslow–Day = 0.016 (*df* = 1), NS					
Cochrane Q = 0.016 (*df* = 1), NS					
Flare-up					
Akman *et al.*, 2001	4/20	5/20	18.9	0.75	0.12, 4.3
De Placido *et al.*, 2006	17/62	14/62	48.1	1.30	0.53, 3.2
Malmusi *et al.*, 2005	3/18	6/24	20.3	0.6	0.08, 3.45
Schmidt *et al.*, 2005	5/13	4/11	12.6	1.1	0.16, 7.96
Pooled odds ratio				1.02	0.56, 1.87
Fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)					
χ^2 (test odds ratio differs from 1) = 0.0049, NS					
Non-combinability of studies					
Breslow–Day = 0.96 (*df* = 3), NS					
Cochrane Q = 0.95 (*df* = 3), NS					

NS = not significant.

Table 6. Meta-analysis of gonadotrophin-releasing hormone-antagonist (GnRH-ant) versus gonadotrophin-releasing hormone agonist (GnRHa) (long protocol and flare-up) treatments in poor responders for clinical pregnancy rate per transfer.

Trial	*Clincial pregnancy rate per transfer*		*% Weights*	*Odds ratio*	*95% CI*
	GnRH-ant	*GnRHa*			
Long protocol					
Cheung *et al.*, 2005	5/19	3/17	57.2	1.67	0.26, 12.7
Marci *et al.*, 2005	5/29	2/26	42.8	2.5	0.36, 28.3
Pooled odds ratio				2.03	0.62, 6.55
Fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)					
χ^2 (test odds ratio differs from 1) = 0.80, NS					
Non-combinability of studies					
Breslow-Day = 0.11 (*df* = 1), NS					
Cochrane Q = 0.11 (*df* = 1), NS					
Flare-up					
Akman *et al.*, 2001	4/18	5/19	18.8	0.80	0.13, 4.6
De Placido *et al.*, 2006	17/62	14/62	50.6	1.30	0.53, 3.2
Malmusi *et al.*, 2005	3/14	6/24	17.3	0.81	0.11, 4.87
Schmidt *et al.*, 2005	5/13	4/11	13.3	1.1	0.16, 7.96
Pooled odds ratio				1.09	0.59, 2.00
Fixed effects (Mantel–Haenszel, Robins–Breslow–Greenland)					
χ^2 (test odds ratio differs from 1) = 0.017, NS					
Non-combinability of studies					
Breslow–Day = 0.46 (*df* = 3), NS					
Cochrane Q = 0.46 (*df* = 3), NS					

NS = not significant.

Discussion

Poor ovarian response to gonadotrophin remains a significant problem in assisted conception. There have been various reports formulating the ideal stimulation protocol for poor ovarian responders. It has been documented that cycle cancellation is common for this particular group of patients, mostly due to premature LH surges or to an inadequate ovarian response (Akman *et al*., 2001).

GnRH analogues have been indicated for this clinical problem as a drug coadjuvant for treatment. GnRH-ant 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 *et al*., 1992). Unlike GnRH-ant, GnRHa exert their effect by binding to the transmembrane receptor and, following a period of flare-up, produce a down-regulation phenomenon (Reissmann *et al*., 1995).

On the other hand, the introduction of GnRH-ant into clinical practice and their addition to ovarian stimulation during the late follicular phase will prevent the premature LH surges while not causing any suppression in the early follicular phase, which is a critical period for those patients with decreased ovarian reserves, and would be a reasonable option for patients with poor ovarian response in previous stimulation cycles (Cheung *et al*., 2005). In a published meta-analysis comparing GnRH-ant with GnRHa protocols in non-selected IVF patients, the use of GnRH-ant was found to be associated with significantly less gonadotrophin consumption and shorter treatment duration, while having an equal effectiveness in the prevention of a premature LH surge (Al-Inany and Aboulghar, 2002). This GnRH-ant characteristic will always be welcome for poor ovarian responders because a high gonadotrophin requirement is commonly associated with high cancellation rates and low numbers of oocytes retrieved.

The lack of a uniform definition of 'poor responders' makes it difficult to compare treatment outcomes and develop and assess protocols for prevention and management (Surrey and Schoolcraft, 2000; Kailasam *et al*., 2004). This fact could be observed in this meta-analysis since the authors used different criteria for the selection of the population defined as poor responders: Akman *et al*. (2001): FSH, oestradiol and number of mature oocytes; De Placido *et al*. (2006): age ≥37 years or day 2 FSH serum concentration ≥9 IU/l; Malmusi *et al*. (2005): no ovarian response when ≥300 IU of FSH were administered for ≥15 days or low number of oocytes ≤4; Marci *et al*. (2005): oestradiol concentrations <600 pg/ml on the day of HCG administration and <3 oocytes retrieved after a previous standard long protocol with GnRHa; Cheung *et al*.: <3 mature follicles in a long GnRHa protocol in their previous IVF cycles or those with repeated high basal concentrations of FSH >10 IU/l; Schmidt *et al*. (2005): serum peak oestradiol concentration ≤850 pg/ml and or ≤4 preovulatory follicles ≥15 mm in average diameter present on the day of HCG administration during a previous cycle. An international standardization of criteria to define poor ovarian responders should be an important future measure. This aspect could be a possible limit for the results of this meta-analysis.

The presence of randomization was the primary criterion used to select all the papers of this meta-analysis. An interesting fact observed was the difference among authors with respect to the randomization process. (Akman *et al*., consecutive number method; De Placido *et al*., computer-generated list; Malmusi *et al*., 2005: randomization list; Marci *et al*., 2005: consecutive number method; Cheung *et al*. (2005): computer-generated randomization; Schmidt *et al*. (2005): computer-generated randomization). True randomization involves selecting patients by a random process, such as the use of a random-numbers table. Quasi-random methods such as sorting by days of the week, birth dates, or medical record numbers, are reasonable in most cases, although investigators need to test for any bias that might result.

This meta-analysis showed no difference between GnRH-ant and GnRHa long protocols with respect to CCR, number of mature oocytes, CPR per cycle initiated, CPR per oocyte retrieval and CPR per embryo transfer. However, the use of GnRH-ant protocols showed a significantly higher number of oocytes retrieved ($P < 0.018$; WMD: 1.12, 95% CI 0.18, 2.05) compared with GnRHa long protocols.

More recently, with the discovery of extrapituitary GnRH receptors in the human ovary (Janssens *et al*., 2000), there has been some concern that the non-physiological concentration of GnRHa given to achieve down-regulation may have a direct, deleterious effect on the ovary, and contribute to the poor response to ovarian stimulation in some patients undergoing IVF/ICSI cycles. To overcome the extra-suppression hypothesis while preventing the premature LH surges, various researchers have advocated decreasing the dosage and the timing of GnRHa, such as in microdose and flare-up regimens (Scott and Navot, 1994; Surrey *et al*., 1998).

In general, trials have compared the short and long GnRHa protocols (Garcia *et al*., 1990; Hughes *et al*., 1992; Cramer *et al*., 1999) and observed that the GnRHa long protocol had preference over the GnRHa flare-up in patients with normal ovarian response in IVF/ICSI cycles. However, although not derived from authentically prospective trials, optimistic data have been presented that suggest the beneficial use of flare-up GnRHa protocols (standard or microdose) along with high doses of gonadotrophins in poor ovarian responders. These regimens seem to have better results compared with those of the standard long luteal protocols. A significant improvement was demonstrated with the use of the low-dose, mid-luteal onset, GnRHa regimens, which are discontinued with the initiation of ovarian stimulation, followed by high doses of gonadotrophins, according to the prospective studies with historical controls. However, well-designed prospective trials failed to confirm this, and showed no significant improvement (Tarlatzis *et al*., 2003).

In this study, when the meta-analysis was performed with the trials that had used flare-up protocols of GnRHa, a significantly higher number of retrieved oocytes was observed in the GnRHa protocol when compared with the GnRH-ant ($P = 0.032$). However, no other difference was observed with the GnRH-ant

protocol in any of the outcomes analysed (CCR due to poor ovarian response, number of mature oocytes, CPR per initiated cycle, CPR per oocyte retrieval, CPR per embryo transfer).

Overall, no conclusions about pregnancy rates have emerged in this meta-analysis, probably due to exiguity of available data or because the number of oocytes represents an intermediate outcome parameter, which is not always predictive of pregnancy rates.

Garcia-Velasco *et al*. (2001) observed that GnRH-ant 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 GnRH-ant than in those treated with GnRHa. In that study, an interesting observation was the fact that, with similar FSH doses (GnRH-ant and GnRHa groups) and with a significantly reduced period of stimulation, fewer oocytes were retrieved in antagonist-treated women. However, this was a matching study and not a randomized controlled clinical trial.

Lindheim and Morales (2003), in a study of 37 donor cycles, starting GnRH-ant administration on day 6 of stimulation, reported that 35% of the donor 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-ant administration. They concluded that the use of GnRH-ant has an unpredictable effect on oestradiol production during follicular recruitment that appears to adversely affect pregnancy outcome if a decline in oestradiol occurs.

At present, the relationship between GnRH-ant and a negative effect on ovarian follicular steroidogenesis (decreased oestradiol concentrations, small number of oocytes) is unclear. Nevertheless, some variables could be interfering with this problem, such as total dose, different length of the down-regulation period, and individual sensitivity to the drug. GnRH-ant 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, which have been recruited in a physiological FSH and LH environment, are dramatically deprived of their LH sustenance (Alviggi *et al*., 2006). Some studies have suggested that the suppression of the endogenous LH secretion does not seem to affect the majority of women undergoing assisted reproduction and stimulation with recombinant FSH. However, other studies have indicated that a group of normogonadotrophic women down-regulated and stimulated with pure FSH preparations may experience LH concentrations so low that parameters of the IVF treatment are compromised (Humaidan *et al*., 2004). Then heterogeneity among studies with respect to type of gonadotrophins (containing or not LH activity) in the GnRH-ant or GnRHa protocols could be an important bias in the results of this meta-analysis.

The data obtained (GnRH-ant versus flare-up protocols, a significantly higher number of retrieved oocytes (P = 0.032; WMD: –0.51, 95% CI –0.99, –0.04) was observed in the GnRHa flare-up protocol), indicating that randomized studies of poor ovarian responders should be planned for comparison of GnRH-ant multidose protocols versus GnRHa flare-up protocols (gold standard).

So far as is known, this is the first meta-analysis comparing GnRH-ant to GnRHa in ovarian stimulation protocols for poor responders. Nevertheless, additional randomized controlled trials with better planning are needed to further confirm these results, since the randomized studies available for this meta-analysis involved reduced sample numbers and varied widely in the definition of poor ovarian response, and heterogeneity (trial GnRH-ant versus GnRHa agonist protocols with respect to number of mature oocytes).

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