



Efficacy of luteal estrogen administration and an early follicular Gonadotropin-releasing hormone antagonist priming protocol in poor responders undergoing *in vitro* fertilization

Hwajeong Lee¹, Hyun Joung Choi¹, Kwang Moon Yang², Min Jung Kim¹, Sun Hwa Cha¹, Hyun Jeong Yi²

Department of Obstetrics and Gynecology, ¹Cheil General Hospital and Women's Healthcare Center, College of Medicine, Dankook University, Seoul, ²Maria Fertility Hospital, Seoul, Korea

Objective

We investigated whether luteal estrogen administration and an early follicular Gonadotropin-releasing hormone antagonist (E/G-ant) priming protocol improves clinical outcomes in poor responders to controlled ovarian stimulation for *in vitro* fertilization (IVF)-embryo transfer, and identified underlying mechanisms.

Methods

This retrospective study consisted of 65 poor responders who underwent the E/G-ant priming protocol. Sixty-four other poor responders undergoing conventional protocols without pretreatment were included as the control group. Clinical outcomes were compared between 2 groups.

Results

The E/G-ant priming protocol group exhibited improvements over the control group in terms of the number of retrieved oocytes (3.58 ± 2.24 vs. 1.70 ± 1.45 ; $P=0.000$), mature oocytes (2.68 ± 2.11 vs. 1.65 ± 1.23 ; $P=0.000$), fertilized oocytes (2.25 ± 1.74 vs. 1.32 ± 1.26 ; $P=0.001$), good embryos (1.62 ± 0.91 vs. 1.14 ± 0.90 , $P=0.021$). Day 3 follicle-stimulating hormone (FSH; 8.40 ± 4.84 vs. 16.39 ± 13.56 ; $P=0.000$) and pre-ovulation progesterone levels (0.67 vs. 1.28 ng/mL; $P=0.016$) were significantly higher in the control group than in the E/G-ant priming group. The overall rate of positive human chorionic gonadotropin tests was higher in the E/G-ant priming group than in the control group (32.3% vs. 16.1% ; $P=0.039$). Also, clinical pregnancy rate (26.2% vs. 12.5% ; $P=0.048$) and the rate of live births (23.1% vs. 7.1% ; $P=0.023$) were significantly higher in the E/G-ant priming group than in the control group.

Conclusion

The E/G-ant priming protocol would lead to promising results in poor responders to IVF by suppressing endogenous FSH and by preventing premature luteinization.

Keywords: Diminished ovarian reserve; Fertilization *in vitro*; Gonadotropin-releasing hormone; Hormone antagonist; Estrogen

Introduction

Controlled ovarian stimulation (COS) is a key determinant of the success of *in vitro* fertilization (IVF)-embryo transfer (ET). Extensive research has established many combinations of medications and COS protocols for IVF-ET. Nevertheless, there are always patients who respond poorly to COS protocols for various reasons, including reduced ovarian reserves and hormonal imbalances [1,2]. The prevalence of poor ovarian response (POR) can vary from 5.6% to 35.1% — depending on

Received: 2017.03.06. Revised: 2017.07.22. Accepted: 2017.07.24.

Corresponding author: Hyun Jeong Yi

Department of Obstetrics and Gynecology, Maria Fertility Hospital, 103-11, Sinseol-dong, Dongdaemun-gu, Seoul, Korea

E-mail: deligh@naver.com

<http://orcid.org/0000-0002-5237-8684>

Articles published in *Obstet Gynecol Sci* are open-access, distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2018 Korean Society of Obstetrics and Gynecology

how the term is defined — and is gradually increasing among IVF patients [3-8]. Numerous studies and modified protocols have been proposed over the past 20 years for poorly responding patients. It is frustrating to note, however, that there has been no efficient treatment ensuring notable improvement of ovarian response in patients [7,9-11]. Women who respond poorly to various COS methods generally have lower numbers of retrieved oocytes and transferable embryos, and inevitably have lower clinical pregnancy rates than those of normal responders [8,12,13].

In a COS cycle, follicles are expected to grow concurrently in response to exogenous gonadotropins in order to accomplish simultaneous maturation. Conversely, marked discrepancies in follicular size in the early follicular phase result in decreased oocyte maturation and fertilization potential, which negatively impact the number of transferable embryos and the probability of conception [14-16]. This heterogeneity of follicular growth in COS cycles has been observed more frequently in POR [3,14,17,18]. Although the physiological mechanism implicated in this process remains poorly understood, a few hypotheses have focused on premature dominant follicle selection after the rise of follicle-stimulating hormone (FSH) in the late luteal phase, or on different developmental levels of each recruited follicle [18,19]. To reduce heterogeneity of follicles, estrogen pretreatment in late luteal phases in COS cycles were proposed, but estrogen pretreatment did not lead to satisfying results in clinical pregnancy rates, regardless of improvement to follicular size homogeneity and increased numbers of retrieved oocytes [17,20-24]. In addition, some authors postulated that applying a Gonadotropin-releasing hormone (GnRH) antagonist in the follicular phase before ovarian stimulation could create an opportunity to lengthen the recruitment phase of the cycle, thereby allowing the rescue of more follicles by suppressing endogenous FSH and preventing corpus luteum formation without compromising the ovarian stimulatory response of poor responders [25-28].

Cakmak et al. [3] suggested a protocol that focused on the heterogeneity of antral follicle responsiveness to gonadotropins during the early follicular phase. In order to synchronize the growth of a pool of follicles they applied estrogen priming during the late luteal phase and following pretreatment with the GnRH antagonist for 7 days of the early follicular phase prior to gonadotropin stimulation. The authors reported improved ovarian responsiveness during COS, although they could not describe clinical pregnancy rates between protocols

due to the study design [3]. Furthermore, the duration of GnRH antagonist pretreatment was relatively longer than other GnRH antagonist pretreatment protocols [25-28], so that total duration of the IVF-ET cycle was relatively prolonged. Although the mechanism was not made clear, prolonged duration of treatment might be implicated as a cause of premature luteinization (PL) in COS [29,30]. PL is understood to be a subtle increase in serum progesterone levels observed during the late follicular phase in COS, which reflects only the total amount of progesterone secreted by maturing follicles in the absence of any premature uncontrolled luteinizing hormone (LH) surge [31]. PL has been observed frequently in elderly or poorly responding patients [32-34], and in addition, recent meta-analyses have found that PL had more adverse effects on IVF outcomes in poor responders than in normal or high responders [35-37]. In line with these trials, we conducted 7–10 days of luteal estrogen administration, followed by 5 consecutive days of early follicular GnRH antagonist (E/G-ant) priming protocol for POR (Fig. 1).

The primary aim of this study was to investigate whether the luteal estradiol (E2) and E/G-ant priming protocol would improve ovarian responses and clinical outcomes in poor responders, and to identify the mechanisms underlying favorable outcomes in poor responders, particularly with respect to changes in hormonal status.

Materials and methods

1. Patient selection

This retrospective, single-center cohort study was conducted at the Infertility Center at Cheil Hospital in Seoul between May 2014 and July 2016. We enrolled 65 consecutive patients who met the Bologna criteria [38]. The Bologna criteria states that POR is defined by at least 2 of the following 3 features that must be present: 1) advanced maternal age (≥ 40 years)

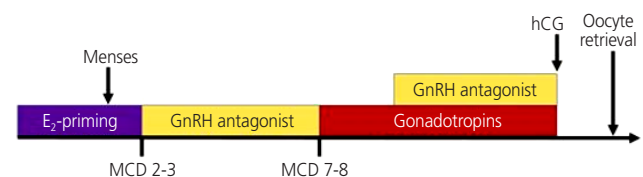


Fig. 1. Diagram of the early follicular Gonadotropin-releasing hormone (GnRH) antagonist (E/G-ant) priming protocol. E₂, estradiol; MCD, menstrual cycle days; hCG, human chorionic gonadotropin.

or any other risk factor for POR; 2) previous POR (≤ 3 oocytes with a conventional stimulation protocol); and 3) an abnormal ovarian reserve test (i.e., antral follicle count, 5–7 follicles or anti-mullerian hormone [AMH], 0.5–1.1 ng/mL) [38]. Sixty-four other women categorized into POR according to the Bologna criteria were selected as controls and underwent a conventional GnRH antagonist protocol during the study period. All patients had been previously diagnosed with female infertility based on an infertility evaluation that included serum basal hormone levels (E2, FSH, LH, thyroid-stimulating hormone [TSH], and prolactin levels on menstrual cycle days [MCD] 2 or 3), as well as a hysterosalpingogram and hysteroscopy or laparoscopy as needed. All patients had normal menstrual cycles with a range of 24–35 days, normal serum TSH and prolactin levels and had no other factors of infertility except for POR. Among 64 patients in the control group, 8 patients were excluded because of high E2 levels or abnormal ultrasound findings on the second or third MCD. Institutional Review Board has been exempted due to retrospective nature of the study.

2. Luteal E2 administration and E/G-ant priming protocol

Patients were put on 4 mg of daily oral E2 valerate (Progynova; Bayer Healthcare Pharmaceuticals, Wayne, NJ, USA) for an estrogen priming cycle beginning a week following their previous ovulation until menses. On MCD 2 or 3, patients visited the center for measurement of baseline FSH, LH, and E2 levels, ultrasound examination for antral follicle counting, and endometrial thickness measurement. Next, the patients began 0.25-mg daily subcutaneous injections of GnRH antagonist (cetorelix acetate) (Merck KGaA, Darmstadt, Germany) on MCD 2 or 3. After 5 days of GnRH antagonist pretreatment, patients started on 375–450 IU of human menopausal gonadotropin (hMG) (Menopur; Ferring Pharmaceuticals, Tarrytown, NY, USA) at the mid-follicular phase of the cycle (MCD 7–8). The GnRH antagonist was added for the second time when the largest follicle measured >12 mm in order to prevent premature ovulation before the human chorionic gonadotropin (hCG) trigger. hCG was administered when the leading follicle measured >18 mm and the remaining follicles were >10 mm.

3. Conventional GnRH antagonist protocol (control group)

The conventional GnRH antagonist protocol in the control group did not apply hormonal pretreatment during the luteal period and subsequent early follicular phase. On MCD

2 or 3, patients visited the center for measurement of their baseline FSH, LH, and E2 levels, for antral follicle counting via ultrasound examination, and for measurement of endometrial thickness. For the conventional antagonist protocol, patients were administered 375–450 IU of hMG (Menopur; Ferring Pharmaceuticals) via daily subcutaneous injection beginning on MCD 3 for ovarian stimulation. The GnRH antagonist was added when the largest follicle measured >12 mm in order to prevent premature ovulation before the hCG trigger. Recombinant hCG (Ovidrel; Merck Serono, Geneva, Switzerland) was administered when the leading follicle measured >18 mm and the remaining follicles were >10 mm.

4. Oocyte retrieval, embryo culture, and transfer procedure

Oocytes were harvested via transvaginal ultrasound-guided follicular puncture 35–36 hours after hCG administration. Metaphase oocytes were fertilized by an intracytoplasmic sperm injection (ICSI) technique 4–6 hours after oocyte retrieval. Embryos were cultured separately and scored according to the modified Gardner and Schoolcraft grading system [39]. The number of embryos transferred was based on the number of good embryos (above grade II) together with the circumstances of the patient. Embryos were transferred on either post-fertilization day 2 or 3 under abdominal ultrasound guidance.

5. Outcome measures related to IVF and pregnancy outcomes

In order to analyze the effectiveness of the E/G-ant priming protocol, we compared the IVF-ET outcome, hormonal status and pregnancy outcome with those of the control group. IVF-ET outcomes included the number of mature (metaphase II [MII]) oocytes, total number of oocytes retrieved, oocyte maturity rate (MII oocyte number/total oocyte number), fertilization rate (percentage of 2PN stage zygotes approximately 16 hours after ICSI treatment) and the number of good embryos (above grade II according to the graduated embryo score [GES] grading system). To analyze serial hormonal status, changes of serum E2 and P4 levels were compared between E/G-ant priming protocol group and the control group on MCD 3, the GnRH antagonist starting day when the largest follicle measured >12 mm, and the day of hCG injection. Serum beta hCG levels were measured 12 days after ovum pick-up, and values above 5.0 IU were considered positive. Clinical

pregnancy was defined as the presence of an intrauterine gestational sac with a yolk sac, a fetal pole, and fetal heart pulsations. The live birth rate was defined as delivery of a viable baby after 24 weeks of gestation.

6. Statistical analysis

Data are statistically presented in terms of the mean±standard deviation, number of cases, and percentage when appropriate. We used Student's *t*-tests to evaluate continuous parameters. Chi-squared tests and Fisher's exact tests, as appropriate, were used to compare clinical pregnancy rates. A *P*-value <0.05 was considered statistically significant. The statistical analysis was performed with SPSS ver. 22.0 (IBM Co., Armonk, NY, USA).

Results

Among the 64 patients enrolled in the control group, 8 patients were excluded because of abnormal baseline hormonal levels or ultrasound findings on MCD 2 or 3, while no one was excluded from the E/G-ant priming group. The E/G-ant priming group and the control group were generally well matched in terms of baseline characteristics known to affect clinical pregnancy rates, such as age, previous pregnancy, body mass index and AMH levels (Table 1). The 2 groups did not differ significantly in the total dose of gonadotropin for stimulation or the duration of stimulation (Table 1). However,

the levels of E2 (E/G-ant priming group vs. control group; 83.15±80.27 vs. 26.99±13.28; *P*<0.001) and FSH (E/G-ant priming group vs. control group; 8.40±4.84 vs. 16.39±13.56; *P*<0.001) in samples taken at the beginning of the cycle differed significantly between groups. Also, E2 level sampled on the day of hCG triggering was higher in the E/G-ant priming group than in the control group (E/G-ant priming group vs. control group; 1,234.05±743.85 vs. 664.96±498.09; *P*<0.001; Table 1).

Serum progesterone levels were initially documented in 52 patients from the E/G-ant priming group and 45 patients in the control group on MCD 2 or 3. As shown in Fig. 2, group progesterone levels did not differ at the beginning of the cycle (E/G-ant priming group vs. control group; 0.26±0.17 vs. 0.40±0.25 ng/mL; *P*=0.156) or on the mid-follicular antagonist administration day (E/G-ant priming group vs. control group; 0.27±0.24 vs. 0.41±0.20 ng/mL; *P*=0.066). However, serum progesterone levels were significantly higher in the control group than in the E/G-ant priming group before ovulation (E/G-ant priming group vs. control group; 0.67±0.53 vs. 1.28±1.12 ng/mL; *P*=0.016).

Clinical outcomes in each group are shown in Table 2. The overall success rate of yielding at least one oocyte after the procedure of ovum pick up in the E/G-ant priming group (90.8% [59/65]) was greater than that in the control group (80.4% [45/56]), although this lacked statistical significance. However, the number of oocytes retrieved (3.58±2.24 vs.

Table 1. Comparison of the clinical characteristics of early follicular Gonadotropin-releasing hormone antagonist priming cycle and control groups

Characteristics	E/G-ant priming cycle (n=65)	Control (n=56)	P-value
Age (yr)	38.93±3.87	39.28±4.31	0.630
BMI (kg/m ²)	21.6±4.07	22.02±3.47	0.608
AMH (ng/mL)	0.46±0.35	0.41±0.32	0.397
Day 3 LH (mIU/mL)	4.83±2.54	7.34±5.39	0.003
Day 3 FSH (mIU/mL)	8.40±4.84	16.39±13.56	0.000
Day 3 E2 (mIU/mL)	83.15±80.27	26.99±13.28	0.000
Middle E2	379.21±241.46	129.13±181.75	0.000
hCG triggering day E2	1,234.05±743.85	664.96±498.09	0.000
Duration of stimulation (day)	9.85±1.94	10.00±1.95	0.992
Gonadotropin dose (IU)	3,031.25±631.58	2,891.25±574.70	0.792

Values are presented as mean±standard deviation. Student's *t*-test for numeric variables. Middle E2: E2 levels on GnRH antagonist start day in the mid-follicular phase.

E/G-ant, early follicular Gonadotropin-releasing hormone antagonist; BMI, body mass index; AMH, anti-mullerian hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; E2, estradiol; hCG, human chorionic gonadotropin.

1.70±1.45; $P=0.000$) and the number of mature oocytes (2.68±2.11 vs. 1.65±1.23; $P=0.000$) were significantly greater in the E/G-ant priming group. The number of successfully fertilized embryos (2.26±1.62 vs. 1.52±0.72; $P=0.012$) and good embryos according to the GES grading system (1.62±0.91 vs. 1.14±0.90; $P=0.021$) was also greater in the E/G-ant priming group than in the control group (Table 2).

The overall rate of positive hCG tests was higher in the E/G-ant priming group than in the control group (32.3% [21/65] vs. 16.1% [9/56]; $P=0.039$). Also, clinical pregnancy rate for overall study group (26.2% [17/65] vs. 12.5% [7/56]; $P=0.048$) and the rate of live births (23.1% [15/65] vs. 7.1% [4/56]; $P=0.023$) were significantly higher in the E/G-ant priming group than in the control group.

Discussion

POR to COS is a serious problem due to a quantitative reduc-

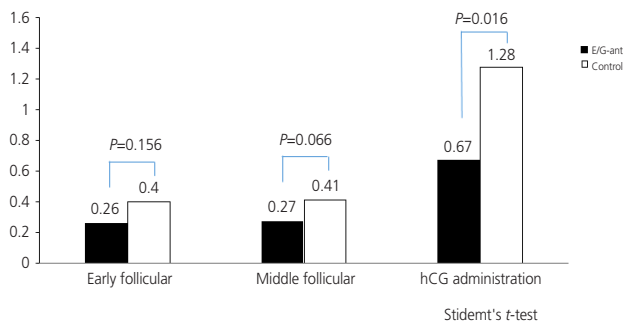


Fig. 2. Serum progesterone level during controlled ovarian stimulation. E/G-ant, early follicular Gonadotropin-releasing hormone antagonist.

tion in the follicular response and a consequent decrease in the retrieved oocyte number, transferable embryo number and pregnancy rate in comparison to normal responders [8,13,40-43]. Therefore, it is essential to identify an optimal stimulation protocol to maximize the quality and number of retrieved oocytes so that designated poor responders can achieve pregnancy.

In a COS cycle, exogenous gonadotropins are administered in order to achieve simultaneous maturation of follicles. Some authors have postulated that FSH elevation during the luteal-follicular transition might cause asynchronous follicular size and, ultimately, may reduce the follicular cohort available for recruitment [17,23,44]. Although the physiological mechanisms implicated in this process remain poorly understood, a few strategies have been proposed in order to suppress this phenomenon in POR [3,17,20-23,25-27]. Taking into account all these considerations, we hypothesized that using estrogen administration in the late luteal phase of the preceding menstrual cycle followed by GnRH antagonist pretreatment in the beginning of the follicular phase could be a promising method for suppressing endogenous FSH during the luteal-follicular transition and early follicular phase, and for promoting and synchronizing follicle development without impairing developmental competence of oocytes. In the present study, we modified the "delayed start" GnRH antagonist protocol in the work of Camak et al. [3] by shortening GnRH antagonist pretreatment to 5 days in the beginning of the follicular phase for in order to optimize patient hormonal status in COS cycles, as well as to minimize costs and maximize patient convenience. The purpose of this retrospective cohort study was to evaluate the impact of luteal estrogen administration and

Table 2. Clinical outcomes of early follicular Gonadotropin-releasing hormone antagonist priming and control groups

Characteristics	E/G-ant priming	Control	P-value
No. of retrieved oocytes	3.58±2.24	1.70±1.45	0.000
No. of mature oocytes	2.68±2.11	1.65±1.23	0.000
No. of fertilized embryos after ICSI/IVF	2.26±1.62	1.52±0.72	0.012
No. of good embryos	1.62±0.91	1.14±0.90	0.021
No. of transferred embryos	2.02±0.83	1.50±0.99	0.002
b-hCG positive rate (%)	32.3 (21/65)	16.1 (9/56)	0.039
Clinical pregnancy rate (%)	26.2 (17/65)	12.5 (7/56)	0.048
Live birth rate (%)	23.1 (15/65)	7.1 (4/56)	0.023

Values are presented as mean±standard deviation. Student's *t*-test for numeric variables, χ^2 test for categorical variables.

E/G-ant, early follicular Gonadotropin-releasing hormone antagonist; ICSI, intracytoplasmic sperm injection; IVF, *in vitro* fertilization; β -hCG, beta human chorionic gonadotropin.

a 5-day course of E/G-ant priming protocol in poor responders. Secondly, we sought to understand the mechanisms that bring favorable outcomes, particularly with regard to changes in hormonal status.

Our study findings are consistent with previous studies that demonstrate greater numbers of retrieved oocytes and transferable embryos by using luteal estrogen or GnRH antagonist pretreatment in comparison to controls [3,17,20,21,23-26]. With respect to hormonal status, basal FSH levels in the E/G-ant priming group were notably lower than basal FSH levels in the control group. Conversely, E2 levels in the E/G-ant priming group were significantly higher than the E2 levels of patients in the control group. In our opinion, these results indicate that FSH elevation was sufficiently suppressed before the start of the follicular phase, and therefore, the E/G-ant priming protocol might effectively inhibit premature dominant follicle selection.

The association of clinical outcomes of COS cycles with PL remains controversial. Many studies have suggested that PL adversely affects IVF-ET cycle outcomes [32,45-47], whereas other authors have reported that this condition does not have any negative effects on the outcomes of COS cycles [48-51]. While clinical observations have had mixed results, several possible mechanisms have been proposed to explain the pre-ovulatory progesterone rise that has been observed among IVF patients. In some findings, the level of pre-ovulatory progesterone was positively related to the number and size of multiple follicles, the amount of exogenous gonadotropin, and the number of oocytes retrieved. Therefore, it was concluded that PL is usually observed in patients displaying a good response to ovarian stimulation [29,51-53]. In contrast, several papers have reported that PL is more prevalent in older or poorly responding patients despite relatively small number of oocytes [32-34]. Furthermore, recent meta-analysis studies have found that PL had a more detrimental effect on the probability of pregnancy achievement in poor responders than in good responders in IVF-Et cycles [35-37]. Although the mechanism was not clear, this could be explained by the fact that poor responders need longer periods of stimulation, and thus a significantly higher total FSH dosage than good responders [29,30]. Also, Younis et al. [34] tried to explain this phenomenon in the context of POR with concepts of increased LH sensitivity. Additionally, PL may influence the endometrium, leading to impaired endometrial receptivity, which has negative effects on clinical pregnancy rates [36,54].

In a recent study suggested that elevated pre-ovulatory progesterone level due to increased progesterone production per follicle (high PFI) was detrimental to IVF outcomes, but not if it is a consequence of additional follicular recruitment [55]. In our study, although the E2 levels of patients in the E/G-ant priming group on hCG triggering day and the number of retrieved oocytes exceeded these values in the control group, P4 levels remained significantly low in the E/G-ant priming group. Also, the E/G-ant priming group showed improved pregnancy outcomes compared to the control group in our study. These data indicate that the E/G-ant priming protocol effectively inhibits PL such that it may have positive effects on clinical pregnancy outcomes.

According to a recent meta-analysis, the clinical pregnancy rate of poor responders in an IVF-ET cycle averages 14.8%, whereas normal patients have a 34.5% pregnancy rate [56]. In our study, the overall rate for positive hCG tests in the E/G-ant priming protocol was 32.3%, and the rate of live births was 23.1%. To the best of our knowledge, these improved clinical pregnancy rates have not yet been reported in earlier studies of POR.

In conclusion, for patients with poor ovarian reserve who plan to undergo IVF, poor quality and a low quantity of oocytes have been major limitations on standard IVF-ET cycles. This luteal E2 administration and E/G-ant priming protocol would yield more mature oocytes and transferable embryos and, consequently, better clinical pregnancy rates than conventional GnRH antagonist IVF protocols. Nevertheless, the total number of analyzed patients was small. Accordingly, further prospective and randomized clinical trials are warranted to verify the findings reported herein.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

References

1. Hendriks DJ, Mol BW, Bancsi LF, Te Velde ER, Broekmans FJ. Antral follicle count in the prediction of poor ovarian response and pregnancy after *in vitro* fertilization: a meta-analysis and comparison with basal follicle-stimulating

- hormone level. *Fertil Steril* 2005;83:291-301.
- Pandian Z, McTavish AR, Aucott L, Hamilton MP, Bhat-tacharya S. Interventions for 'poor responders' to controlled ovarian hyper stimulation (COH) in *in-vitro* fertilisa-tion (IVF). *Cochrane Database Syst Rev* 2010:CD004379.
 - Cakmak H, Tran ND, Zamah AM, Cedars MI, Rosen MP. A novel "delayed start" protocol with gonadotropin-releasing hormone antagonist improves outcomes in poor responders. *Fertil Steril* 2014;101:1308-14.
 - Polyzos NP, Devroey P. A systematic review of random-ized trials for the treatment of poor ovarian responders: is there any light at the end of the tunnel? *Fertil Steril* 2011;96:1058-1061.e7.
 - Keay SD, Liversedge NH, Mathur RS, Jenkins JM. Assisted conception following poor ovarian response to gonadotro-phin stimulation. *Br J Obstet Gynaecol* 1997;104:521-7.
 - Surrey ES, Schoolcraft WB. Evaluating strategies for improving ovarian response of the poor responder un-dergoing assisted reproductive techniques. *Fertil Steril* 2000;73:667-76.
 - Vollenhoven B, Osianlis T, Catt J. Is there an ideal stimu-lation regimen for IVF for poor responders and does it change with age? *J Assist Reprod Genet* 2008;25:523-9.
 - Jenkins JM, Davies DW, Devonport H, Anthony FW, Gadd SC, Watson RH, et al. Comparison of 'poor' responders with 'good' responders using a standard buserelin/human menopausal gonadotrophin regime for *in-vitro* fertiliza-tion. *Hum Reprod* 1991;6:918-21.
 - Cheung LP, Lam PM, Lok IH, Chiu TT, Yeung SY, Tjer CC, et al. GnRH antagonist versus long GnRH agonist pro-tocol in poor responders undergoing IVF: a randomized controlled trial. *Hum Reprod* 2005;20:616-21.
 - Ubaldi F, Vaiarelli A, D'Anna R, Rienzi L. Management of poor responders in IVF: is there anything new? *BioMed Res Int* 2014;2014:352098.
 - Kyrou D, Kolibianakis EM, Venetis CA, Papanikolaou EG, Bontis J, Tarlatzis BC. How to improve the probability of pregnancy in poor responders undergoing *in vitro* fertil-ization: a systematic review and meta-analysis. *Fertil Steril* 2009;91:749-66.
 - Kligman I, Rosenwaks Z. Differentiating clinical profiles: predicting good responders, poor responders, and hyper-responders. *Fertil Steril* 2001;76:1185-90.
 - Saldeen P, Källen K, Sundström P. The probability of suc-cessful IVF outcome after poor ovarian response. *Acta Obstet Gynecol Scand* 2007;86:457-61.
 - Rosen MP, Shen S, Dobson AT, Rinaudo PF, McCulloch CE, Cedars MI. A quantitative assessment of follicle size on oocyte developmental competence. *Fertil Steril* 2008;90:684-90.
 - Devreker F, Pogonici E, De Maertelaer V, Revelard P, Van den Bergh M, Englert Y. Selection of good embryos for transfer depends on embryo cohort size: implications for the 'mild ovarian stimulation' debate. *Hum Reprod* 1999;14:3002-8.
 - Opsahl MS, Blauer KL, Black SH, Lincoln SR, Thorsell L, Sherins RJ. The number of embryos available for transfer predicts successful pregnancy outcome in women over 39 years with normal ovarian hormonal reserve testing. *J Assist Reprod Genet* 2001;18:551-6.
 - Fanchin R, Cunha-Filho JS, Schonäuer LM, Kadoch IJ, Co-hen-Bacri P, Frydman R. Coordination of early antral fol-licles by luteal estradiol administration provides a basis for alternative controlled ovarian hyperstimulation regimens. *Fertil Steril* 2003;79:316-21.
 - Klein NA, Battaglia DE, Fujimoto VY, Davis GS, Bremner WJ, Soules MR. Reproductive aging: accelerated ovar-ian follicular development associated with a monotropic follicle-stimulating hormone rise in normal older women. *J Clin Endocrinol Metab* 1996;81:1038-45.
 - Chun SY, Eisenhauer KM, Minami S, Billig H, Perlas E, Hsu-eh AJ. Hormonal regulation of apoptosis in early antral follicles: follicle-stimulating hormone as a major survival factor. *Endocrinology* 1996;137:1447-56.
 - Dragisic KG, Davis OK, Fasouliotis SJ, Rosenwaks Z. Use of a luteal estradiol patch and a gonadotropin-releasing hor-mone antagonist suppression protocol before gonadotro-pin stimulation for *in vitro* fertilization in poor responders. *Fertil Steril* 2005;84:1023-6.
 - Fanchin R, Salomon L, Castelo-Branco A, Olivennes F, Frydman N, Frydman R. Luteal estradiol pre-treatment coordinates follicular growth during controlled ovarian hyperstimulation with GnRH antagonists. *Hum Reprod* 2003;18:2698-703.
 - Van Soom A, Mateusen B, Leroy J, De Kruif A. Assess-ment of mammalian embryo quality: what can we learn from embryo morphology? *Reprod Biomed Online* 2003;7:664-70.
 - Frattarelli JL, Hill MJ, McWilliams GD, Miller KA, Bergh PA, Scott RT Jr. A luteal estradiol protocol for expected poor-

- responders improves embryo number and quality. *Fertil Steril* 2008;89:1118-22.
24. Chang EM, Han JE, Won HJ, Kim YS, Yoon TK, Lee WS. Effect of estrogen priming through luteal phase and stimulation phase in poor responders in *in-vitro* fertilization. *J Assist Reprod Genet* 2012;29:225-30.
 25. Copperman AB. Antagonists in poor-responder patients. *Fertil Steril* 2003;80 Suppl 1:S16-24.
 26. Frankfurter D, Dayal M, Dubey A, Peak D, Gindoff P. Novel follicular-phase gonadotropin-releasing hormone antagonist stimulation protocol for *in vitro* fertilization in the poor responder. *Fertil Steril* 2007;88:1442-5.
 27. Younis JS, Soltsman S, Izhaki I, Radin O, Bar-Ami S, Ben-Ami M. Early and short follicular gonadotropin-releasing hormone antagonist supplementation improves the meiotic status and competence of retrieved oocytes in *in vitro* fertilization-embryo transfer cycles. *Fertil Steril* 2010;94:1350-5.
 28. Blockeel C, Devroey P. Optimisation of the follicular phase in IVF/ICSI. *Facts Views Vis Obgyn* 2012;4:203-12.
 29. Fanchin R, Hourvitz A, Olivennes F, Taieb J, Hazout A, Frydman R. Premature progesterone elevation spares blastulation but not pregnancy rates in *in vitro* fertilization with coculture. *Fertil Steril* 1997;68:648-52.
 30. Fanchin R, Righini C, Olivennes F, Ferreira AL, de Ziegler D, Frydman R. Consequences of premature progesterone elevation on the outcome of *in vitro* fertilization: insights into a controversy. *Fertil Steril* 1997;68:799-805.
 31. Hugues JN. Impact of 'LH activity' supplementation on serum progesterone levels during controlled ovarian stimulation: a systematic review. *Hum Reprod* 2012;27:232-43.
 32. Shulman A, Ghetler Y, Beyth Y, Ben-Nun I. The significance of an early (premature) rise of plasma progesterone in *in vitro* fertilization cycles induced by a "long protocol" of gonadotropin releasing hormone analogue and human menopausal gonadotropins. *J Assist Reprod Genet* 1996;13:207-11.
 33. Lidor AL, Goldenberg M, Cohen SB, Seidman DS, Mashiach S, Rabinovici J. Management of women with polycystic ovary syndrome who experienced premature luteinization during clomiphene citrate treatment. *Fertil Steril* 2000;74:749-52.
 34. Younis JS, Matilsky M, Radin O, Ben-Ami M. Increased progesterone/estradiol ratio in the late follicular phase could be related to low ovarian reserve in *in vitro* fertilization-embryo transfer cycles with a long gonadotropin-releasing hormone agonist. *Fertil Steril* 2001;76:294-9.
 35. Venetis CA, Kolibianakis EM, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: facts and fiction. *Hum Reprod Update* 2008;14:538.
 36. Xu B, Li Z, Zhang H, Jin L, Li Y, Ai J, et al. Serum progesterone level effects on the outcome of *in vitro* fertilization in patients with different ovarian response: an analysis of more than 10,000 cycles. *Fertil Steril* 2012;97:1321-1327. e1-4.
 37. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60 000 cycles. *Hum Reprod Update* 2013;19:433-57.
 38. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, et al. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for *in vitro* fertilization: the Bologna criteria. *Hum Reprod* 2011;26:1616-24.
 39. Jansen R, Mortimer D. Towards reproductive certainty: fertility and genetics beyond 1999: the plenary Proceedings of the 11th World Congress. London: CRC Press; 1999.
 40. Garcia JE, Jones GS, Acosta AA, Wright G Jr. Human menopausal gonadotropin/human chorionic gonadotropin follicular maturation for oocyte aspiration: phase II, 1981. *Fertil Steril* 1983;39:174-9.
 41. Templeton A, Morris JK, Parslow W. Factors that affect outcome of *in-vitro* fertilisation treatment. *Lancet* 1996;348:1402-6.
 42. Ulug U, Ben-Shlomo I, Turan E, Erden HF, Akman MA, Bahceci M. Conception rates following assisted reproduction in poor responder patients: a retrospective study in 300 consecutive cycles. *Reprod Biomed Online* 2003;6:439-43.
 43. Hendriks DJ, te Velde ER, Looman CW, Bancsi LF, Broekmans FJ. Expected poor ovarian response in predicting cumulative pregnancy rates: a powerful tool. *Reprod Biomed Online* 2008;17:727-36.
 44. Gougeon A, Lefèvre B. Evolution of the diameters of the largest healthy and atretic follicles during the human menstrual cycle. *J Reprod Fertil* 1983;69:497-502.
 45. Bosch E, Valencia I, Escudero E, Crespo J, Simón C, Remohí J, et al. Premature luteinization during gonadotropin-releasing hormone antagonist cycles and its relationship with *in vitro* fertilization outcome. *Fertil Steril*

- 2003;80:1444-9.
46. Harada T, Yoshida S, Katagiri C, Takao N, Ikenari T, Toda T, et al. Reduced implantation rate associated with a subtle rise in serum progesterone concentration during the follicular phase of cycles stimulated with a combination of a gonadotrophin-releasing hormone agonist and gonadotrophin. *Hum Reprod* 1995;10:1060-4.
 47. Fanchin R, de Ziegler D, Taieb J, Hazout A, Frydman R. Premature elevation of plasma progesterone alters pregnancy rates of *in vitro* fertilization and embryo transfer. *Fertil Steril* 1993;59:1090-4.
 48. Hofmann GE, Khoury J, Johnson CA, Thie J, Scott RT Jr. Premature luteinization during controlled ovarian hyperstimulation for *in vitro* fertilization-embryo transfer has no impact on pregnancy outcome. *Fertil Steril* 1996;66:980-6.
 49. Fanchin R, Righini C, Olivennes F, de Ziegler D, Selva J, Frydman R. Premature progesterone elevation does not alter oocyte quality in *in vitro* fertilization. *Fertil Steril* 1996;65:1178-83.
 50. Saleh HA, Omran MS, Draz M. Does subtle progesterone rise on the day of HCG affect pregnancy rate in long agonist ICSI cycles? *J Assist Reprod Genet* 2009;26:239-42.
 51. Requena A, Cruz M, Bosch E, Meseguer M, García-Velasco JA. High progesterone levels in women with high ovarian response do not affect clinical outcomes: a retrospective cohort study. *Reprod Biol Endocrinol* 2014;12:69.
 52. Kolibianakis EM, Albano C, Camus M, Tournaye H, Van Steirteghem AC, Devroey P. Prolongation of the follicular phase in *in vitro* fertilization results in a lower ongoing pregnancy rate in cycles stimulated with recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonists. *Fertil Steril* 2004;82:102-7.
 53. Fleming R, Jenkins J. The source and implications of progesterone rise during the follicular phase of assisted reproduction cycles. *Reprod Biomed Online* 2010;21:446-9.
 54. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. *Hum Reprod Update* 2006;12:731-46.
 55. Shufaro Y, Sapir O, Oron G, Ben Haroush A, Garor R, Pinkas H, et al. Progesterone-to-follicle index is better correlated with *in vitro* fertilization cycle outcome than blood progesterone level. *Fertil Steril* 2015;103:669-674. e3.
 56. Oudendijk JF, Yarde F, Eijkemans MJ, Broekmans FJ, Broer SL. The poor responder in IVF: is the prognosis always poor?: a systematic review. *Hum Reprod Update* 2012;18:1-11.