OVULATION INDUCTION

Supplementation with a recombinant human chorionic gonadotropin microdose leads to similar outcomes in ovarian stimulation with recombinant follicle-stimulating hormone using either a gonadotropin-releasing hormone agonist or antagonist for pituitary suppression

Mario Cavagna, M.D., Ph.D.,[a,b,c] *Luiz Guilherme Louzada Maldonado, M.D.,*[a] *Tatiana Carvalho de Souza Bonetti, B.Sc., M.Sc.,*[a,b] *Daniela Paes de Almeida Ferreira Braga, D.V.M., M.Sc.,*[a,b] *Assumpto Iaconelli, Jr., M.D.,*[a] *and Edson Borges, Jr., M.D., Ph.D.*[a,b]

[a] Fertility-Assisted Fertilization Center, [b] *Sapientiae* Institute—Educational and Research Center in Assisted Reproduction, and [c] Perola Byington Hospital, São Paulo, Brazil

Objective: To compare the outcomes of protocols for ovarian stimulation with recombinant hCG microdose, with GnRH agonists and antagonists for pituitary suppression.

Design: Prospective nonrandomized clinical trial.

Setting: A private assisted reproduction center.

Patient(s): We studied 182 patients undergoing intracytoplasmic sperm injection (ICSI) cycles, allocated into two groups: GnRH agonist group, in which patients received a GnRH agonist (n = 73), and a GnRH antagonist group, in which patients were administered a GnRH antagonist for pituitary suppression (n = 109).

Intervention(s): Pituitary suppression with GnRH agonist or GnRH antagonist. Ovarian stimulation carried out with recombinant FSH and supplemented with recombinant hCG microdose.

Main Outcome Measure(s): Total dose of recombinant FSH and recombinant hCG administered; E_2 concentrations and endometrial width on the day of hCG trigger; number of follicles aspirated, oocytes and mature oocytes retrieved; fertilization, pregnancy (PR), implantation, and miscarriage rates.

Result(s): The total dose of recombinant FSH and recombinant hCG administered were similar between groups, as were the E_2 concentrations and endometrial width. The number of follicles aspirated, oocytes, and metaphase II oocytes collected were also comparable. There were no statistically significant differences in fertilization, PR, implantation, and miscarriage rates in the GnRH agonist and GnRH antagonist groups.

Conclusion(s): When using recombinant hCG microdose supplementation for controlled ovarian stimulation (COS), there are no differences in laboratory or clinical outcomes with the use of either GnRH antagonist or agonist for pituitary suppression. (Fertil Steril® 2010;94:167–72. ©2010 by American Society for Reproductive Medicine.)

Key Words: GnRH agonists, GnRH antagonists, recombinant FSH, recombinant hCG, ICSI

Pituitary suppression is a well-established strategy in the protocols of ovarian stimulation for assisted reproductive technologies (ART). During the past 20 years GnRH agonists were used for this purpose, and more recently GnRH antagonists were introduced for the same purpose (1, 2). Although GnRH agonists promote a flare-up effect and require a longer time for pituitary suppression, which is achieved by desensitization of hypophyseal receptors, GnRH antagonists act by direct competition with hypophyseal receptors and promote a rapid blockage of the hypothalamus–hypophyseal axis. The result is a shorter duration of treatment and a friendlier treatment strategy. Comparative studies between GnRH agonists and antagonists for pituitary suppression have suggested that the use of antagonists is associated with a shorter duration of stimulation with FSH (3).

When using antagonists for pituitary suppression, it is not clear whether or not the supplementation with LH is beneficial for ovarian stimulation, but there is evidence that LH activity may be useful, particularly when antagonists are used (4, 5). In addition, in vitro evidence supports a role for LH in improving embryo cleavage and blastocyst formation, and LH supplementation may improve cycle outcome by creating better embryo quality (6).

It is well established that granulosa cells (GC) of larger follicles (>10 mm in diameter) express LH receptors, and thus become sensitive to LH stimulation (7). Even when FSH is withdrawn and ovarian stimulation is performed by LH activity alone, the outcomes are comparable to recombinant FSH administration throughout the stimulation cycle (8). It has been demonstrated that, after recombinant FSH priming, a low dose of hCG is able to stimulate the development of preovulatory follicles with little or no addition of FSH (7, 8).

Based on these findings we designed this investigation to compare the outcomes of protocols of ovarian stimulation using a GnRH agonist and an antagonist for pituitary suppression, with supplementation of LH activity achieved by the administration of a recombinant hCG microdose in the late follicular phase.

MATERIALS AND METHODS

This is a prospective nonrandomized clinical trial comparing the outcomes of ovarian stimulation for assisted reproduction cycles with a recombinant hCG microdose. We studied 182 patients undergoing intracytoplasmic sperm injection (ICSI) cycles, for the first time, in a private clinic, the Fertility-Assisted Fertilization Center, São Paulo, Brazil, between June 2003 and December 2007. To be selected for the present study, patients had to meet the inclusion criteria as follows: age <37 years; body mass index (BMI) ≥ 20 and ≤ 30 kg/m²; basal FSH <10 mIU/mL; eumenorrheic cycles (menstrual cycles consistently every 25–35 days); and negative for HIV and hepatitis B and C markers in serum.

The investigation was submitted to, and approved by, the Ethics Committee of the Women's Health Reference Center, São Paulo, Brazil. Written informed consent to share the outcomes for research purposes was obtained from the couples.

Ovarian Stimulation Protocols

All patients received oral contraceptives (OC; Gynera; Schering, Rio de Janeiro, Brazil), for 2–3 weeks before ovarian stimulation. The patients were divided in two groups according to the use of agonist or antagonist in the protocol of pituitary suppression.

In the agonist group (GnRH-a, n = 73), a half-dose of tryptorelin (Gonapeptyl 3.75; Ferring, São Paulo, Brazil) equivalent to 1.875 mg, was administered IM 18–20 days after the menstrual period induced by the OC. After 12–14 days, ovarian stimulation was commenced with 225 IU of recombinant FSH (Gonal F; Serono, Geneva, Switzerland) daily (day 1 of ovarian stimulation = S1), for 3 days. On S4, the recombinant FSH dose was reduced to 150 IU, until the visualization of at least one follicle ≥ 14 mm, when the recombinant FSH dose was reduced to 75 IU and it was concomitantly administered with the recombinant hCG microdose (7.7 μg, equivalent to 200 IU hCG), which was obtained by the dilution of one ampule of 250 μg of recombinant hCG (Ovidrel; Serono), SC for 2 days. After that, the recombinant hCG microdose was administered alone until the day of ovulation trigger.

In the antagonist group (GnRH-ant, n = 109), ovarian stimulation was performed as follows: 3–5 days after discontinuing OC, ovarian stimulation was commenced with 225 IU of recombinant FSH on a daily basis (day 1 of ovarian stimulation = S1). On S4, the recombinant FSH dose was reduced to 150 IU until the visualization of at least one follicle ≥ 14 mm, at which time, we began the administration of cetrorelix acetate (Cetrotide; Serono) 0.25 mg SC. The day after beginning the antagonist therapy, the recombinant FSH dose was reduced to 75 IU and the concomitant SC administration of the recombinant hCG microdose was initiated and continued for 2 days. After that, the recombinant hCG microdose was exclusively administered until the day of ovulation trigger.

With this approach, both protocols were exactly the same, except for the method of pituitary suppression. In both groups, the ovulation trigger was given by SC injection of 250 μg of recombinant hCG when at least three follicles ≥ 17 mm were observed, and oocyte retrieval was performed 35–36 hours later by transvaginal ultrasound (TVS) guided aspiration. The luteal phase was supplemented with a vaginal administration of 90 mg of P gel (Crinone; Serono).

The recovered oocytes were assessed for their nuclear status, and those in metaphase II were submitted to ICSI following routine procedures (9). Fertilization, indicated by the presence of two clearly distinct pronuclei (PN), was assessed 18 hours after ICSI. Embryo morphological quality was evaluated daily under an inverted microscope (Eclipse TE 300; Nikon, Tokyo, Japan); and one to three high-quality embryos were transferred per patient on the second or third day of development.

The implantation rate was defined as the number of gestational sacs per number of embryos transferred per patient. Clinical pregnancy was defined as the presence of a gestational sac with heart beat visualized by ultrasound 4–6 weeks after embryo transfer. Miscarriage was defined as the spontaneous loss of a pregnancy before 12 weeks' gestation.

End Points

The end points of this study were total dose of recombinant FSH administered; total dose of recombinant hCG administered; E_2 concentrations and endometrial width on day of hCG administration; number of follicles aspirated; number of oocytes collected and number of metaphase II oocytes retrieved; and fertilization, pregnancy (PR), implantation, and miscarriage rates.

Statistical Analysis

The categorical variables (e.g., PRs and miscarriage rates), were analyzed by performing χ^2 or Fisher's test as

appropriate. The Shapiro-Wilk test was used to test the distribution of numerical variables, and Student's *t*-tests were used for the analysis when the numerical variables had a normal distribution, and results were shown as mean and SD. Nonparametric analysis (*Mann*-Whitney's test) was performed when the data did not show a normal distribution, and results showed as median and range. Values of P $><.05$ were considered to be significant.

RESULTS

Despite the nonrandomization, the two groups of patients were well-balanced. The women's ages and BMI were similar (Table 1) and most couples used ejaculated sperm for ICSI (GnRH-a: 94.5% vs. GnRH-ant: 93.5%; $P=.794$) in both groups. The causes of infertility of the two treatment groups are equally distributed, except for the higher incidence of unexplained infertility in the GnRH-a group (Table 2).

In addition, the total dose of recombinant FSH and recombinant hCG administered, the E_2 concentration and the endometrial width measures did not differ among the groups (Table 1).

The number of follicles aspirated, and the number of oocytes and metaphase II oocytes collected were also similar in the groups. The fertilization rates were 68.06% and 68.87% in the groups GnRH-a and GnRH-ant, respectively ($P=.741$). In the GnRH-a group, three women (4.11%) did not receive an embryo transfer because of total fertilization failure, and the same fact occurred in six women (5.5%) of the GnRH-ant group ($P=.671$). The clinical outcomes in terms of PRs per transfer and implantation rates were not statistically different. There were four miscarriages in the GnRH-a group and three miscarriages in the GnRH-ant group (Table 3).

DISCUSSION

In the protocols of ovarian stimulation for ART, the use of FSH is recognized to play an important role in multifollicular development. The role of LH is much more controversial, but it is well known that LH acts on the theca cells by inducing an androgen substrate for aromatization (10). In the midfollicular phase, GCs acquire LH receptors, therefore this hormone can stimulate folliculogenesis independently of FSH activity (7).

Filicori et al. (7, 8, 11) have demonstrated that low-dose urinary hCG may be used to replace the LH activity in the protocols of ovarian stimulation. Our group has demonstrated that a microdose of recombinant hCG may be used for this purpose (12), and other groups of investigators have used the same approach (13, 14). These findings demonstrate that the addition of LH activity in controlled ovarian stimulation can be performed through the administration of highly purified hMG, recombinant LH, and a urinary or recombinant hCG microdose (7, 8, 15–17). It is important to emphasize that in late follicular phase, recombinant hCG microdose is able to complete follicular maturation in the absence of FSH, a fact that was confirmed by the results of this investigation.

Classically, pituitary desensitization for ovarian stimulation is performed through the administration of GnRH analogues, especially agonists, which were first introduced for this purpose in 1984 (1). More recently GnRH antagonists were introduced for the same purpose, and the efficient dose is reported to be 0.25 mg, administered daily (2, 18). Initially, GnRH antagonists were related to lower PRs and implantation rates (19–22), but recent studies did not confirm this finding (23–26), especially when care was taken to avoid prolongation of the follicular phase and initiation of ovarian stimulation with high P levels (27, 28).

Recently, Lainas et al. (29) published a study showing higher PRs in poor responders with the use of GnRH antagonists, compared with use of the flare-up protocol with GnRH agonists. However, the investigation was performed in patients with very poor prognosis, as demonstrated by the low PRs achieved (12.2% vs. 4.4%). In addition, a recent meta-analysis did not show superiority of any analogue in patients with previous poor response to ovarian stimulation (30, 31).

TABLE 1

The age, body mass indices, total dose of recombinant FSH administered, total dose of recombinant hCG administered, E_2 concentrations, and endometrial thickness for patients from groups GnRH agonist (GnRH-a) and GnRh antagonist (GnRH-ant).

Variable	GnRH-a group	GnRH-ant group	*P* value
Age	30.45 ± 3.61	30.48 ± 3.65	.950
Body mass indices	23.8 ± 3.64	23.9 ± 3.61	.779
Total dose of recombinant FSH	1,774.7 ± 468.6	1,766.3 ± 404.6	.900
Total dose of recombinant hCG	614.3 ± 208.0	565.7 ± 209.2	.131
E_2 concentrations	2,722 ± 1,642	2,696 ± 1,805	.927
Endometrial thickness	13.09 ± 2.74	12.56 ± 2.01	.169

Note: Values expressed as average ± SD.

Cavagna. Recombinant hCG microdose for ICSI cycles. Fertil Steril 2010.

The introduction of GnRH antagonists to promote pituitary suppression brought about a new discussion as to whether or not supplementation with LH could be beneficial with this new approach. The GnRH antagonists promote a rapid inhibition of LH release from competitive binding to hypophyseal GnRH receptors, and this profound suppression of LH might have a deleterious effect on the outcome of ovarian stimulation (32). Thus, when using GnRH antagonists to promote pituitary suppression, supplementation with LH in controlled ovarian stimulation could be beneficial to ovarian stimulation outcomes (4, 5). It was demonstrated that supplementation of recombinant FSH regimens with LH activity may be useful in GnRH antagonist cycles, by diminishing FSH requirements (33) and by increasing the oocyte maturation rate (34). In the present investigation we analyzed the parameters that are considered to be the major end points of a cycle of ovarian stimulation for assisted reproduction, in a group of women who used a GnRH agonist and in another group who used a GnRH antagonist for pituitary suppression. In both groups, LH activity was introduced in the late follicular phase, through the administration of recombinant hCG microdose.

In some patients a reduction in E_2 is observed after administration of the antagonist; this reduction is related to the LH decrease that may occur due to the inhibitory action of the antagonist (35). Recent studies have shown that the addition of LH activity to protocols with GnRH antagonists may prevent the decrease in E_2 after the antagonist administration, but does not seem to positively influence the PRs and implantation rates (4, 5, 36). In the present study, a possible bias cannot be excluded as the patients were not randomized but were assigned to each group on the basis of the clinician judgment. However, the parameters evaluated indicated homogeneity between the groups, except for the higher incidence of unexplained infertility in the GnRH agonist group. We cannot find a reasonable explanation for this fact; nevertheless, although this finding is statistically significant it is not necessarily clinically relevant. In both groups in this investigation we supplemented recombinant FSH with LH activity through the administration of recombinant hCG microdose in the late follicular phase. We observed that the concentrations of E_2 on the day of ovulation triggering were not statistically different between the groups. This can be attributed to recombinant hCG supplementation, because several investigations have

TABLE 3

Number of follicles aspirated, oocytes and MII oocytes collected, and clinical outcomes in GnRH-a and GnRH-ant groups.

Parameter	GnRH-a group	GnRH-ant group	*P* value
No. of follicles aspirated	16 (1–54)	17 (2–50)	.831
No. of oocytes collected	11 (1–47)	10 (1–44)	.756
No. of MII oocytes collected	9 (1–40)	9 (1–38)	.499
E_2 concentrations			
No. of transferred embryos	1.68 (1–3)	1.78 (1–3)	.427
Pregnancy rate per transfer	35.71%	33.98%	.814
Implantation rate	26.07%	20.34%	.317
Miscarriage rate	16.00%	8.57%	.377

Note: Numbers of follicles, oocytes and MII oocytes are expressed as the median with the range between parentheses. MII = metaphase II; GnRH-a = GnRH agonist; GnRH-ant = GnRh antagonist.

Cavagna. Recombinant hCG microdose for ICSI cycles. Fertil Steril 2010.

demonstrated that the use of antagonists for pituitary suppression is related to lower concentrations of E_2 when ovarian stimulation is performed with recombinant FSH alone (4–6, 36).

A recent study did not reveal any significant differences with the use of gonadotropins with LH activity (highly purified hMG) or recombinant FSH in controlled ovarian stimulation protocols with GnRH antagonists, with regard to PRs and implantation rates. Nevertheless, more oocytes were obtained from patients receiving recombinant FSH than hMG and E_2 was higher at the end of stimulation with hMG, whereas P was higher in patients stimulated with recombinant FSH (6). It is possible that there is an "LH concentration window," below which the ovarian stimulation would not be optimal, with inadequate production of E_2, and above which follicular development would be impaired (37). In our study we did not observe any significant differences in the amounts of recombinant FSH required, the number of follicles aspirated, or the number of metaphase II oocytes collected between groups. Fertilization, PRs, and implantation rates did not show statistically significant differences. These data suggest that the supplementation of recombinant FSH with LH activity obtained through the administration of recombinant hCG microdose leads to similar outcomes in ovarian stimulation with recombinant FSH using either a GnRH agonist or antagonist for pituitary suppression.

Nevertheless, it has to be noted a lower implantation rate in the antagonist group (20.34% vs. 26.07%), although statistical significance was not achieved. This finding is in accord with other investigations that suggest that the clinical efficacy of GnRH antagonists may be slightly reduced, and discretely lower implantation rates should be expected (34, 38). On the other hand, we observed a slightly augmented miscarriage rate in the agonist group (16.0% vs. 8.57%), without statistical significance; this fact led to ongoing PRs being very similar between the groups (31% in the antagonist group vs. 30% in the agonist group).

With regard to the exact role of LH in ovarian stimulation, more evidence is needed to allow for definitive conclusions. However, we can stress that LH is able to act synergistically with FSH to complete follicular development, and that recombinant hCG microdose is an adequate source of LH activity. In stimulated cycles, low dose hCG is able to promote follicular development in the late follicular phase, even with withdrawal of FSH administration, as demonstrated by several investigations (7, 17).

With regard to pituitary suppression, it is now clear that the use of GnRH antagonists is a simple and patient-friendly option, with shorter treatment duration and reduced costs, as registered by Fauser and Devroey (38). The same investigators report that many European IVF centers have shifted their clinical protocols almost entirely toward the use of GnRH antagonists, without a noticeable decrease in PRs (38). Maybe the ideal protocol with GnRH antagonist co-treatment has not yet been identified.

In a recently published meta-analysis (4) we concluded that the association of recombinant LH with recombinant FSH may prevent the E_2 concentration decrease after antagonist administration, and may significantly increase the number of mature retrieved oocytes. Therefore, together with our present findings, these evidences suggest that the use of LH in controlled ovarian stimulation protocols, commencing at the time of antagonist administration is recommended. If its positive role regarding PRs and implantation rates remains controversial, it seems clear that LH supplementation does not have any negative effects on E_2 synthesis, providing a more physiological hormonal milieu. Our data also confirm that the recombinant hCG microdose is an efficient source of LH activity, and this strategy can be used to reduce the FSH amounts required in controlled ovarian stimulation protocols, independently of the type of GnRH analogue used.

REFERENCES

1. Porter RN, Smith W, Craft IL, Abdulwahid NA, Jacobs HS. Induction of ovulation for in-vitro fertilisation using buserelin and gonadotropins. Lancet 1984;2:1284–5.
2. Albano C, Smitz J, Camus M, Riethmuller-Winzen H, Van Steirteghem A, Devroey P. Comparison of different doses of gonadotropin-releasing hormone antagonist Cetrorelix during controlled ovarian hyperstimulation. Fertil Steril 1997;67:917–22.
3. Tarlatzis BC, Kolibianakis EM. GnRH agonists vs antagonists. Best Pract Res Clin Obstet Gynaecol 2007;21:57–65.
4. Baruffi RL, Mauri AL, Petersen CG, Felipe V, Martins AM, Cornicelli J, et al. Recombinant LH supplementation to recombinant FSH during induced ovarian stimulation in the GnRH-antagonist protocol: a meta-analysis. Reprod Biomed Online 2007;14:14–25.
5. Levi-Setti PE, Cavagna M, Bulletti C. Recombinant gonadotrophins associated with GnRH antagonist (cetrorelix) in ovarian stimulation for ICSI: comparison of r-FSH alone and in combination with r-LH. Eur J Obstet Gynecol Reprod Biol 2006;126:212–6.
6. Bosch E, Vidal C, Labarta E, Simon C, Remohi J, Pellicer A. Highly purified hMG versus recombinant FSH in ovarian hyperstimulation with GnRH antagonists—a randomized study. Hum Reprod 2008;23:2346–51.
7. Filicori M, Cognigni GE, Gamberini E, Parmegiani L, Troilo E, Roset B. Efficacy of low-dose human chorionic gonadotropin alone to complete controlled ovarian stimulation. Fertil Steril 2005;84:394–401.
8. Filicori M, Cognigni GE, Tabarelli C, Pocognoli P, Taraborrelli S, Spettoli D, et al. Stimulation and growth of antral ovarian follicles by selective LH activity administration in women. J Clin Endocrinol Metab 2002;87:1156–61.
9. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17–8.
10. Sullivan MW, Stewart-Akers A, Krasnow JS, Berga SL, Zeleznik AJ. Ovarian responses in women to recombinant follicle-stimulating hormone and luteinizing hormone (LH): a role for LH in the final stages of follicular maturation. J Clin Endocrinol Metab 1999;84:228–32.
11. Filicori M, Cognigni GE, Taraborrelli S, Spettoli D, Ciampaglia W, de Fatis CT, et al. Luteinizing hormone activity supplementation enhances follicle-stimulating hormone efficacy and improves ovulation induction outcome. J Clin Endocrinol Metab 1999;84:2659–63.
12. Borges E Jr, Rossi LM, Busato WC, Bibancos M, Maldonado LGL, Iaconelli A Jr. Better outcomes using microdose of recombinant chorionic gonadotropin (r-hCG microdose) to support ovarian folliculogenesis in good prognosis patients. Fertil Steril 2005;84(Suppl 1):S107.
13. Bukulmez O, Rehman KS, Langley M, Carr BR, Nackley AC, Doody KM, et al. Precycle administration of GnRH antagonist and microdose HCG decreases clinical pregnancy rates without affecting embryo quality and blastulation. Reprod Biomed Online 2006;13:465–75.

14. Berkkanoglu M, Isikoglu M, Aydin D, Ozgur K. Clinical effects of ovulation induction with recombinant follicle-stimulating hormone supplemented with recombinant luteinizing hormone or low-dose recombinant human chorionic gonadotropin in the midfollicular phase in microdose cycles in poor responders. Fertil Steril 2007;88:665–9.

15. The European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: a dose-finding study. J Clin Endocrinol Metab 1998;83:1507–14.

16. Serafini P, Yadid I, Motta EL, Alegretti JR, Fioravanti J, Coslovsky M. Ovarian stimulation with daily late follicular phase administration of low-dose human chorionic gonadotropin for in vitro fertilization: a prospective, randomized trial. Fertil Steril 2006;86:830–8.

17. Filicori M, Cognigni GE, Samara A, Melappioni S, Perri T, Cantelli B, et al. The use of LH activity to drive folliculogenesis: exploring uncharted territories in ovulation induction. Hum Reprod Update 2002;8:543–57.

18. Lee TH, Lin YH, Seow KM, Hwang JL, Tzeng CR, Yang YS. Effectiveness of cetrorelix for the prevention of premature luteinizing hormone surge during controlled ovarian stimulation using letrozole and gonadotropins: a randomized trial. Fertil Steril 2008;90:113–20.

19. Hernandez ER. Embryo implantation and GnRH antagonists: embryo implantation: the Rubicon for GnRH antagonists. Hum Reprod 2000;15:1211–6.

20. Ludwig M, Katalinic A, Diedrich K. Use of GnRH antagonists in ovarian stimulation for assisted reproductive technologies compared to the long protocol. Meta-analysis. Arch Gynecol Obstet 2001;265:175–82.

21. Al-Inany H, Aboulghar M. GnRH antagonist in assisted reproduction: a Cochrane review. Hum Reprod 2002;17:874–85.

22. Lindheim SR, Morales AJ. GnRH antagonists followed by a decline in serum estradiol results in adverse outcomes in donor oocyte cycles. Hum Reprod 2003;18:2048–51.

23. Bodri D, Vernaeve V, Guillen JJ, Vidal R, Figueras F, Coll O. Comparison between a GnRH antagonist and a GnRH agonist flare-up protocol in oocyte donors: a randomized clinical trial. Hum Reprod 2006;21:2246–51.

24. Kolibianakis EM, Collins J, Tarlatzis BC, Devroey P, Diedrich K, Griesinger G. Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. Hum Reprod Update 2006;12:651–71.

25. Moraloglu O, Kilic S, Karayalcin R, Yuksel B, Tasdemir N, Isik A, et al. Comparison of GnRH agonists and antagonists in normoresponder IVF/ICSI in Turkish female patients. Adv Ther 2008;25:266–73.

26. Wei AY, Mijal KA, Christianson MS, Schouweiler CM, Lindheim SR. Comparison of GnRH antagonists and flareup GnRH agonists in donor oocyte cycles. J Reprod Med 2008;53:147–50.

27. Kolibianakis EM, Zikopoulos K, Smitz J, Camus M, Tournaye H, Van Steirteghem AC, et al. Elevated progesterone at initiation of stimulation is associated with a lower ongoing pregnancy rate after IVF using GnRH antagonists. Hum Reprod 2004;19:1525–9.

28. Kolibianakis EM, Bourgain C, Papanikolaou EG, Camus M, Tournaye H, Van Steirteghem AC, et al. Prolongation of follicular phase by delaying hCG administration results in a higher incidence of endometrial advancement on the day of oocyte retrieval in GnRH antagonist cycles. Hum Reprod 2005;20:2453–6.

29. Lainas TG, Sfontouris IA, Papanikolaou EG, Zorzovilis JZ, Petsas GK, Lainas GT, et al. Flexible GnRH antagonist versus flare-up GnRH agonist protocol in poor responders treated by IVF: a randomized controlled trial. Hum Reprod 2008;23:1355–8.

30. Franco JG Jr, Baruffi RL, Mauri AL, Petersen CG, Felipe V, Cornicelli J, et al. GnRH agonist versus GnRH antagonist in poor ovarian responders: a meta-analysis. Reprod Biomed Online 2006;13:618–27.

31. Sunkara SK, Tuthill J, Khairy M, El-Toukhy T, Coomarasamy A, Khalaf Y, et al. Pituitary suppression regimens in poor responders undergoing IVF treatment: a systematic review and meta-analysis. Reprod Biomed Online 2007;15:539–46.

32. Fleming R, Lloyd F, Herbert M, Fenwick J, Griffiths T, Murdoch A. Effects of profound suppression of luteinizing hormone during ovarian stimulation on follicular activity, oocyte and embryo function in cycles stimulated with purified follicle stimulating hormone. Hum Reprod 1998;13:1788–92.

33. Levi-Setti PE, Cavagna M, Baggiani A, Zannoni E, Colombo GV, Liprandi V. FSH and LH together in ovarian stimulation. Eur J Obstet Gynecol Reprod Biol 2004;115(Suppl. 1):S34–9.

34. Koichi K, Yukiko N, Shima K, Sachiko S. Efficacy of low-dose human chorionic gonadotropin (hCG) in a GnRH antagonist protocol. J Assist Reprod Genet 2006;23:223–8.

35. Olivennes F. GnRH antagonists: do they open new pathways to safer treatment in assisted reproductive techniques? Reprod Biomed Online 2002;5(Suppl 1(3)):20–5.

36. Van Horne AK, Bates GW Jr, Robinson RD, Arthur NJ, Propst AM. Recombinant follicle-stimulating hormone (rFSH) supplemented with low-dose human chorionic gonadotropin compared with rFSH alone for ovarian stimulation for in vitro fertilization. Fertil Steril 2007;88:1010–3.

37. Balasch J, Fabregues F. LH in the follicular phase: neither too high nor too low. Reprod Biomed Online 2006;12:406–15.

38. Fauser BC, Devroey P. Why is the clinical acceptance of gonadotropin-releasing hormone antagonist cotreatment during ovarian hyperstimulation for in vitro fertilization so slow? Fertil Steril 2005;83:1607–11.