HALF-DOSE DEPOT TRIPTORELIN COMPARABLE TO REDUCED DAILY BUSERELIN: A RANDOMIZED CLINICAL TRIAL

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Abstract- Pituitary suppression by depot GnRH agonist may be excessive for ovarian stimulation. This study compares the efficacy of a single half-dose depot triptorelin and reduced-dose daily buserelin in a long protocol ICSI/ET. METHODS: A total of 182 patients were randomized into two groups using sealed envelopes. Pituitary desensitization was obtained in group 1 (91 patients) with half-dose (1.87 mg) depot triptorelin in the mid-luteal phase of their menstrual cycle, and in group 2 (91 patients) with standard daily dose (0.5 mg) buserelin, which was then reduced to 0.25 mg at the start of human menopausal gonadotropin (HMG) stimulation. RESULTS: No significant differences were found among those who received HCG in terms of clinical pregnancy rate (34.4% in both groups), implantation rate (14.8% in group 1 versus 11.1% in group 2), fertilization rate (93.3 versus 95.6%), poor response rate (11.1 versus 6.7%), and miscarriage rate (11.1 versus 7.8%). No significant differences were seen in number of HMG ampoules used, follicles at HCG administration, and oocytes retrieved. The number of days of stimulation was significantly reduced in group 2 (11.2 +/- 1.8 in group 1 versus 10.6 +/- 1.9, p = 0.030). CONCLUSION: A half-dose of depot triptorelin can be successfully used in ovarian stimulation instead of reduced-dose daily buserelin, with more patient comfort and reduced stress and cost of injections.

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INTRODUCTION

Gonadotropin releasing hormone agonists (GnRHa) have been used in controlled ovarian stimulation (COS) cycles for more than ten years.

However, significant doubts remain about which type of GnRHa administration to be used.

A single depot long-acting GnRHa instead of a daily low dose preparation in in-vitro fertilization (IVF) cycles increases the number of gonadotropins ampoules and the duration of the COS cycle without improving pregnancy rates or other clinical outcomes (1). Although the specific role of GnRHa-induced ovarian quiescence on subsequent ovarian responsiveness to gonadotropins is yet to be determined and a direct ovarian action of GnRHa on steroidogenesis, folliculogenesis, and embryo quality is still controversial, it is postulated that the hypophyseal desensitization induced by GnRHa is greatly dependent on the administration dose. These putative deleterious effects of GnRH agonists have led some authors to recommend a reduction of both

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dose and duration of GnRH agonist administration (2), yielding ease and economy for patients (3).

Research studies on pituitary desensitization in COS cycle, comparing half-dose depot triptorelin (1.87 mg) against its full-dose (3.75 mg) administration, date back to 1992 (4-6). They have reported rather similar outcomes or some differences in immediate COS cycle statistics between the two depot-doses, although none of them observed significant difference in the pregnancy rates between the two study groups. Comparison of half-dose depot GnRHa to its standard daily injections in long GnRHa protocol has also existed since 1995. Two similar studies compared a single half-dose (1.88 mg) of leuprolide acetate with daily administration of its short-acting form at the standard dose of 0.5 mg/d (7,8). They neither found statistical difference in the cycle outcomes between the groups. Reducing the daily doses of short acting GnRHa has been advocated to have equivalent results to standard doses (9,10). To our knowledge, however, the reduced daily doses have not been evaluated against half dose depot forms in long GnRHa protocols. Respecting that different GnRHa formulations may behave differently on pituitary desensitization and cycle outcomes, we originally compared a half-dose depot triptorelin with reduced daily doses of short-acting buserelin in a long protocol for intracytoplasmic sperm injection and embryo transfer (ICSI/ET) cycles.

MATERIALS AND METHODS

Patients

After institutional review board approval and written informed consent, 182 patients undergoing ICSI/ET were enrolled in this randomized clinical trial between June 2006 and January 2007. Patients were required to be 35 years old or younger with both ovaries present and have serum FSH less than 10 IU/L on day three of the previous menstrual cycle. They needed to have no more than two previous IVF/ICSI attempts, no planned percutaneous epididymal sperm aspiration (PESA) or testicular sperm extraction (TESE), no known history or risk of severe hyperstimulation, no evidence of hydrosalpinx, no major systemic disease, no uterine abnormality, and no previous ovarian surgery.

Stimulation protocol and ICSI procedure

All patients came to the hospital on the second or third day of menstruation. On the third day, a blood sample was drawn to determine serum concentrations of LH, FSH, estradiol (E_2). The COS cycle initiated for every patient with pituitary desensitization by a course of monophasic low dose oral contraceptive pills (ethinyl estradiol 30 μg + levonorgestrel 150 μg) from the day 3 to 21 of the menstruation period (11,12).

Pituitary desensitization was enhanced by GnRHa medicaments in the mid-luteal phase, on the 21st day. On this day, the patients were randomly assigned into two study groups of 91 patients. The patients in the Triptorelin Group received half-dose (1.87 mg) of triptorelin depot (Diphérélin 3.75, BEAUFOUR IPSEN, Paris, France) in a single intramuscular injection. On the same day, the patients in the Buserelin Group received a standard dose of 0.5 mg buserelin acetate (Suprefact® 1 mg/ml, Aventis Pharma Deutschland Gmbh, Frankfurt, Germany), injection subcutaneously. This dose was repeated daily until the day of human menopausal gonadotropin (HMG). From this day, the daily doses reduced to 0.25 mg and continued until the day before human chorionic gonadotropin (HCG) administration. We asked all the patients to come to the hospital to receive daily injections from their 21st day of menstruation until the day of HCG administration. On the third day of menstruation, ovarian stimulation with HMG (Menogon®, 75 IU FSH / 75 IU LH, FERRING Gmbh, Kiel, Germany) was started at a usual dose of 2-3 ampoules daily. We adjusted the initial and ongoing dosage by the patient's age, body mass index, clinical state, current follicular growth response, and history of poor response. We used transvaginal ultrasound (TVS) to track follicular growth and ovarian hyperstimulation syndrome (OHSS), starting at day 4 of gonadotropin stimulation, and if necessary, two other TVS examinations every 48 hours. An experienced faculty professor performed TVS examinations using a Siemens SONOLINE G20™ ultrasound system with Endovaginal (EV9-4) Transducer and user-selectable MultiHertz multiple-frequency imaging. The mean maximal follicular diameter was calculated from four measurements of the leading follicles in two perpendicular directions. Follicles with 16 mm diameter or more were considered

mature. On day 6, if all follicles had 14 mm diameter or less, we tried doubling the HMG dose for the next 48 hours. Eventually, on day 8, if less than three follicles were seen the cycle was cancelled. Whenever two or more follicles grew to a diameter of 16-20 mm, the patient received an intramuscular injection of 10,000 IU HCG (Profasi, 10,000 IU; Serono Benelux, Den Haag, The Netherlands). Transvaginal oocyte retrieval under TVS guidance was performed 36 hours after HCG administration.

Fresh spermatozoa were prepared early in the morning of oocyte retrieval by the double-wash swim-up method (13). The ICSI procedure was carried out 40-42 hours after HCG administration, as described elsewhere (14). Pronucleated oocytes were assessed and embryo grading was performed according to published criteria (14). When the embryos were at the 4-8 cells stage (around 72 hours after HCG), a maximum of five embryos per woman were then replaced, guided by TVS, using a soft catheter (15,16).

For luteal phase support, all the patients received a daily dose of 400 mg vaginal progesterone pessaries (Cyclogest 200 mg, Alpharma Limited, Barnstaple, England), added by a daily dose of 25 mg intramuscular progesterone in oil (Gestone; Paines & Byrne, Surrey, UK) starting from the day of oocyte retrieval. In the case of a clinical pregnancy, vaginal progesterone was continued until the 11^{th} week after ET, and intramuscular progesterone until documentation of fetal heart beat by TVS. All patients received a daily dose of 100 mg aspirin from the day of ET until the beta-HCG check. Adjuvant prednisolone was prescribed 20 mg/day orally in four divided doses since the day of ET for 5 days. Every patient received just one cycle and was followed up until 12^{th} week after ET.

Assignment and blinding

Allocations were randomly assigned using the Random Allocation Software (17) in 9 permutated blocks of 20 patients. Opaque, sealed envelopes were serially numbered from 1 to 180, containing the name of one of the two medications (Triptorelin or Buserelin) according to the randomization list. Envelopes, not allowed to be opened in advance, were opened sequentially to assign the eligible patients to one of the two study groups in their mid-luteal phase visit.

A single nurse, blinded to the patients, was requested to open the envelopes and prepare medications accordingly. Injections were made by different assistants blinded to the study. Patients were kept blinded to the medications.

Definitions

Poor responded cycles included cancelled cycles due to evolution of less than three mature follicles, in addition to cycles with three or less oocytes retrieved after HCG administration.

The poor response rate was defined as the number of poorly responded patients per those received HCG. Intervention was regarded as ineffective when the cycle was cancelled for any reason, the response to COS cycle was poor, or fertilization was failed. The rate of ineffective intervention was therefore calculated per COS cycle. A clinical pregnancy was identified by the presence of one or more gestational sacs or fetal heartbeat on TVS, performed at least 4 weeks after ET, or pathologic confirmation of trophoblast tissue, in the event of a spontaneous abortion or ectopic pregnancy. Implantation rate was defined as the proportion of the sum of gestational sacs to the sum of replaced embryos among each group. Beta-HCG tests were performed on day 16 after ET, using a commercial ELISA kit. Those patients with only serologic rise of beta-HCG tests, without further evidence of clinical pregnancy, were not considered pregnant but merely reported as biochemical pregnancies.

Sample Size and Statistical Analysis

The primary endpoint was the number of oocytes retrieved from patients who received HCG. According to our pilot study, a sample size of 90 patients in each group would have 90% power and a significance level of 0.05 to detect a difference in the mean number of oocytes of 1.21 (SD 3.52). The SPSS 13.0 software was used to analyze data of all randomized patients who received COS.

The comparisons of means for continuous variables were performed using independent–Samples t-test (or Mann–Whitney U Test when appropriate). Comparisons of dichotomous variables between groups were analyzed by χ^2 Test for (Fisher's Exact Test when appropriate).

Table 1. Patient's characteristics undergoing COS cycle with half-dose triptorelin (1.87 mg im) and standard daily buserelin (0.5 mg sc)

	Study groups		*P*-value
	Triptorelin Group	Buserelin Group	
Number of patients	91	91	
Age (y)*	28.47 (3.61)	27.41 (4.09)	0.064**
Type of infertility per COS cycle***			0.823****
Primary	80/91 (87.9)	79/91 (86.8)	
Secondary	11/91 (12.1)	12/91 (13.2)	
Duration of infertility (y)*	6.92 (3.25)	6.99 (3.13)	0.889

* Data are presented as mean (SD)

** Using Independent Samples T-Test

*** Data are presented as n/N (%); n = number of patients with the quality; N = total number of the patients with the mentioned common feature

**** Using Chi square Test

COS = controlled ovarian stimulation

RESULTS

Between January and December 2006, we enrolled 182 patients at the assisted reproductive technologies (ART) department of a famous referral university-hospital in Tehran, Iran. Ninety one patients were randomly allocated to the Triptorelin Group and 91 patients to the Buserelin Group.

The patients' age as well as their type and duration of infertility were comparable between two groups (Table 1). Two patients withdrew from the protocol after administration of GnRHa and before starting gonadotropin stimulation: one in the Triptorelin Group for personal reasons and one in the Buserelin Group for non-compliance to the study protocol.

Therefore, gonadotropin stimulation was instituted on the remaining 180 patients (90 in each group). No cycles were cancelled because every cycle produced three or more mature follicles. Therefore, all the 180 patients received HCG. Three patients in the Buserelin Group and four in the Triptorelin Group did not produce oocytes. These seven patients, therefore, were excluded from oocyte retrieval. Added to the three patients in the Buserelin Group and six in the Triptorelin Group who produced 3 oocytes or less on retrieval, the poor responders constituted 6 patients in the Buserelin Group and ten in the Triptorelin Group. Interestingly, one patient in the Buserelin Group and three patients in the Triptorelin Group, who finally became clinically pregnant, were among the

Table 2. Results of ovarian stimulation with half-dose triptorelin (1.87 mg im) and standard daily buserelin (0.5 mg sc)

	Study groups		*P*-value
	Triptorelin Group	Buserelin Group	
No. of patients receiving COS cycle	91	91	
No. of patients receiving HCG	90	90	
No. of days of gonadotropin stimulation*	11.19 (1.81)	10.58 (1.94)	0.030**
No. of HMG ampoules	35.66 (12.00)	32.94 (11.51)	0.124**
No. of follicles at HCG administration	10.27 (4.37)	11.12 (4.16)	0.180**
No. of patients undergoing oocyte retrieval	86	87	
No. of retrieved oocytes per HCG administration	7.64 (3.79)	7.70 (3.56)	0.919**
Quality of oocytes per oocyte retrieval***			0.968****
Mature	67/86 (77.9)	68/87 (78.2)	
Immature	19/86 (22.1)	19/87 (21.8)	
No. of patients undergoing ET	84	86	
Quality of embryos per ET***			0.966****
grade 1	78/84 (92.9)	80/86 (93.0)	
grade 2	6/84 (7.1)	6/86 (7.0)	

* Data are presented as mean (SD) ** Using Independent Samples T-Test *** Data are presented as n/N (%); n = number of patients with the quality; N = total number of the patients with the mentioned common feature **** Using Chi square Test

Table 3. Comparison of clinical outcomes with half-dose triptorelin (1.87 mg im) and standard daily buserelin (0.5 mg sc)

	Study groups		*P*-value
	Triptorelin Group	**Buserelin Group**	
No. of patients receiving COS cycle	91	91	
No. of patients receiving HCG	90	90	
Poor response rate per HCG administration*	10/90 (11.1)	6/90 (6.7)	0.295**
Oocyte fertilization rate			
per HCG administration	84/90 (93.3)	86/90 (95.6)	0.515**
per oocyte retrieval	84/86 (97.7)	86/87 (98.9)	0.555***
Ineffective intervention rate per COS cycle	12/91 (13.2)	8/91 (8.8)	0.343**
Clinical pregnancy rate			
per HCG administration	31/90 (34.4)	31/90 (34.4)	1.000**
per oocyte retrieval	31/86 (36.0)	31/87 (35.6)	0.955**
per ET	31/84 (36.9)	31/86 (36.0)	0.907**
Implantation rate**	45/304 (14.80)	36/325 (11.08)	0.163**
Biochemical pregnancy rate			
per HCG administration	37/90 (41.1)	33/90 (36.7)	0.541**
per oocyte retrieval	37/86 (43.0)	33/87 (37.9)	0.495**
per ET	37/84 (44.0)	33/86 (38.4)	0.452**
Multiple pregnancy rate			
per HCG administration	5/90 (5.6)	10/90 (11.1)	0.178**
per oocyte retrieval	5/86 (5.8)	10/87 (11.5)	0.184**
per ET	5/84 (6.0)	10/86 (11.6)	0.192**
Miscarriage rate			
per HCG administration	10/90 (11.1)	7/90 (7.8)	0.445**
per oocyte retrieval	10/86 (11.6)	7/87 (8.0)	0.429**
per ET	10/84 (11.9)	7/86 (8.1)	0.413**
Ectopic pregnancy rate			
per HCG administration	1/90 (1.1)	2/90 (2.2)	0.562***
per oocyte retrieval	1/86 (1.2)	2/87 (2.3)	0.568***
per ET	1/84 (1.2)	2/86 (2.3)	0.575***

* Data are presented as n/N (%); n = number of patients with the quality; N = total number of the patients with the mentioned common feature
** Using Chi square Test *** Using Mann-Whitney Test **** Expressed as sum of gestational sacs / sum of transferred embryos
COS = controlled ovarian stimulation; HCG = human chorionic gonadotropin; ET = embryo transfer

poor responders. ICSI was done for the 173 patients who underwent oocyte retrieval. However, the oocytes of one patient in the Buserelin Group and two patients in the Triptorelin Group did not fertilize. As the result, 170 patients completed the study up to embryo transfer.

From 37 biochemical pregnancies in the Triptorelin Group, 31 became clinically pregnant, 1 developed ectopic pregnancy, and 5 miscarried. Among those 31 clinically pregnancies, again 5 patients had a miscarriage, comprising a total of 10 miscarriages in this group. From 33 biochemical pregnancies in the Buserelin Group, 31 became clinically pregnant and 2 developed ectopic pregnancy. All the 7 miscarriages in this group were among those 31 clinically pregnancies.

There were no significant differences in the results of ovarian stimulation (Table 2) or the clinical outcomes (Table 3) between the groups, except longer duration of ovarian stimulation by HMG in the Triptorelin Group with the mean difference of 0.611 days (CI 95% 0.060 – 1.163). No instances of ovarian hyperstimulation syndrome occurred in the study patients.

DISCUSSION

For better appraisal of our findings, it is sound to analyze different aspects of the protocol individually. Although the most reasonable primary outcome should be live birth rate or take home baby rate, since our center is referral, we may lose following up of many of our patients in a long period. Therefore, we submitted the primary outcome to the number of oocytes retrieved from patients who received HCG. Among the pituitary

desensitization protocols, the long protocol is the one that presents the best clinical pregnancy rates per cycle initiated, according to Daya's meta-analysis (18). However, there is insufficient evidence to show advantage of either type of GnRHa administration (daily doses or a single depot dose) to another in the long protocol for pituitary desensitization. The use of depot GnRHa requires more gonadotropin ampoules needed for ovarian stimulation and a significantly longer duration of ovarian stimulation than using daily GnRHa (1). GnRH agonists also cause extra-pituitary side effects, including the direct inhibition of ovarian steroidogenesis (19). GnRH agonists affect in the differentiation of granulosa cells in vitro (20), as well as on GnRH receptor messenger ribonucleic acids in these cells (21). In whatever way, depot formulations seem to excel over short-acting GnRHa to some extent, as Tsai NM et al. has indicated that a higher incidence of DNA fragmentation and apoptotic bodies in granulosa cells is seen with short-acting buserelin than with leuprolide depot treatment (22).

Optimal doses of GnRHa for COS cycles are those that prevent a premature endogenous LH surge before oocyte retrieval, but allow pituitary LH secretion to be restored immediately after oocyte retrieval so that steroid hormones, necessary to support the luteal phase, may be stimulated. GnRHa doses used in IVF seem to be immoderate as they are derived from treatment schedules used in disseminated prostate cancer, which aim at complete gonadal suppression under all circumstances (23). The fear that these doses may adversely affect oocyte quality and endometrial receptivity is reflected through the recent trend for a reduction in both dose and duration of GnRHa administration. Although some studies have reported a slight improvement in ovarian response with reduced doses of GnRHa, further investigations are needed to evaluate the effects of the GnRHa reduction on pregnancy rate (2).

In contrast to earlier studies in favor of recombinant FSH (rFSH) over HMG, recently, higher clinical pregnancy rates have been associated with urinary HMG compared to rFSH, but there were no significant differences in ongoing pregnancy rates or live births per women treated. Therefore, considering additional factors including safety, cost, and drug availability, we chose the mentioned HMG protocol (24).

Monitoring of COS cycles merely by TVS has been suggested by Murad in 1998 (25). As measurements of plasma immunoreactive LH cannot reflect LH bioactivity, it is not adequate to measure hypophyseal desensitization (2). Even in low-dose GnRHa long protocols it is shown that elevated LH either does not occur (7,26) or occurs in a small subset of women at the time of HCG administration and this event does not appear to alter cycle fecundity nor induce premature luteinization (27,28). We monitored for OHSS only with clinical presentations and TVS, though we did not find any occurrence. A systematic review is being investigated by Kwan et al (29) to quantify the effect of monitoring COS cycle in IVF/ICSI with ultrasound only versus ultrasound plus serum estradiol concentration.

Adjuvant pretreatment with oral contraceptives (OC) in GnRHa protocols may suppress the persistent function of the corpus luteum from a previous cycle (30), abolish functional ovarian cyst formation, shorten the time required to achieve pituitary suppression and decrease gonadotropin requirements, without having a deleterious effect on pregnancy rates (11). Furthermore, pretreatment with OC could cut costs by improving the ovarian response, help to synchronize follicular development and facilitate cycle scheduling (12). A systematic review is also being researched by Haojie He on this issue (31). We used prednisolone as an adjuvant treatment, not only to treat women with history of poor response, but also to reduce the occurrence of a poor response in the first cycle of ovarian stimulation (32). We prescribed aspirin, despite the controversies regarding its beneficial role on implantation in IVF/ICSI cycles. We found no more miscarriage rate than reported by other studies. A systematic review is being carried out on this issue (33). As the optimal route of progesterone administration has not yet been established, and there is evidence of benefit of the intramuscular over the vaginal route for the outcomes of ongoing pregnancy and live birth (34) we employed both the routes for feasibility as mentioned. Our findings are

in an overall agreement with similar studies evaluating half-dose depot GnRHa or reduced daily administration of short-acting forms (4,5,7,35-37). Differences in the study populations and protocols may contribute to some variations in the surrogate results between different studies. Underlying diseases and rate of egg dysfunction also differ among IVF, ICSI, or donor egg patients. Some of the outcomes have different challenging definitions among the studies. For example, or 'poor response' still lacks a universally accepted definition (38), as does 'implantation rate'. In this study, we observed that half-dose depot triptorelin did not increase the required number of HMG ampoules compared to daily standard doses of short acting buserelin; although full-dose depot preparations would do so. Nevertheless, the mean duration of gonadotropin stimulation is still longer with half-dose depot triptorelin, akin to full-dose regimen, contrasted to daily short acting buserelin. However, this difference was statistically significant but clinically trivial. Contrarily, the insignificant difference of multiple pregnancy rates between the groups seems to have some clinical importance. Including other minor differences between the groups, one can think of replicating this study with higher power to resolve such discrepancies, and also with improved protocols and to assess the optimal dose of GnRHa required for pituitary desensitization.

We conclude that half-dose depot triptorelin demonstrates comparable clinical outcomes to reduced daily doses of short-acting buserelin, and has advantage of better compliance and convenience for patients with less stress of injections and risk of infection, and reducing costs in long protocols.

REFERENCES

1. Albuquerque LE, Saconato H, Maciel MC. Depot versus daily administration of gonadotrophin releasing hormone agonist protocols for pituitary desensitization in assisted reproduction cycles. Cochrane Database Syst Rev. 2005; CD002808.
2. Hugues J, Cedrin-Durnerin I. Revisiting gonadotrophin-releasing hormone agonist protocols and management of poor ovarian responses to gonadotrophins. Hum Reprod Update. 1998; 4:83-101.
3. Dal Prato L., Borini A, Coticchio G, et al. Half-dose depot triptorelin in pituitary suppression for multiple ovarian stimulation in assisted reproduction technology: a randomized study. Hum Reprod. 2004; 19:2200-2205.
4. Balasch J, Gomez F, Casamitjana R, et al. Pituitary-ovarian suppression by the standard and half-doses of D-Trp-6-luteinizing hormone-releasing hormone depot. Hum Reprod. 1992; 7:1230-1234.
5. Yim SF, Lok IH, Cheung LP, et al. Dose-finding study for the use of long-acting gonadotrophin-releasing hormone analogues prior to ovarian stimulation for IVF. Hum Reprod. 2001; 16:492-494.
6. Dal Prato L., Borini A, Coticchio G, et al. Half-dose depot triptorelin in pituitary suppression for multiple ovarian stimulation in assisted reproduction technology: a randomized study. Hum Reprod. 2004; 19:2200-2205.
7. Tsai HD, Chen CM, Lo HY, Chang CC. Subcutaneous low dose leuprolide acetate depot versus leuprolide acetate for women undergoing ovarian stimulation for in-vitro fertilization. Hum Reprod. 1995; 10:2909-2912.
8. Hsieh YY, Tsai HD, Chang CC, et al. Comparison of a single half-dose, longacting form of gonadotropin-releasing hormone analog (GnRH-a) and a shortacting form of GnRH-a for pituitary suppression in a controlled ovarian hyperstimulation program. Fertil Steril. 2000; 73:817-820.
9. Dal Prato L., Borini A, Trevisi MR, et al. Effect of reduced dose of triptorelin at the start of ovarian stimulation on the outcome of IVF: a randomized study. Hum Reprod. 2001; 16:1409-1414.
10. Lenton E, Mohamed K. Optimizing assisted reproduction: impact of low-dose gonadotropin-releasing hormone agonist on in vitro fertilization outcome. Fertil Steril. 2005; 84:1783-1785.
11. Biljan MM, Mahutte NG, Dean N, et al. Effects of pretreatment with an oral contraceptive on the time required to achieve pituitary suppression with gonadotropin-releasing hormone analogues and on subsequent implantation and pregnancy rates. Fertil Steril. 1998;70:1063-1069.
12. Gonen Y, Jacobson W, Casper RF. Gonadotropin suppression with oral contraceptives before in vitro fertilization. Fertil Steril. 1990; 53:282-287.
13. Lampiao F, du Plessis SS. Comparing the Multi-ZSC one-step standardized swim-up method to the double-wash swim-up method with regard to the effects of sperm separation on morphology, head morphometry, and acrosome reaction inducibility. Fertil Steril. 2006; 86:739-741.

14. Scott LA, Smith S. The successful use of pronuclear embryo transfers the day following oocyte retrieval. Hum Reprod. 1998; 13:1003-1013.
15. Wilson A, Proctor M, Garzo G, et al. Techniques for intrauterine embryo transfer. Cochrane Database Syst Rev. 2006; P.4.
16. Pandian Z, Bhattacharya S, Ozturk O, et al. Number of embryos for transfer following in-vitro fertilisation or intra-cytoplasmic sperm injection. Cochrane Database Syst Rev. 2004; P.CD003416.
17. Saghaei M. Random allocation software for parallel group randomized trials. BMC Med Res Methodol. 2004; 4:26.
18. Daya S. Gonadotropin releasing hormone agonist protocols for pituitary desensitization in in vitro fertilization and gamete intrafallopian transfer cycles. Cochrane Database Syst Rev. 2000; P.CD001299.
19. Dor J, Bider D, Shulman A, et al. Effects of gonadotrophin-releasing hormone agonists on human ovarian steroid secretion in vivo and in vitro - results of a prospective, randomized in-vitro fertilization study. Hum Reprod. 2000; 15:1225-1230.
20. Gerrero HE, Stein P, Asch RH, et al. Effect of a gonodotropin-releasing hormone agonist on luteinizing homone receptors and steroidogenesis in ovarian cells. Fertil Steril. 1993; 59:803-808.
21. Peng C, Fan NC, Ligier M, et al. Expression and regulation of gonadotropin-releasing hormone (GnRH) and GNRH receptor messenger ribonucleic acids in human granulosa-luteal cells. Endocrinology 1994; 135:1740-1746.
22. Tsai NM, Hsieh RH, Au HK, et al. Effects of gonadotrophin-releasing hormone agonists on apoptosis of granulosa cells. Ann N Y Acad Sci. 2005; 1042:531-537.
23. Janssens RM, Lambalk CB, Vermeiden JP, et al. Dose-finding study of triptorelin acetate for prevention of a premature LH surge in IVF: a prospective, randomized, double-blind, placebo-controlled study. Hum Reprod. 2000; 15:2333-2340.
24. Van WM, Westergaard LG, Bossuyt PM, Van d, V. Human menopausal gonadotropin versus recombinant follicle stimulation hormone for ovarian stimulation in assisted reproductive cycles. Cochrane Database Syst Rev. 2003; P.CD003973.
25. Murad NM. Ultrasound or ultrasound and hormonal determinations for in vitro fertilization monitoring. Int J Gynaecol Obstet. 1998; 63:271-276.
26. Hsieh YY, Tsai HD, Chang CC, et al. Comparison of a single half-dose, longacting form of gonadotropin-releasing hormone analog (GnRH-a) and a shortacting orm of GnRH-a for pituitary suppression in a controlled ovarian hyperstimulation program. Fertil Steril. 2000; 73:817-820.
27. Lincoln SR, Long CA, Whitworth NS, et al. Elevated luteinizing hormone on the day of human chorionic gonadotropin administration does not reduce cycle fecundity in a low-dose flare-up in vitro fertilization protocol. Fertil Steril. 1995; 63:563-565.
28. Cabrera RA, Stadtmauer L, Mayer JF, et al. Follicular phase serum levels of luteinizing hormone do not influence delivery rates in in vitro fertilization cycles down-regulated with a gonadotropin-releasing hormone agonist and stimulated with recombinant follicle-stimulating hormone. Fertil Steril. 2005; 83:42-48.
29. Kwan I, Bhattacharya S, McNeil A, van Rumste MME. Monitoring of stimulated cycles in assisted reproduction (IVF and ICSI). Cochrane Database Syst Rev. 2006; P.4.
30. Lindheim SR, Barad DH, Witt B, et al. Short-term gonadotropin suppression with oral contraceptives benefits poor responders prior to controlled ovarian hyperstimulation. J Assist Reprod Genet. 1996; 13:745-747.
31. He H, Bolarinde O, Pan L, Li Sh. Pretreatment with oral contraceptives in a GnRHa program for couples undergoing assisted reproductive techniques. Cochrane Database Syst Rev. 2006; P.4.
32. Tan BK, Vandekerckhove P, Keay SD. Glucocorticoid supplementation during ovarian stimulation for IVF or ICSI. Cochrane Database Syst Rev. 2006; P.4.
33. Poustie VJ, Dodd S, Drakeley A. Low-dose aspirin for in vitro fertilisation. Cochrane Database Syst Rev. 2006; P. 4.
34. Daya S, Gunby J. Luteal phase support in assisted reproduction cycles. Cochrane Database Syst Rev. 2004; P.CD004830.
35. Dal Prato L., Borini A, Coticchio G, et al. Half-dose depot triptorelin in pituitary suppression for multiple ovarian stimulation in assisted reproduction technology: a randomized study. Hum Reprod. 2004; 19: 2200-2205.
36. Hsieh YY, Tsai HD, Chang CC, et al. Comparison of a single half-dose, longacting form of gonadotropin-releasing hormone analog (GnRH-a) and a shortacting form of GnRH-a for pituitary suppression in a controlled ovarian hyperstimulation program. Fertil Steril. 2000; 73:817-820.
37. Dal Prato L., Borini A, Trevisi MR, et al. Effect of reduced dose of triptorelin at the start of ovarian stimulation on the outcome of IVF: a randomized study. Hum Reprod. 2001; 16:1409-1414.
38. Fasouliotis SJ, Simon A, Laufer N. Evaluation and treatment of low responders in assisted reproductive technology: a challenge to meet. J Assist Reprod Genet. 2000; 17:357-373.