Arch Gynecol Obstet (2010) 281:741–746
DOI 10.1007/s00404-009-1252-4

REPRODUCTIVE MEDICINE

Comparison of mild stimulation and conventional stimulation in ART outcome

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Received: 6 July 2009 / Accepted: 25 September 2009 / Published online: 16 October 2009

Abstract
Purpose To provide a treatment for particular condition that is the most effective treatment with the least risk and cost for the patient we compared the efficacy of using clomiphene 100 mg + delayed low dose gonadotropin + flexible GnRH antagonist administration for ovarian stimulation protocol and GnRH agonist + gonadotropin for stimulation protocol in IVF outcome.
Methods Clinical outcome of 243 women with regularly menstruation who were candidate for IVF. They had undergone stimulation with GnRH agonist and gonadotropin (group A) or clomiphene citrate, gonadotropin and GnRH antagonist (group B). Main outcome was ongoing pregnancy.
Results There were no significant difference in mean age, cause of infertility, basal FSH, BMI, duration of infertility, endometrial thickness on the day HCG administration in two groups. The number of recovered oocytes, obtained embryos, transferred embryos, peak of estradiol on the day HCG administration and OHSS were significantly higher in group A. Significantly more patients in control group had embryos for cryopreservation. There were no significant difference in clinical pregnancy rate and ongoing pregnancy rate between two groups.
Conclusion Clomiphene + delayed low dose gonadotropin + flexible GnRH − antagonist stimulation is an acceptable alternative protocol for IVF in patients with regularly menstruation.

Keywords Clomiphene citrate · GnRH agonist · GnRH antagonist · In vitro fertilization · Pregnancy rate

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Introduction

The first ovarian stimulation protocols in the early 1980s were done using clomiphene citrate alone or in combination with gonadotropins. Elevated basal levels of luteinizing hormone (LH) were shown to have a poor correlation with the success of invitro fertilization (IVF) cycle [1]. These protocols were later changed, since the use of gonadotropin releasing hormone (GnRH) analogues allowed a better timing of oocytes retrieval and the maturation of more oocytes within one cycle [2, 3]. But ovarian stimulation for IVF with the use of GnRH agonist co-treatment is not without health risks. Between 0.1 and 5% of women receiving ovarian stimulation will develop ovarian hyperstimulation syndrome (OHSS) [4]. Furthermore, IVF combined with multiple embryo transfer (ET) is associated with a high incidence of multiple pregnancy [5]. Apart from health risk, standard IVF treatment can be an economic burden to patient, physical discomfort [6] and increased risk in aneuploidy in the preimplantation embryo [7]. Then there is a renewed interest in the use of mild ovarian stimulation protocol. Many ovarian stimulation protocols, such as

clomiphene citrate alone and in combination with follicle stimulating hormone (FSH) and/or human menopausal gonadotropin (HMG) [8–10], and letrozole with FSH have been reported [11]. One of the problems of mild stimulation is the possibility of ovulation before oocyte retrieval. GnRH antagonists are used to suppress natural ovulation [12]. Clomiphene citrate is a selective estrogen receptor modulator that induces follicle growth via hypothalamic and pituitary effects [13] and if administrated shortly after menstruation begins, will stimulate the growth of a number of follicles. HMG and r-FSH act directly at the level of ovary [14] and administration of them will then sustain the growth of this cohort of recruited follicles. GnRH antagonist rapidly and efficaciously controls the LH surge [6] and prevent of natural ovulation. An ovarian stimulation protocol combining CC/gonadotropin/GnRH antagonist could lead to a reduction in the amount gonadotropins due to the combined synergistic effects. Additionally, because gonadotropins may counterbalance the undesired anti-estrogenic effects of the CC on the endometrium [15] which is responsible for the relatively low embryo implantation rates, observed that this combination might lead to improve pregnancy rates compared with CC alone. The combination of these drugs into treatment protocols not only increased efficacy and pregnancy rates, but costs as well. Mild ovarian stimulation is effective in two groups of patient with decreased and normal ovarian reserve [16]. In this randomized clinical trial, GnRH agonist + gonadotropin stimulation and CC + delayed low dose gonadotropin + flexible GnRH antagonist stimulation is compared in the regularly menstruation patients that are candidate for IVF.

Materials and methods

Study design

This study is a prospective randomized controlled trial, performed at a university reproduction center between 1 January 2008 and 30 December 2008, including 243 patients who were candidate for ART. This study was approved by ethics committee of Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Science and prior to starting the study, informed consent was obtained from each patient. Inclusion criteria were female patient age 18–35 years, presence of a regular and proven ovulatory menstruation cycle with a length of 26–35 days, basal FSH <10 IU/L, body mass index (BMI) of 18–30 kg/m^2 and first IVF attempt. Indication for IVF were unexplained infertility, male factor, tubal factor, early stage endometriosis and cervical factor.

Treatment protocols

The patients were randomized in to one of two treatment groups using a computer-generated randomization schedule assigned via numbered sealed envelops. In group A, the patients were stimulated conventional. They desensitized with buserelin (suprefact, Aventis, Frankfurt, Germany) 500 μg subcutaneously (S.C.) everyday for menstrual cycle 21, until the baseline evaluation, which takes place in the first few days of menstruation. If baseline levels of estradiol (<50 pg/ml) had been achieved, then the dose of buserelin would be reduced to 250 μg and ovarian stimulation would commence with 150–225 IU recombinant FSH (r_FSH) (Gonal F, Serono, Aubnne, Switzerland) S.C. Patients in group B were stimulated clomiphene citrate (Iran hormone, Tehran, Iran) 100 mg from cycle day three through seven and continuous gonadotropin stimulation with of r_FSH 75 IU daily from cycle day 5. Ultrasound in two group was performed on 8 cycle day. In group B 0.25 mg GnRH antagonist (Ganirelix, Organon, Netherland) daily was started with dominant follicle ≥12 mm and in this day 75 IU human menopausal gonadotropin (HMG) (Menogon, ferring, pharmaceuticals, Germany) increased to the initial gonadotropin. LH assessment on the day of starting antagonist was performed and if premature LH surge was occurred (LH >10 IU/L), cycle was cancelled [17]. Human chorionic gonadotropin 10,000 IU (pregnyl, Organon, Oss, the Netherlands) was given when one to three follicles reached 18 mm. On that day, endometrial thickness was measured ultrasonographically and venous blood samples were obtained to determine the serum levels of E2. Oocyte pick-up was performed 34–36 h after HCG injection by transvaginal ultrasound-guided puncture (TVS) of follicles and IVF or ICSI was performed. All the embryos were scored by the number of blastomers, the size, the shape, the symmetry and cytoplasmic appearance of blastomeres, and the presence of anucleate cytoplasmic fragments on the third day after oocyte collection [18]. ET was done on the day 2 or 3, under ultrasound guidance, with a CCD embryo transfer catheter (Laboratory C.C.D., Paris, France). Luteal support with progesterone in oil (Progesterone, Aburaihan Co., Tehran, Iran) 100 mg daily IM was started on the day of oocyte retrieval and continued until the documentation of fetal heart activity on ultrasound. Pregnancy was confirmed by measuring serum ß-hCG levels 12 days after ET. Clinical pregnancy was considered as the presence of gestational sac with fetal heart activity by TVS that performed 3 weeks after positive ß-hCG. Primary outcome measures including clinical pregnancy rate per cycle and ongoing pregnancy; later were defined as pregnancy proceeding beyond the 12th gestational week. Secondary outcome included OHSS, defined by ≥15 follicles with a mean diameter ≥14 mm

per each ovary at the end of the follicular phase of stimulation, and/or E2 levels on the day of hCG administration >3,000 pg/mL and/or presence of ascites after hCG administration in ultrasonography.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science version 15.0 for windows (SPSS Inc., Chicago. IL). The data were analyzed by student's *t* test and chi-squared test. A *P* value of <0.05 was considered statistically significant.

Results

The results were reported in accordance with the Consort statement. 243 patients were enrolled and randomized. In control group (GnRH agonist/gonadotropin) 6 were excluded, and 13 patients did not come back, and we lost follow up in 3 patients. In study group (CC/gondotropin/antagonist) 2 were excluded, and 12 patients did not come back, and we lost follow up in 7 too. Therefore we analysed 100 patients in each group. The Consort statement flow diagram is presented in Fig. 1. Demographic and infertility characteristics for both groups are presented in Table 1. Age, BMI, basal FSH, duration of infertility and diagnosis of the patients, were comparable in both groups. The results of the ovarian stimulation are shown in Table 2. ET in two cycles were cancelled in the group A due to OHSS. 4 cycles were cancelled in group B due to premature LH surge. The number of days per stimulation (11.2 ± 1.3 vs. 11.4 ± 1.8) were statistically similar in both groups. However, the mean total ampoule gonadotropin consumption was significantly lower in group B than group A (22 ± 3.6 vs. 12.1 ± 4.3; CI 95%: 8.78–11.01). There was no difference in endometrial thickness between the groups (10.4 ± 0.4 vs. 10.4 ± 0.5 mm). The number of recovered oocytes (9 ± 2.2 vs. 5.4 ± 1.5; CI 95%: 3.12–4.21), mature oocytes (7.5 ± 1.9 vs. 4.8 ± 1.4; CI 95%: 2.21–3.17), embryos obtained (6.3 ± 1.8 vs. 4.2 ± 1.4; CI 95%: 1.66–2.59), E2 peak levels on the day HCG administration (1,314.8 ± 331.4 vs. 990.6 ± 199 pg/ml; CI 95%: 279.25–435.17) and OHSS (6 vs. 0%; CI 95%: 0.01–0.10) were higher in group A. The results of insemination of oocytes, embryological characteristics and ETs are given in Table 3. Conventional IVF, ICSI and combined insemination were used in the same percentage of cycles in both groups. The percentage of good quality embryos was 75.5% in group A and 80% in the group B. The mean number of transferred embryos was 2.7 ± 0.7 in group A and 2.2 ± 0.7 in group B ($P = 0.00$; CI 95%; 0.35–0.77). There were not significant statistically difference in clinical pregnancy per cycle (31 vs. 37%; OR: 0.76; CI 95% 0.42–1.37) and per transfer (30.6 vs. 37.5%; OR: 0.73; CI 95%: 0.40–1.33) and ongoing pregnancy (26.5 vs. 33.3%; OR: 0.72; CI 95%: 0.39–1.3) but there was a trend to high pregnancy in group B. 63% of cycles in

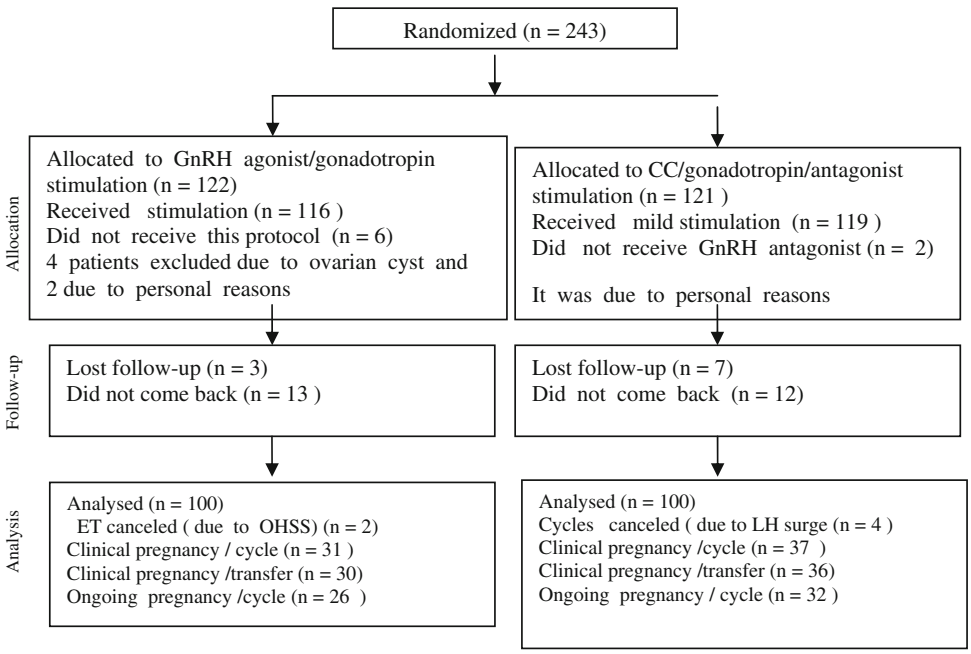

Fig. 1 Recruitment follow-up and drop outs over the course of the study

Table 1 Demographic and infertility characteristics of IVF patients

Variables	Group A (GnRH agonist/ gonadotropin)	Group B (CC/gonadotropin/ antagonist)
Age (years)	30.0 ± 2.3	29.4 ± 2.4
BMI (kg/m^2)	25.9 ± 2.3	25.3 ± 1.9
Basal FSH (IU/L)	6.5 ± 1.2	5.9 ± 1
Duration of infertility (years)	5.8 ± 1.0	5.6 ± 0.9
Infertility causes		
Male factor	55 (55%)	64 (64%)
Tubal factor	13 (13%)	15 (15%)
Mild endometriosis	5 (5%)	6 (6%)
Unexplain	5 (5%)	2 (2%)
Cervical	5 (5%)	2 (2%)
Mixed	17 (17%)	11 (11%)

BMI body mass index, *FSH* follicular stimulating hormone

Values are mean ± SD

Statistically significant values ($P < 0.05$)

Table 2 Results of ovarian stimulation of the two different group

Variables	Group A (GnRH agonist/ gonadotropin)	Group B (CC/gonadotropin/ antagonist)	*P* value
No. oocyte retrieval	9 ± 2.2	5.4 ± 1.5 CI 95%: 3.12–4.21	0.00[a]
No. oocyte M 2	7.5 ± 1.9	4.8 ± 1.4 CI 95%: 2.21–3.17	0.00[a]
Endometrial thickness (mm)	10.4 ± 0.4	10.4 ± 0.5	0.41
E2 day HCG (pg/ml)	1,314.8 ± 331.4	990.6 ± 199 CI 95%: 279.25–435.17	0.00[a]
No. of OHSS	6 (6%)	0 CI 95%: 0.01–0.10	0.02[a]
Total cycle cancellation	0 (0%)	4 (4%)	0.12
Total ET cancellation	2 (2%)	0 (0%)	0.49
Total ampule (75 IU) of gonadotropin	22 ± 3.6	12.1 ± 4.3 CI 95%: 8.78–11.01	0.00[a]
Days of stimulation	11.2 ± 1.3	11.4 ± 1.8	0.58

M2 metaphase 2, *E2* estradiol, *HCG* human chorionic gonadotropin, *OHSS* ovarian hyperstimulation syndrome

[a] Statistically significant value

group A and 30.5% of cycles in group B had embryos for cryopreservation (CI 95%: 2.13–7.03; significant statistically difference).

Discussion

The aim of this study was to provide a treatment for particular condition that is the most effective treatment with the least risk and cost for the patient.

One of the most severe and potentially life-threatening complication of IVF is the development of the OHSS. One randomized controlled study showed that CC/gonadotropin regimen with GnRH antagonist co-treatment resulted significantly reducing the number of ampoules HMG used, the number of treatment days and the number of oocytes retrieved and lower risk of OHSS [19, 20]. This fact was reflected in the present study in that E2 levels on the day of hCG injection were lower, and oocytes obtained were fewer, with CC/r-FSH/ganirelix protocol.

A previous report suggested that GnRH antagonist administration resulted in more mature oocytes and embryos of better quality compared with long protocol [21]. Lee et al. suggested that GnRH antagonists do not have a detrimental effect on embryo quality [22]. Other studies have shown that there is no cancellation as a result of ovulation because LH surge is controlled by the GnRH antagonist [10]. In our study there was no statistically difference in embryos quality and transfer cancellation in two groups.

Two retrospective analyses concluded that equally high pregnancy rates could obtained with CC/gonadotropin protocol with GnRH antagonist co-treatment compared with standard ovarian stimulation [10, 23]. In other report although more oocytes and more embryos obtain in long protocol, there was no difference in clinical pregnancy with mild ovarian stimulation protocol when compared with the conventional long protocol [24]. In contrast, Mansour et al. in a non randomized comparative study reported significantly lower pregnancy rates following ovarian stimulation with CC/HMG/GnRH antagonist compared with a long GnRH agonist protocol [25]. But in a recent meta analysis that was combining the results of three RCTs performed by Verberg et al. and suggested that retrieval of modest number of oocytes following mild stimulation is associated with higher implantation rate compared with the same number of oocytes is retrieved following conventional stimulation [26]. In our study, there was a trend to higher pregnancy in CC/gonadotropin/antagonist stimulation. Chen et al. reported that marker of endometrial receptivity were reduced in stimulation cycle compared with natural cycles, and more in high response compared with moderate response cycles [27]. In the largest of the three trials, the authors suggested that mild stimulation with GnRH antagonist co-treatment and single ET would be more tolerate than conventional stimulation with transfer of two embryos [28] and maybe these reports can explain

Table 3 Results of the insemination, embryological, embryo transfer in two groups

Variable	Group A (GnRH agonist/ gonadotropin)	Group B (CC/gonadotropin/ antagonist)	*P* value
Conventional IVF	18%	21.9%	0.59
ICSI	41%	36.5%	0.55
Combined IVF-ICSI	41%	41.6%	1
No. embryos obtained	6.3 ± 1.8	4.2 ± 1.4	0.00[a]
		CI 95%: 1.66–2.59	
No. embryos transferred	2.7 ± 0.7	2.2 ± 0.7	0.00[a]
		CI 95%: 0.35–0.77	
Top embryo quality %	75.5%	80%	0.49
Total ET cancellation	2 (2%)	0 (0%)	0.49
Cycles of cryopreserved embryos %	63%	30.5%	0.00[a]
		CI 95%: 2.13–7.03	
Clinical Pregnancy rate/cycle	31%	37%	0.45
	OR: 0.76	CI 95%: 0.42–1.37	
Clinical Pregnancy rate/transfer	30.6%	37.5%	0.45
	OR: 0.73	CI 95%: 0.40–1.33	
Ongoing pregnancy/transfer	26.5%	33.3%	0.34
	OR: 0.72	CI 95%: 0.39–1.3	

IVF in vitro fertilization, *ICSI* intra cytoplasmic sperm injection, *ET* embryo transfer, *OR* odd ratio, *CI* confidence interval

[a] Statistically significant difference

low pregnancy rate in agonist/gonadotropin protocol to CC/gonadotropin/antagonist protocol in our research.

But an advantage of agonist/gonadotropin stimulation over to CC/gonadotropin/antagonist stimulation is that more fresh embryos will be obtained and therefore more embryos available for cryopreservation [29], that present study confirmed it.

Conclusion

In our study CC/gonadotropin/antagonist protocol is as good as GnRH agonist/gonadotropin protocol in results of IVF; but if sample size was larger, maybe it was better than standard protocol in pregnancy rate. Then it possible to develop CC/gonadotropin/GnRH antagonist using instead of long GnRH agonist protocol in couples with male factor, tubal factor, mild endometriosis and unexplained infertility when ovarian reserve is normal and women have regularly menstruation.

Acknowledgments The authors are grateful to the nursing and embryology staff of the Research and Clinical Center for Infertility.

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