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The addition of gonadotrophin releasing hormone agonist to routine luteal phase support in intracytoplasmic sperm injection and embryo transfer cycles: a randomized clinical trial

Gülşah Aynaoğlu Yıldız [a,*], Yavuz Emre Şükür [a], Can Ateş [b], Ruşen Aytaç [a]

[a] Ankara University School of Medicine, Department of Obstetrics and Gynecology, Ankara, Turkey
[b] Ankara University School of Medicine, Department of Biostatistics, Ankara, Turkey

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ABSTRACT

Objectives: To determine the effects of addition of different dosages of gonadotrophin releasing hormone agonist (GnRH-a) to routine luteal phase support (LPS) on implantation and pregnancy rates.
Study design: Three hundred infertile couples who were treated by intracytoplasmic sperm injection and embryo transfer (ICSI-ET) following controlled ovarian stimulation (COS) with long luteal GnRH agonist protocol were enrolled. All women received 600 mg/day vaginal micronized progesterone plus 4 mg 17β estradiol for LPS starting from the day of oocyte retrieval. Patients (n = 300) were randomized into three treatment groups. Group A (n = 100) received leuprolide acetate 1 mg s.c. injection 3 days after ET in addition to routine LPS. Group B (n = 100) received two sequential doses of leuprolide acetate 1 mg s.c. injections 3 and 6 days after ET in addition to routine LPS. Control group (n = 100) received only the routine LPS.
Results: A total of 279 patients completed the study. The groups were comparable in terms of baseline demographic parameters including age, duration of infertility and day 3 levels of FSH and estradiol. The cycle parameters of the groups were also comparable regarding the E_2 level on day of hCG, number of retrieved oocytes, number of day 3 embryos, number of embryos transferred, and endometrial thickness on both days of OPU and ET. The implantation rates were similar in between the Groups A, B, and control group (20.7% and 25.8% vs. 13.3%, respectively; P = .099). The clinical pregnancy rates and miscarriage rates were similar in between the groups. The ongoing pregnancy rates were 27.4% in control group, 36% in Group A and 42.9% in Group B (P = .093). The OHSS rates were comparable in between the groups. The multiple pregnancy rates were significantly higher in Groups A and B than in control group (12% and 17.9% vs. 4.2%, respectively; P = .014).
Conclusions: The implantation, clinical pregnancy and ongoing pregnancy and multiple pregnancy rates seem to be increased with the addition of GnRH-a to routine luteal phase support.

Introduction

In a natural menstrual cycle the luteal phase is characterized by the formation of corpus luteum in which its main product, progesterone, is essential for progression of a pregnancy. Progesterone provides endometrial receptivity by secretory transformation of endometrium which is under the influence of estrogen [1]. This hormone prepares the endometrium for nidation and implantation of embryo. Human chorionic gonadotropin (hCG) which is secreted from the blastocysts maintains the persistence of corpus luteum during the early pregnancy period [2]. Luteal phase deficiency is a frequent problem in artificial reproductive technology (ART) cycles as a result of gonadotrophin releasing hormone agonist (GnRH-a) usage. The aim of pituitary down-regulation with GnRH-a in in vitro fertilization (IVF) treatment cycles is to reduce the cycle cancelation risk due to premature luteinization [3]. The advantages of GnRH-a usage prior to gonadotrophin stimulation are increased number of mature oocytes and improved pregnancy rates [4]. However, this method also results with luteal phase deficiency in almost all patients by inhibiting corpus luteum [5,6].

* Corresponding author at: Numune Taining and Research Hospital, Sıhhıye, Ankara 06100, Turkey. Tel.: +90 5324811908.
E-mail address: gulsahayna@gmail.com (G.A. Yıldız).

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Luteal phase support (LPS) is considered essential to counter luteal phase deficiency that may have a negative effect on early pregnancies [7]. It improved implantation rates, pregnancy rates and delivery rates [8,9]. The first LPS modalities were administering hCG and progesterone and both had similar effects on pregnancy rates [10]. However, as the usage of hCG induced ovarian hyperstimulation syndrome (OHSS), progesterone has become the primary choice for LPS in IVF cycles [11,12]. Although hCG supports the luteal phase indirectly by stimulating corpus luteum, progesterone induces secretory transformation of the endometrium in the luteal phase and improves endometrial receptivity [1]. Progesterone for LPS can be administered via oral, intramuscular or vaginal routes, but optimal route of progesterone has not yet been established [10]. Some other LPS modalities such as estrogen, steroids, ascorbic acid and acupuncture have been also tried but none of these were found to be effective [13–15].

In recent studies GnRH agonists have been evaluated for LPS. Both subcutaneous and intranasal routes of GnRH-a were found to be effective to support luteal phase in different studies [16–19]. The possible mechanism for LPS is stimulatory effects on corpus luteum in certain doses, by stimulation of LH secretion from hypophysis and activation of local GnRH receptors on endometrium [17,18]. However, contrary results were also achieved regarding the beneficial effects of GnRH-a administration for LPS [12]. In an attempt to increase the pregnancy rates, different doses, durations and combinations of treatments for LPS, including GnRH-a have been evaluated. However, a consensus on the best LPS regimen has not yet consisted.

The aim of the present prospective randomized controlled study was to determine the effects of addition of different dosages of subcutaneous GnRH-a to routine LPS with progesterone on implantation and pregnancy rates.

Materials and methods

A randomized controlled trial (RCT) was conducted after obtaining approval from the Institutional Review Board of Ankara University. A written informed consent was signed by all enrolled patients. Participants were recruited at the infertility out-patients clinic of a university-based tertiary care hospital between November 2008 and January 2010. Three hundred infertile couples treated by ICSI-ET following COS with long GnRH agonist protocol were enrolled in the study. The inclusion criteria were ICSI-ET cycles without gamete donation, COS with long GnRH-a down regulation, day 3 FSH level $\leq$10 IU/l, age between 20 and 40 years, body mass index (BMI) between 20 and 30 kg/m^2, and presence of both ovaries. The exclusion criteria were presence of polycystic ovary syndrome (PCOS), endometriosis documented by laparoscopy, hydrosalpinx, abnormal uterine cavity documented by hysterosalpingography and receiving any COS treatment other than long luteal protocol.

All patients underwent a standardized pituitary down-regulation protocol using GnRH-a leuprolide acetate 1 mg s.c. (Lucrin; Abbott, Istanbul, Turkey) which commenced on 21st day of preceding menstrual cycle. Leuprolide acetate dose was reduced to half on commencement of menstrual bleeding and continued at the same dose until hCG injection (Pregnyl; Schering-Plough, Istanbul, Turkey). Ovarian stimulation with recombinant human follicle stimulating hormone (rFSH) (Gonal-F; Serono, Bari, Italy) was started in all patients on day 3 of menstrual bleeding. The daily dose ranged between 150 and 300 IU, depending on body mass index, age of the patient and the anticipated ovarian response. Dose adjustment was done according to follicular development and serum estradiol and LH levels. When at least three follicles reached a mean diameter of 18 mm, a single dose of 10,000 IU of hCG was administered. After 36 h, transvaginal ultrasonography guided oocyte retrieval was performed. Fertilization was achieved with ICSI in all couples and ET was performed on day 3. A maximum of three embryos were transferred under ultrasound guidance. All women received 600 mg/day vaginal micronized progesterone (Progestan; Kocak Farma, Istanbul, Turkey) plus 4 mg 17β estradiol (Estrofem; Novo Nordisk, Bagsvaerd, Denmark) for LPS starting from the day of oocyte retrieval and continued until the pregnancy test performed 12 days after ET. Women with a positive test continued to take only micronized progesterone until 10th week of gestation.

After obtaining informed consent, all included patients (n = 300) were randomized into three treatment groups using a computer-generated randomization model. Study subjects were randomized in blocks of 15; i.e. of every 15 subjects included, five were allocated to control group, five were allocated to Group A, and 5 were allocated to Group B in a random manner. Women allocated to Group A (n = 100) received leuprolide acetate 1 mg s.c. injection 3 days after ET in addition to routine LPS mentioned above. Women allocated to Group B (n = 100) received two sequential doses of leuprolide acetate 1 mg s.c. injections 3 and 6 days after ET in addition to routine LPS. Women allocated to control group (n = 100) received only the routine LPS.

Pregnancy was confirmed by measuring serum β-hCG levels two weeks after ET. Clinical pregnancy was defined as the presence of a fetus with a heart beat at 6th gestational week and ongoing pregnancy was defined as pregnancy proceeding beyond 20 weeks of gestation. Implantation rate was calculated separately for each woman as number of gestational sacs divided by number of transferred embryos multiplied by 100.

Statistical analyses

In a previous RCT evaluating GnRH agonist administration as LPS 570 subjects were randomized to placebo and GnRH-a arms, and the ongoing pregnancy rates were found to be 29.5% and 31.2% respectively [12]. Considering the difficulty of recruiting so many participants to a single center study and as the trial was aimed to be finished within two years we performed a post hoc test. With reference to that, sample size estimation based on to detect a 10% increase of ongoing pregnancy from an assumed 30% in control group was performed using a one-way ANOVA test. A total sample of 252 subjects achieved 80% power to detect differences among the means versus the alternative of equal means using an F test with an alpha error level of 0.05 and beta error level of 0.2.

Statistical Package for the Social Sciences (SPSS) 15.0 for Windows was used for all statistical analyses. Shapiro–Wilk test was used for test distribution of normality. According to the results, non-parametric tests were preferred. Continuous variables were compared with Kruskal–Wallis test. Categorical variables were compared with Chi-square test or Fisher's exact test where appropriate. A P value of $<$0.05 was considered statistically significant.

Results

Fig. 1 shows the flowchart of patients assessed, excluded, randomized, treated and followed-up. During the study period 362 infertile couples were assessed for eligibility. However, 54 patients did not meet the inclusion criteria and eight patients refused to participate in the study. In Group A all patients received the allocated intervention and completed the follow-up period. In Group B 12 patients failed to receive the allocated intervention as they forgot or misunderstood the second dose of GnRH-a. In the control group all patients received the allocated intervention. There were a total of nine patients lost during the follow-up period (4 from Group B and 5 from control group). At the end of the study a