Evaluation of pretreatment with Cetrotide in an antagonist protocol for patients with PCOS undergoing IVF/ICSI cycles: a randomized clinical trial

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ABSTRACT
Objective: This study aimed to evaluate the effect of three days of GnRH antagonist pretreatment on the pregnancy outcomes of women with polycystic ovarian syndrome (PCOS) on GnRH antagonist protocols for IVF/ICSI.

Methods: Fifty women with PCOS in the control group received conventional antagonist protocols, starting on day 2 of the cycle. In the pretreatment group (n=38), a GnRH antagonist was administered from day 2 of the menstrual cycle for three days.

Results: Controlled ovarian stimulation (COS) duration and gonadotropin dosages were similar in both groups. The number of metaphase II (MII) oocytes, 2PN oocytes, embryos, along with implantation and clinical pregnancy rates, were higher in the pretreatment group when compared with controls, although the increment was not significant (p value ≥0.05). The chemical pregnancy rate was significantly higher in the pretreatment group. The rate of OHSS was significantly lower in the pretreatment than in the control group.

Conclusion: Women with PCOS offered early follicular phase GnRH antagonist pretreatment for three consecutive days had significantly fewer cases of OHSS and higher chemical pregnancy rates. There were trends toward greater numbers of MII oocytes, 2PN oocytes, and embryos, and higher clinical pregnancy rates in the pretreatment group.

Keywords: assisted reproductive technology, gonadotropins, polycystic ovarian syndrome

INTRODUCTION
Polycystic ovary syndrome (PCOS) is a very common endocrine disorder. It affects 5-7% women of reproductive age and is the leading cause of anovulatory infertility in this age range (Singh et al., 2014; Kalem et al., 2017). Irregular menstruation, hirsutism, acne, and infertility are common clinical features. Forty percent of infertile women have anovulation/oligoovulation and PCOS accounts for 80% of these cases (Singh et al., 2014). Assisted reproductive technology (ART) protocols are indicated when infertile women with PCOS are unable to become pregnant through standard ovulation induction methods. Ovarian stimulation includes two methods to prevent premature LH surges, GnRH agonist and GnRH antagonist protocols (Toftager et al., 2016). However, issues such as increased risk of ovarian hyperstimulation syndrome (OHSS), increased immature oocyte rates, lower fertilization rates, lower embryo quality, and lower implantation rates are observed in the IVF cycles of women with PCOS when the two protocols are compared (Kalem et al., 2017).

GnRH antagonists have been extensively used in ART clinics during the past years and a variety of GnRH antagonist protocols have been suggested. In spite of GnRH agonist protocol cycles, GnRH antagonists cause immediate suppression of gonadotropin secretion, which results in shorter treatments and less patient distress (European and Middle East Orgalutran Study Group, 2001; Al-Inany et al., 2006; Devroye et al., 2009). Moreover, the use of GnRH antagonists has yielded significantly lower chances of hospitalization due to OHSS (Kolbianakis et al., 2006). Despite the benefits associated with GnRH antagonists, GnRH agonist protocols remain as the treatment of choice in controlled ovarian hyperstimulation (COH) in the majority of ART clinics. There are some reasons for this practice. First, some investigators have reported uncoordinated antlfollicle growth during ovarian stimulation with GnRH agonists, leading to an asynchrony of the follicular cohort (Fanchin et al., 2003), which in turn may raise concerns over the outcome of the treatment. The flexibility of GnRH agonist protocols also permits more controlled oocyte retrievals, significantly decreasing and even preventing the need to perform retrievals, whereas the initiation of ovarian stimulation in GnRH antagonist protocol relies on the random incidence of spontaneous menses (Guivarc’h-Lévéque et al., 2010; Levy et al., 2009; Tremellen & Lane, 2010). Therefore, pretreatment with oral contraceptive pills (OCP) is frequently used in GnRH antagonist protocols to schedule the start of gonadotropin stimulation, although it considerably increases the consumption of gonadotropins and the duration of ovarian stimulation (Griesinger et al., 2008). A recent meta-analysis detected a considerable decrease in the ongoing pregnancy rate of patients prescribed pretreatment with OCP (Griesinger et al., 2010). Comparisons between a pituitary down-regulation protocol and a GnRH antagonist protocol at the beginning of ovarian stimulation found that women with PCOS in particular had higher serum gonadotropin and E2 levels. Consequently, in these women the unsuppressed level of FSH at the start of a GnRH antagonist cycle in contrast with a long GnRH agonist protocol allows the initial growth of a few leading follicles before the addition of exogenous rFSH (Blockeel et al., 2011a). We hypothesized that short pituitary suppression in the early follicular phase might
mimic the pre-stimulation hormonal environment of long GnRH agonist protocols, challenging the idea that high endogenous FSH levels cause developmental asynchrony of early antral follicles while maintaining the benefits of short antagonist protocols.

Furthermore, stable and early suppression of LH levels during the entire period of stimulation may be advantageous for implantation and pregnancy outcomes (Blockeel et al., 2011a). Occasional elevated baseline progesterone levels at the beginning of ART cycles and their association with reduced pregnancy outcomes is another problem in GnRH antagonist protocols (Kolibianakis et al., 2004a; Blockeel et al., 2011b). Based on these data, administration of GnRH antagonists for three consecutive days before the start of COS may normalize raised progesterone levels.

In agreement with the above findings, we posited that pretreatment with GnRH antagonists might allow follicular cohort synchronization and scheduling of ART treatment in women with PCOS. The purpose of this prospective randomized trial was to evaluate the effects of a 3-day course of GnRH antagonist pretreatment before the initiation of ovarian stimulation with gonadotropins on pregnancy outcomes.

MATERIALS AND METHODS

Study Design

This randomized clinical trial enrolled 88 women with PCOS based on the Rotterdam criteria, participating in an ART program at the Yazd Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, from March 2015 to March 2016. The Ethics Committee of the university approved the study. Informed written consent was obtained from all participating couples.

Patients with at least two of the following findings were included in the study: oligoovulation or anovulation; clinical or biochemical hyperandrogenism; and polycystic ovaries on ultrasound examination. Women aged 40 years or older, presence of severe male factor or systemic disease, use of hormone medication other than OCP, individuals on systemic drug therapy or with recurring IVF failure, recurrent pregnancy loss or uterine anomalies were excluded.

Two GnRH antagonist protocols for ovarian stimulation were compared. The patients were randomly (Random Digit Software) allocated to two groups. The 50 individuals assigned to the control group were prescribed a standard GnRH antagonist protocol. Controls were administered Gonalf 150 IU (SA Merck Serono, Geneva, Switzerland) on cycle day 2 subcutaneously, and later 0.25 mg of cetorelix (Cetrodote; Asta Medica, Frankfurt, Germany) daily when the leading follicle reached 14 mm in diameter until the HCG injection. The 38 women allocated in the pretreatment group were offered a modified protocol with antagonist administration for three days (starting on day 2 of the cycle) before the start of recombinant FSH (rFSH) therapy (Figure 1). Final oocyte maturation was triggered with HCG 10,000 IU (Pregnyl; Schering Plough) when the three larger follicles reached a mean diameter of 17 mm. Serum estrogen (E2) and endometrial thickness (ET) were measured on triggering day. Ultrasound-guided transvaginal oocyte retrieval was performed 36 hours later. Follicles measuring ≥14 mm were aspirated and the physicians performing follicular aspiration were blinded to the stimulation protocol. The IVF and ICSI procedures were performed, and the embryos were transferred on the third day after retrieval with a catheter (Labotec, Gotting, Germany).

Embryo quality was assessed based on the morphology criteria described by Dokras et al.; cleavage-stage embryos were given grades A, B, C, or D. Embryos graded as D were not transferred. Grade A included embryos with no fragmentation and equal size homogenous blastomeres; grade B included embryos with fragmentation <20% and equal size homogenous blastomeres; grade C included embryos with fragmentation ranging from 20% to 50% and unequal size blastomeres; grade D included embryos with fragmentation >50% and unequal size blastomeres (Dokras et al., 1993).

The number of transferred embryos depended on embryo quality, patient age, and risk of OHSS. Women at moderate or severe risk of OHSS had their embryos frozen as described in Table 1.

All patients were given 400 mg progesterone suppositories (Cox Pharmaceuticals, Barnstaple, UK) twice a day for luteal support, initiated on the day of oocyte retrieval. Serum B-hCG was checked 14 days after embryo transfer. If the patient became pregnant, then progesterone was continued until the 10th week of pregnancy. The implantation rate was calculated as the ratio between the number of gestational sacs and transferred embryos; chemical pregnancy was defined by serum B-hCG levels ≥50 IU/L 14 days after embryo transfer; clinical pregnancy was established as the presence of a gestational sac with fetal heartbeat identified by ultrasound examination five weeks after embryo transfer; miscarriages were defined as clinically recognized pregnancy losses before 20 weeks of gestation; and ongoing pregnancies were defined as pregnancies continued after 20 weeks of gestation.

The two groups had different sizes on account of patients lost to follow-up (Figure 2).

Outcome Measures

The primary endpoints in this study were the number of cumulus oocyte complexes (COCs), metaphase II oocytes (MII), and 2-pronuclei (2PN) oocytes in each group. Secondary endpoints included fertilization, implantation, and pregnancy rates in each treatment group. Tertiary endpoints were risk of OHSS and miscarriage rates in each group. Demographic and clinical characteristics such as age, baseline serum hormone levels, AMH, duration of stimulation, and total cumulative dose of rFSH were also collected.

Statistical Analysis

SPSS software (Statistical Package for the Social Sciences version 16.0, SPSS Inc., Chicago, IL, USA) was used for all statistical calculations. Student's t-test was used to compare quantitative variables. Statistical significance was attributed to differences with a p value<0.05.

RESULTS

Eighty-eight patients were randomly assigned to either control (n=50) or pretreatment groups (n=38). A flowchart diagram with the phases of the trial is shown in Figure 2. Baseline characteristics of the study groups are presented in Supplemental Table 2. The groups did not significantly differ with regard to demographic and cycle parameters. Table 2 summarizes the outcome parameters of both treatment protocols. Embryos from 18 women in the control group and 15 in the pretreatment group were frozen due to risk of OHSS. The implantation rates in the pretreatment and control groups were 20.5±27.3% and 11.8±20.6%, respectively. The difference was not statistically significant. Chemical and clinical pregnancy rates in the pretreatment group were higher (p<0.05) (Table 3). Ongoing pregnancy rates were higher in the pretreatment group, although not statistically different from the rates seen in the control group (Table 3).

DISCUSSION

The study showed that pretreatment with GnRH antagonists for three consecutive days before the start of ovar-
In conclusion, pretreatment with GnRH antagonists for women with PCOS for three consecutive days before the beginning of ovarian stimulation was associated with improved pregnancy results. Further investigation is needed.
Figure 2. Consort flowchart.

Table 2. Baseline and cycle characteristics of patients in both groups

|                                | Pretreatment group (n=38) | Control group (n=50) | p-value |
|--------------------------------|---------------------------|----------------------|---------|
| Age (years)                    | 28.07±3.85                | 29.04±4.76           | .313    |
| Duration of infertility (years)| 6.57±3.77                 | 5.99±3.74            | .468    |
| AMH                            | 6.64±1.29                 | 6.46±1.01            | .367    |
| End. Thickness at triggering day (mm) | 10.27±1.92            | 10.15±1.98           | .770    |
| Estradiol level at triggering day (ng/ml) | 3323±2670              | 2967±2149            | .491    |
| Gonadotropin Dose (IU)         | 1598±1932                 | 1515±1475            | .771    |
| Cycle duration (days)          | 12.36±1.54                | 13.66±2.23           | .003    |
| COC number                     | 17.68±9.29                | 16.46±9.76           | .554    |
| Number of M2 oocytes           | 14.65±8.30                | 14.10±8.79           | .764    |
| Number of 2PN oocytes          | 8.84±6.67                 | 7.40±6.41            | .308    |
| Total number of embryos       | 7.94±6.16                 | 6.94±6.06            | .446    |
| Number of embryos transferred  | 2.80±0.7                  | 2.69±0.6             | .676    |
| Number of embryos frozen       | 3.56±2.10                 | 4.13±2.01            | .454    |

Data are presented as mean value ± SD or number (%).
Table 3. Pregnancy outcomes of patients in both groups

| Pretreatment group (n=38) | Control group (n=50) | p-value |
|--------------------------|----------------------|---------|
| Fertilization rate       | 51.7%                | 50.6%   | 0.49    |
| Implantation rate        | 20.5±27.3%           | 11.8±20.6% | .091   |
| Chemical pregnancy rate  | 9 (41%)              | 3 (13%) | .035    |
| Clinical pregnancy rate  | 7 (32%)              | 2 (9%)  | .050    |
| Ongoing pregnancy rate   | 6 (28%)              | 2 (9%)  | .103    |
| Moderate, severe risk of | 15 (39%)             | 18 (36%) | .739   |
| OHSS (n, %)*             |                      |         |         |
| Miscarriage rate (n, %)  | 3 (37%)              | 1 (33%) | .898    |

Student’s t test.

to find whether GnRH antagonist pretreatment leads to better coordination of multifollicular development in high responders.

Conflict of Interest

The authors of this article have no conflicts of interest to declare.

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