A comparison of the effects of three different luteal phase support protocols on in vitro fertilization outcomes: a randomized clinical trial

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Objective: To evaluate the effects of three different luteal phase support protocols on pregnancy and implantation rates, as well as luteal phase hormone profile in intracytoplasmic sperm injection–ET cycles.
Design: A prospective, randomized study.
Setting: A tertiary teaching and research hospital.
Patient(s): Two hundred eighty-eight patients who were undergoing intracytoplasmic sperm injection with a long protocol of controlled ovarian hyperstimulation.
Intervention(s): Group 1 (E_2 + P) received daily P plus 4 mg of E_2, group 2 (hCG + P) received P plus 1,500 IU of hCG, and group 3 (P only) received daily vaginal P gel. Blood samples were drawn on the day of hCG administration, as well as 7 and 10 days after the hCG for the E_2 and P measurements.
Main Outcome Measure(s): The clinical pregnancy rate.
Result(s): No difference existed between the E_2 + P and hCG + P groups with respect to pregnancy rate, but it was significantly lower in the P-only group.The implantation rate was significantly lower in the P-only group than in the other groups.The highest miscarriage rate was in the P-only group (38%).
Conclusion(s): In assisted reproductive technology cycles including treatment with GnRH agonist, adding 4 mg of oral E_2 to P during the luteal phase significantly increased the pregnancy and implantation rates and decreased the miscarriage rate compared with the use of P only. (Fertil Steril® 2011;95:985–9. ©2011 by American Society for Reproductive Medicine.)

Key Words: Luteal phase support, progesterone, estradiol, hCG, luteal phase hormone profile, ICSI, pregnancy rate

Luteal phase insufficiency generally stems from an insufficiency of E_2 and P production after ovulation. This insufficiency is due to the inhibition of the LH in the early luteal phase by steroids secreted in supraphysiologic doses (1). If luteal phase hormonal support is not present in assisted reproduction technique cycles, the serum E_2 and P levels drop, thus leading to a decrease in the implantation rates and pregnancy rates (PRs) (2).

The only consensus is that luteal phase supplementation improves outcomes in IVF cycles (3). However, different ideas exist about the agents to be used for ideal luteal phase support in these stimulated cycles, and their doses and timing.

The addition of P and hCG with the aim of luteal support has been shown to increase PRs in many randomized studies (4, 5). On the other hand, ideas about using E_2 for luteal phase support are conflicting. Some reports favored the addition of E_2 supplementation (6–9), whereas others failed to observe any beneficial effects (10–13). In both meta-analyses with these studies (14, 15), it was reported that supplementing P with E_2 does not contribute to IVF outcomes. However, both studies stressed that larger series were needed to determine the importance of E_2 in luteal phase support, depending on its dose and administration. In this prospective randomized study, we compared the effects of using P gel only with using P gel with an oral 4-mg dose of E_2 or with using P gel and IM hCG for luteal phase support of the intracytoplasmic sperm injection outcomes.

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MATERIALS AND METHODS

Patient Population

A total of 464 women undergoing treatment with intracytoplasmic sperm injection at the Zekai Tahir Burak Women's Health Education and Research Hospital IVF Unit between January 2007 and January 2008 were included in the study. The IVF indications included the tubal factor, subfertile male factor (≥5 million total progressive motile spermatozoa per milliliter), unexplained infertility, and stage I and II endometriosis.

The study inclusion criteria were as follows: [1] patients between 19 and 39 years of age, [2] patients on a first IVF cycle, and [3] long protocol with GnRH agonist and recombinant FSH. The exclusion criteria were the following: [1] an hCG day E_2 level above 3,000 pg/mL (because of ovarian hyperstimulation syndrome [OHSS] risk), [2] diminished ovarian reserve (FSH >12 IU/mL), [3] endometriosis greater than stage II, [4] severe male factor (<5 million motile spermatozoa per milliliter requiring testicular sperm extraction, [5] endocrine disorders, [6] polycystic ovary syndrome, or [7] frozen-thawed cycles.

Informed consent was obtained from all patients, and the investigation was approved by the Institutional Review Board. This study was conducted in accordance with the basic principles of the Helsinki Declaration.

Ovulation Induction

Ovarian stimulation was performed with the GnRH-a Lucrin (Lucrin Daily; Abbott, Johannesburg, South Africa) in a standard long protocol, and a step-down regimen was used for ovulation induction with a starting daily recombinant FSH (Gonal F; Serono Laboratories, Bari, Italy) dose of 225 IU.

Dose alterations were performed on the fourth day of stimulation and on continuing days according to the sonographic findings and circulating E_2 levels. Once three follicles, at least 18 mm in diameter, were observed, ovulation was induced by an SC injection of 250 μg of recombinant hCG (Ovitrelle; Merck Serono, Darmstadt, Germany). Oocyte pickup was performed 34 to 36 hours after the hCG injection. Intracytoplasmic sperm injection was performed for all metaphase II oocytes. Embryo transfer was performed under ultrasound guidance on day 3 for all patients. On the day of oocyte retrieval, all patients began supplementation with a vaginal gel form of P (Crinone 8%; Merck Serono) at 90 mg once daily, which was continued at least until pregnancy was ruled out by a negative serum β-hCG measurement performed on day 14 after the ET.

Before randomization, 170 patients who did not comply with the inclusion criteria were excluded from the study. Starting from the day of oocyte pickup, 294 patients were allocated randomly to three groups to receive three different luteal support protocols, and they remained on the same allocation throughout the study. A further 6 patients were excluded from the study during the analysis stage (4 patients did not come to give blood on days 7 and 10, and 2 patients had fertilization failure). A total of 288 patient results were assessed.

In addition to vaginal P gel, group 1 (E_2 + P, n = 96) subjects received 4 mg of micronized E_2 (Estrofem; Novo Nordisk, Bagsvaerd, Denmark), with 2 mg ingested orally twice daily starting on the day of oocyte pickup until the day of the pregnancy test. Group 2 (hCG + P, n = 95) received 1,500 IU of hCG IM on the ET day, as well as 3 and 6 days after the transfer along with vaginal P gel. Group 3 (P only, n = 97) received only daily vaginal P gel for luteal support from the day of oocyte pickup to the day of the pregnancy test. The vaginal P gel was continued in all patients who became pregnant in all three groups until the 12th week of pregnancy.

Pregnancies were confirmed 2 weeks after ET, when the serum hCG level was elevated. Clinical pregnancies were detected with the confirmation of positive fetal cardiac activities by transvaginal sonography. The implantation rate was the proportion of embryos transferred resulting in an intrauterine gestational sac. Multiple pregnancies were defined as two or more gestational sacs in the uterine cavity. Miscarriage was defined as a loss of a clinical pregnancy before the 13th week of gestation.

Blood samples were drawn on the day of hCG and on days 7 and 10 after the hCG for the E_2 and P measurements. No drug-related side effects emerged.

Laboratory Methods

The E_2 and P levels were determined with an electrochemiluminescence immunoassay (Elecsy and cobas e analyzers; Roche Diagnostics GmbH, Mannheim, Germany). The results were determined via a calibration curve that was generated specifically for the instrument by a two-point calibration and a provided master curve. The analysis sensitivity of the assay was 5 pg/mL, and the linear interval of the test was 5 to 4,300 pg/mL for estrogen. The E_2 levels were assayed with intra-assay and interassay coefficients of variation of <3.3% and <4.9%, respectively. The analysis sensitivity of the assay was 0.21 ng/mL, and the linear interval of the test was 0.21 to 60 ng/mL for P. The P levels were assayed with intra-assay and interassay coefficients of variation of <8% and <9.1%, respectively.

Sample Size

We estimated that 90 patients per group would be needed to show a 20% difference in the clinical PR among the three groups, assuming a statistical power of 98% at an alpha level of 0.05. A power analysis was performed by using the NCSS-PASS package (Kysville, UT).

Randomization

On the oocyte pickup day, patients were assigned randomly into three groups by a systematic randomization. A clinician who was not included in this study allotted the participants to their treatment groups according to their application numbers. The investigators were blinded to the treatment allocation.

Statistical Analysis

The data were evaluated with use of SPSS for Windows release 15.0 (SPSS, Inc., Chicago, IL). Continuous data were expressed as means ± SD and were analyzed with one-way ANOVA tests for normally distributed data and with the Kruskal-Wallis test for other data. Categorical data were analyzed with Pearson's χ^2 test. If statistical difference was found, we compared the groups by using χ^2 test with Bonferroni correction. Also, the E_2, P, and E_2/P rates for ongoing pregnancies in all groups were analyzed with the Mann-Whitney *U* test.

A probability value of <.05 represented statistical significance. A no-stopping rule was defined, and there was no need for an interim analysis because of the lack of adverse effects.

RESULTS

No difference existed among the three groups with respect to the mean age, body mass index, cause of infertility, duration of infertility, day 3 E_2 and FSH levels, gonadotropin dose, or endometrial thickness. The clinical characteristics in the different luteal phase support groups are shown in Table 1. At the same time, no differences were found in the number of oocytes retrieved and the number of embryos transferred. Although the implantation rates were similar between group 1 (16.8%) and group 2 (20.0%), the rate was significantly lower in group 3 (7.9%) (*P*=.001). Although no differences existed between group 1 (40.6%) and group 2 (38.9%), the PR in group 3 (21.6%) was significantly lower (*P*=.01). The miscarriage rate was significantly higher in group 3 (38%) than in group 1 and group 2 (*P*=.02). Multiple pregnancies were not observed in group 3, whereas they were highest in group 2 (14.7%), and a significant difference existed between group 2 and the other groups (*P*=.001) (Table 2). Ovarian hyperstimulation syndrome developed in two patients in group 2. Both OHSS cases were mild ones.

After the hCG trigger, the E_2 levels on days 7 and 10 were significantly higher in groups 1 and 2 than in group 3 (*P*=.001, *P*=.001). No significant difference was observed between groups 1 and 2 with respect to the E_2 levels. Considering the percentages of the decrease from the peak E_2 level to those on days 7 and 10, group 3 had a statistically significant decrease compared with the other two (*P*=.001, *P*=.001). The percentages of the E_2 decreases in groups 1 and 2 were similar on days 7 and 10.

As for the P levels on days 7 and 10 after the hCG trigger, no statistical difference existed among the three groups (*P*=.22, *P*=.63). The E_2/P rates on days 7 and 10 had significantly lower E_2 rates in group 3 than in the other two groups (*P*=.001, *P*=.001). The E_2/P rates in groups 1 and 2 were similar (Table 3).

In all three groups, the day 7 and 10 E_2 levels and the percentages of decrease from the peak E_2 level to the day 7 and 10 E_2 levels were determined for pregnant and nonpregnant patients. In groups 1 and 2, the day 7 and 10 E_2 levels were significantly higher in the pregnant patients. Similarly, the percentages of E_2 decrease were significantly lower in pregnant patients. In group 3, the day 7 E_2 levels and day 7 percentage of E_2 decrease were similar in pregnant and nonpregnant patients. In this group, the day 10 E_2 levels were higher and the E_2 decrease was smaller than in pregnant patients (Table 4).

DISCUSSION

Multiple studies have demonstrated the need for luteal phase supplementation in IVF cycles suppressed with GnRH agonist (16–18). The role of P supplementation in the luteal phase of down-regulated cycles is well established. Progesterone supplementation is a routine treatment throughout the world with different doses and types of administration (6).