Comparison of serum and follicular fluid hormone levels with recombinant and urinary human chorionic gonadotropin during in vitro fertilization

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Objective: To study serum and follicular fluid (FF) hormone levels after the administration of urinary or recombinant hCG to initiate the final stages of oocyte maturation during IVF.
Design: Prospective randomized study between 250 μg of recombinant hCG and 7,500 IU of urinary hCG as the final trigger of ovulation during IVF.
Setting: Private IVF center.
Patient(s): Infertile women undergoing IVF/intracytoplasmic sperm injection (ICSI) using the long protocol and recombinant FSH.
Intervention(s): IVF treatment. Serum and FF hormone measurements on the day of oocyte collection.
Main Outcome Measure(s): Serum and FF E_2, P, hCG, and T levels.
Result(s): Stimulation parameters, serum and follicular E_2, P, T, and hCG levels were similar in the recombinant and urinary hCG groups. The number of oocytes retrieved from follicles >14 mm, the proportion of mature oocytes, fertilization rate, and pregnancy rate (PR) were also comparable.
Conclusion(s): Recombinant and urinary hCG provided similar serum and follicular hormonal environments during the final stages of oocyte maturation. The IVF outcome parameters were also comparable. The two medications appear to be equally effective. (Fertil Steril® 2008;90:2133–7. ©2008 by American Society for Reproductive Medicine.)

Key Words: Recombinant, urinary, human chorionic gonadotropin, follicular fluid, steroid, serum

During a spontaneous menstrual cycle a preovulatory LH surge initiates the ovulatory process. As a result of the LH surge the oocytes complete meiosis I and enter meiosis II, the cumulus–oocyte complex separates from the follicle wall and the process resulting in the release of the oocyte–cumulus complex is started (1).

During an IVF cycle premature luteinization could negatively affect treatment outcome. If the follicles rupture prematurely, oocytes cannot be collected. To prevent premature LH surges GnRH agonist or antagonist is combined with gonadotropins. Both GnRH preparations successfully prevent premature ovulation. Therefore to collect mature eggs for fertilization the LH surge needs to be induced medically (2, 3). In cycles without down-regulation and in cycles where a GnRH antagonist is used to prevent the premature LH surge both GnRH agonist or hCG can be administered for this purpose (4, 5). In GnRH agonist down-regulated cycles hCG has to be administered before the retrieval. Human chorionic gonadotropin and LH have a lot of similarities in their structure, therefore hCG is capable of inducing the necessary changes through the LH receptor (6).

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The growing follicle requires a unique endocrine environment to develop a mature egg. Steroid hormones and various peptides produced both locally and at distant sites are responsible for providing an optimal nourishing milieu. Changes in this environment might lead to oocytes that are less likely to fertilize successfully or might result in poorer quality embryos. Previous studies have shown that an environment rich in androgens is unfavorable for the developing oocyte. Xia and Younglai (7) found that when the follicular fluid (FF) E_2-to-T ratio exceeded 200, significantly more high quality (grades 3, 4) oocytes were obtained. Higher FF E_2-to-T ratio was reported in IVF cycles that resulted in a pregnancy (8). Significantly higher FF P-to-E_2 ratio was measured in follicles from which the oocyte was successfully fertilized (9). Kreiner et al. (10) observed that FF E_2 and P levels tended to be within a given range in IVF cycles that resulted in a pregnancy. More recently a follicular environment richer in E_2 was reported with the use of highly purified hMG when it was compared to recombinant FSH. This group also reported that the FF E_2-to-T ratio was higher in those follicles that provided an egg resulting in a pregnancy (11).

Although the follicular environment is primarily influenced by the type of gonadotropin the follicle is exposed to during the follicular phase, the hCG injection that triggers the luteinizing changes also has a significant influence on this environment.

For decades hCG derived from urine was available and was used during IVF cycles. More recently recombinant hCG became available. Several clinical studies have shown that the two hCG preparations result in a similar number of oocytes, clinical pregnancies, ongoing pregnancies, delivery and miscarriage rates (12, 13).

In our study we were interested in the follicular hormonal environment with the available urinary and recombinant hCG preparations. As secondary outcomes, we were also interested in any possible association between the hormonal values/ratios and oocyte quality, fertilization, embryo development, and pregnancy rates (PR).

MATERIALS AND METHODS

In this prospective, randomized multicenter trial, Institutional Review Board (IRB) approval was obtained (SZTE Albert Szent-Gyorgyi School of Medicine 106/2006) and all participants signed informed consent before entering the study. Sixty women were randomized to receive either 250 μg of recombinant hCG SC (Ovitrelle, Serono, Italy) or 7,500 IU of urinary hCG IM (Choragon, Ferring, Germany) to induce the final maturation of the oocytes. Block randomization (blocks of 2) was used to allocate patients to the two hCG preparations. Patients were randomized when they came in for the suppression check during the GnRH agonist. The study was conducted between December 2006 and April 2007.

Infertile women less than 40 years of age with regular menstrual cycles were eligible to participate. The baseline FSH level had to be $<$12 IU/L and the uterine cavity had to be intact. Couples with severe male factor infertility (need for surgical sperm extraction or use of donor sperm) were excluded. Couples with previous poor response to stimulation ($<$3 oocytes, cancelled cycle, use of high dose of gonadotropins [$>$300 IU/day]) and those with more than two failed previous IVF cycles were also excluded.

Multiple ovarian follicle development was achieved using the GnRH agonist long protocol and recombinant FSH. The GnRH agonist (0.5 mL of Suprefact SC; Aventis Pharma, Germany) was started in the luteal phase of the previous cycle. After 10–12 days of GnRH agonist administration, once suppression was confirmed, stimulation with 150 IU of recombinant FSH (Gonal-F, Serono, Italy) was started. The first ultrasound was performed on day 6 of stimulation. The dose of the gonadotropin could be increased or decreased at this point based on response. Ultrasound and, if required, serum E_2 measurements were used to monitor follicle growth. Once two leading follicles reached 17 mm in diameter recombinant or urinary hCG was administered to induce final follicular maturation. Transvaginal oocyte retrieval was scheduled 35–36 hours after this final injection. After the retrieval the oocytes were evaluated for maturity and conventional insemination or if needed intracytoplasmic sperm injection (ICSI) was used for fertilization. Fertilization was assessed the next day. The embryos were cultured for 3–5 days before the transfer. The day of transfer and the number of embryos transferred were decided by the physician based on patient and cycle characteristics. The luteal phase was supported by 600 mg of vaginal micronized P in divided doses (Utrogestan, Lab Besius, France). Twelve days after the transfer a serum β-hCG was measured. At 6 weeks and at 8 weeks a pelvic ultrasound was performed to monitor the early pregnancy. Progesterone supplementation was continued until week 9 of pregnancy.

Serum and Follicular Fluid Samples

On the day of the oocyte retrieval serum and FF were collected. Blood was drawn from the antecubital vein. The specimen was spun for 10 minutes at 3,000 rpm to separate the serum and cellular components. Serum measurement for E_2, P, T, and hCG were carried out with commercially available ELISA assays using an automated system. Follicular fluid was collected from several follicles. Pooled samples were obtained. All bloody samples were discarded. Follicular fluid was also spun for 10 minutes at 3,000 rpm, first to separate the cellular components. Estradiol, P, T, and hCG levels were determined using commercially available ELISA assays. For E_2 and P measurements the samples had to be diluted 1,000 times manually.

Data Collection

Data was collected for baseline characteristics (age, baseline FSH, E_2), stimulation parameters (dose of gonadotropin, type of hCG, number of follicles $>$14 mm, number of oocytes, rate of mature oocytes [mature MII oocytes/total oocytes retrieved], fertilization rate based on all oocytes and mature oocytes only [fertilized oocytes/all oocytes retrieved, fertilized oocytes/mature MII oocytes retrieved], number of available embryos, proportion of top quality embryos [$\geq$6 cells on day 3, $<$20% fragmentation; top quality embryos/all embryos], number of embryos transferred and cryopreserved), and for treatment outcome. When a positive serum pregnancy test was obtained 12 days after the embryo transfer it was considered a "pregnancy." When a pregnancy progressed beyond week 8 and was discharged from our care we considered it an "ongoing pregnancy." In addition to serum and FF measurements, the ratios of E_2-to-T and E_2-to-P were compared.

Statistical Methods

Student's *t*-test was used to compare quantitative variables and χ^2 test to compare proportions between the different outcomes with recombinant and urinary hCG. A $P<.05$ was considered significant.

RESULTS

Sixty patients were randomized. Baseline characteristics were comparable in the two groups (Table 1). None of the cycles were cancelled during stimulation and all cycles ended with embryo transfer.