The Need to Step up the Gonadotropin Dosage in the Stimulation Phase of IVF Treatment Predicts a Poor Outcome

DOREEN L. HOCK,^{1,2} HELEN LOUIE,¹ ROBERT M. SHELDEN,¹ CANDE V. ANANTH,¹ and EKKEHARD KEMMANN¹

Submitted: January 13, 1998

Accepted: March 6, 1998

Purpose: It is a common practice to increase the gonadotropin dose during ovarian stimulation when the estradiol (E_2) rise is found to be inadequate. The prognostic impact of the use of this step-up regimen on the outcome of the affected in vitro fertilization (IVF) cycle is the subject of this study.

Methods: This is a retrospective analysis of IVF cycles in a series of consecutive patients who required an increase in the gonadotropin dosage during the stimulation phase because of inadequate E_2 rise. Controls consisted of patients in whom the dose was not increased. After 4 days of stimulation, the gonadotropin dosage was increased if E_2 levels failed to rise by 70% every 2 days. Outcome was defined in terms of maximum E_2 level, number of follicles at aspiration, number of oocytes obtained, fertility rate, and pregnancy rate and was compared in study and control patients. Pregnancy was defined by sonographic demonstration of cardiovascular activity.

Results: One hundred forty-five patients were analyzed. A step-up regimen was used in 35 patients (24.1%). Patients who required the step-up dosing had significantly lower peak E_2 levels (1373 vs 1828 pg/ml; $P < 0.005$), fewer follicles measuring greater than 16 mm (7.2 vs 9.7; $P < 0.003$), and fewer oocytes recovered (8.3 vs 11.2; $P < 0.009$). The fertilization rate (67.6 vs 64.2%) was not significantly different. The pregnancy rate (8.5 vs 32.7%; $P < 0.004$) was significantly lower in the group requiring the step-up regimen.

Conclusions: The utilization of a step-up regimen during an IVF treatment cycle is a predictor of a poor outcome for the specific IVF cycle. As this information is available before

retrieval, consideration of cycle cancellation may be appropriate.

KEY WORDS: gonadotropin dosage; in vitro fertilization; ovarian stimulation.

INTRODUCTION

Ovarian stimulation by exogenous gonadotropins has become a common feature of treatment plans in various causes of infertility. The stimulation of the ovary typically is monitored by vaginal ultrasonographic assessment of follicular growth and measurement of serum estradiol (E_2) levels. During stimulation, physicians adjust the gonadotropin dosage to optimize follicular development. However, the consequence of increasing the dose (step-up) during stimulation is controversial. It has been argued that this approach does not improve the pregnancy rate (1) or may even have a deleterious effect (2). The purpose of this study was to determine the prognostic significance of the use of the step-up regimen. We hypothesized that patients requiring this treatment in the stimulation phase of an in vitro fertilization (IVF) cycle have a poor prognosis for pregnancy.

MATERIALS AND METHODS

This is a retrospective analysis of patients having had IVF and embryo transfer (ET) from May 1994 to December 1995. Only patients undergoing their first cycle of IVF-ET without micromanipulation were included for analysis. Patients with elevated day-3 follicle-stimulating hormone (FSH) (>20 IU/ml) and/or E_2 (>60 pg/ml) levels were excluded. All patients received individualized doses of gonadotropins for ovarian stimulation after suppression with a gonado-

¹ Department of Obstetrics, Gynecology and Reproductive Medicine, University of Medicine and Dentistry of New Jersey (UMDNJ)—Robert Wood Johnson Medical School, New Brunswick, New Jersey.

² To whom correspondence should be addressed at UMDNJ—University Center for Reproductive Endocrinology and Fertility (UCREF), 303 George Street, New Brunswick, New Jersey 08901.

tropin-releasing hormone (GnRH) agonist. Suppressive therapy with leuprolide acetate (LA; Lupron; Tap Pharmaceuticals, Dearborn IL) was initiated in the preceding cycle on cycle day 21 at a dose of 1 mg/day. After at least 2 weeks of receiving leuprolide acetate at this dosage, it was decreased to 0.25 mg/day and gonadotropin therapy was begun. The initial dosage was between 225 and 450 IU/day of Metrodin (Serono Laboratories, Inc., Norwell, MA) depending on the patient's age, serum FSH level, and, if applicable, previous response to gonadotropins. Subsequent dosing was individualized based on the patient's response as determined by serum E₂ levels. Serum E₂ levels were obtained every other day, and gonadotropin dosage was increased if E₂ levels failed to increase by 70% every 2 days. Any increase in the daily gonadotropin dosage was termed the "step-up regimen." When E₂ levels exceeded 400 pg/ml, serial transvaginal sonography was performed to determine follicular size. Treatment with menotropins and leuprolide ceased and 10,000 U of human chorionic gonadotropin (hCG) was administered intramuscularly when follicles exceeded a diameter of 18 mm. Transvaginal aspiration of the follicles was performed 36 hr after hCG administration. Oocytes were cultured in vitro for 6 hr in Ham's F-10 medium (GIBCO, Grand Island, NY) with 10% human serum and oocytes were inseminated with approximately 200,000 motile sperm per dish. One to three two- to eight-cell-stage embryos were transferred to the uterus 48 hr after retrieval. Patients aged 38 and older received more than three embryos when available. Remaining embryos were placed into cryopreservation at the two-pronuclei stage if in agreement with patient preference. Cycle outcome was defined in terms of maximum E₂ level prior to hCG administration, number of follicles at aspiration, number of oocytes obtained, fertilization rate, and clinical pregnancy rate. Pregnancy was identified by serially rising β hCG levels and confirmed by transvaginal sonographic demonstration of fetal cardiovascular activity.

For analysis, the patients were divided into two groups based on the use of the step-up regimen. Group A consisted of those in whom the gonadotropin dose was increased, while group B patients received a steady or declining dosage. Statistical analysis was performed using the *t* test for continuous variables and the chi-square and Fisher's exact tests for discrete variables. A *P* value of less than 0.05 was considered statistically significant. Relative risks with 95% confidence intervals were computed to quantify the association between the pregnancy rate and the dosage groups. Because the relationship between pregnancy rate and

maternal age was nonlinear (results not shown), we performed a restricted cubic-spline transformation (3) on these continuous variables. The restricted cubic splines are nonparametric smoothing procedures that do not impose any restriction on the shape of the distribution between a continuous variable and the outcome. We fit a logistic regression model for the rate of pregnancy using a four-piece cubic spline transformation for maternal age.

RESULTS

During the period from May 1994 to December 1995, 145 patients were analyzed. A step-up regimen was used in 35 patients (group A), while 110 patients represented the controls (group B). The pregnancy rate (8.5 vs 32.7%; *P* < 0.004) was significantly lower in patients who received the step-up dosing. This corresponded to an unadjusted relative risk (RR) of 3.8 (95% CI; 1.2–11.5). Group A also had significantly lower peak E₂ levels (1373 vs 1828 pg/ml; *P* < 0.005), fewer follicles measuring greater than 16 mm (7.2 vs. 9.7; *P* < 0.003), and fewer oocytes recovered (8.3 vs 11.2; *P* < 0.009) than group B. The fertilization rate (67.6 vs 64.2%) was not significantly different.

We noted no significant difference in parity, the distribution of diagnoses or the number of embryos transferred; however, a significant difference in age was noted between the two groups. The mean age of group A was 36 years, whereas the mean age of the controls was 33 years (*P* < 0.01). We used a logistic regression model for the rate of pregnancy using a four-piece cubic spline transformation for maternal age to determine whether maternal age accounted for the effect of the need to increase the gonadotropin dosage on the RR of achieving a pregnancy. The adjusted RR of achieving pregnancy was 4.0 (95% CI; 1.1–14.5) for patients who required the step-up dosing after accounting for age. Thus, the difference in age between the patients requiring the step-up regimen and those not requiring this regimen was not sufficient to account for much of the prognostic effect of the need to increase gonadotropin dosage.

DISCUSSION

In a large series of patients undergoing IVF, we found that in those patients in whom the physician increased the gonadotropin dosage during the stimulation cycle, the chance of achieving pregnancy was

significantly lower compared with controls, whose gonadotropin dosage remained constant or decreased. Further, the study group had lower peak E_2 levels, fewer follicles measuring greater than 16 mm, and fewer oocytes recovered at retrieval. The difference between the study group and controls remained significant after accounting for age.

It is common practice for physicians who monitor serum E_2 levels during the stimulation phase to increase gonadotropin support if the E_2 is deemed unsatisfactory. However, recently this approach has been challenged. van Hoof *et al.* (1) performed a prospective, randomized study in which the dose of gonadotropins was increased in 22 patients with low E_2 levels after 5 days of ovarian stimulation, while in 25 controls exhibiting the same E_2 response the dose remained unchanged. No significant improvement of high-dose gonadotropin treatment on pregnancy rate was found. The effect of high-dose gonadotropin stimulation has also been questioned by Stadtmayer *et al.* (2). They suggested that excess gonadotropin stimulation may have a deleterious effect on the cycle, with an inverse relationship demonstrated between the dosage of medication and the pregnancy rate. Investigators have proposed that this decline in pregnancy rate may be secondary to a resultant short luteal phase and an increase in fractured zona (4), and a higher incidence of chromosomal aberrations and sister chromatid exchange (5). However, in an analysis of intermediate responders, Simon *et al.* (6) did not confirm that a high dosage of gonadotropins has a detrimental effect on IVF outcome. Their study suggested that the poor outcome of patients requiring higher doses of gonadotropins was inherent in that these patients were poor responders and not secondary to the elevation in gonadotropin dose.

Our study differs from these others in that it evaluates the need to implement the step-up regimen as a prognostic indicator of pregnancy. As the results with the step-up regimen indicate that this traditional attempt to compensate for unsatisfactory E_2 rises lacks effectiveness and its use does indeed predict a poor outcome, cancellation of the cycle needs to be considered. Alternative stimulation protocols for a subsequent cycle may be more useful. However, initiating the stimulation with a high gonadotropin dosage from the onset of the subsequent cycle may not be effective. Manzi *et al.* (7) performed a retrospective study that examined patients who responded poorly in a previous gonadotropin/IUI cycle. No increase in cycle fecundity was noted in patients receiving higher gonadotropin doses in the subsequent IUI cycle. Similarly, in a retro-

spective study by Land *et al.* (8), it was demonstrated that increasing the gonadotropin dosage in a subsequent IVF stimulation cycle did not improve pregnancy rate. Other protocols have been proposed. Padilla *et al.* (9) studied 53 patients considered to be at risk for a poor response. They concluded that using a GnRH agonist at the onset of therapy to produce a flare effect with high-dose gonadotropin therapy is a good alternative for these patients. Antoine *et al.* (10) reviewed several stimulation approaches for poor responders. They concurred that high gonadotropin doses in the beginning of stimulation does not improve pregnancy rate. They also found results of growth hormone cotreatment to be disappointing. However, they suggest the use of a GnRH agonist in a flare protocol or the addition of clomiphene citrate to the gonadotropin support as methods to improve outcome, including oocyte and embryo quality. Lindheim *et al.* (11) suggest that pretreatment with exogenous estrogens may improve cycle outcome in patients who respond to gonadotropins with an inadequate rise in serum E_2 .

In conclusion, the practice of increasing gonadotropin support during the stimulation phase of the IVF cycle is associated with a poor outcome. Therefore, it may be reasonable to consider cancellation of the current cycle with the utilization of an alternative induction regimen in subsequent stimulation. In patients who continue to respond poorly, despite changes in the stimulation technique, it may be necessary to consider using donor oocytes.

REFERENCES

1. van Hooff MHA, Alberda AT, Huisman GJ, Zeilmaker GH, Leentveld RA: Doubling the human menopausal gonadotropin dose in the course of an in-vitro fertilization treatment cycle in low responders: A randomized study. *Hum Reprod* 1993;8:369-373
2. Stadtmayer L, Ditkoff EC, Session D, Kelly A: High dosages of gonadotropins are associated with poor pregnancy outcomes after in vitro fertilization-embryo transfer. *Fertil Steril* 1994;61:1058-1064
3. Durrelman S, Simon R: Flexible regression models with cubic splines. *Stat Med* 1989;8:551-561
4. Beauchamp PJ: Human menopausal gonadotropin for follicular recruitment in in vitro fertilization and embryo transfer. In *Human In Vitro Fertilization and Embryo Transfer*, DP Wolf, MM Quigley (eds). New York, Plenum Press, 1984, p 99
5. Sato F, Marrs RP: Effect of ovarian hyperstimulation on chromosome aberration and sister chromatid exchange on in vivo fertilized oocytes. *Fertil Steril* 1984;41:245-247
6. Simon T, Oehninger S, Toner J, Jones D, Muasher S: High doses of gonadotropins have no detrimental effect on in vitro fertilization outcome in normal responders. *Fertil Steril* 1995;63:1333-1336

7. Manzi DL, Thorton KL, Scott LB, Nulsen JC: The value of increasing the dose of human menopausal gonadotropins in women who initially demonstrate a poor response. *Fertil Steril* 1994;62:251-256
8. Land JA, Yarmolinskaya MI, Dumoulin JCM, Evers JLH: High-dose human menopausal gonadotropin stimulation in poor responders does not improve IVF outcome. *Fertil Steril* 1996;65:961-965
9. Padilla SL, Dugan K, Maruschak V, Shalika S, Smith RD: Use of the flare-up protocol with high dose human follicle stimulation hormone and human menopausal gonadotropins for in vitro fertilization in poor responders. *Fertil Steril* 1996;65:796-799
10. Antoine JM: Which stimulation for poor responders? Critical study of results and methods of research. *Contracept Fertil Sex* 1996;24:386-390
11. Lindheim SR, Barad DH, Witt B, Ditkoff E, Sauer MV: Short-term gonadotropin suppression with oral contraceptives benefits poor responders prior to controlled ovarian hyperstimulation. *J Assist Reprod Genet* 1996;13:745-747