Pregnancy rates in varying age groups after in vitro fertilization: A comparison of follitropin alfa (Gonal F) and follitropin beta (Follistim)

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OBJECTIVE: Our purpose was to assess the efficacy of two recombinant follicle-stimulating hormones, follitropin beta (Follistim, Organon, West Orange, NJ) and follitropin alfa (Gonal F, Serono, Norwell, Mass) on pregnancy rates in varying age groups of women undergoing in vitro fertilization (IVF).

STUDY DESIGN: Three hundred sixty-five IVF cycles were retrospectively compared, 233 by use of follitropin beta and 132 by use of follitropin alfa, both after gonadotropin-releasing hormone agonist down-regulation. Assignment to each medication was indiscriminate. The primary outcome measured was pregnancy evidenced by fetal heartbeat on ultrasonography. Secondary outcomes included days of stimulation, ampules per patient cycle, estradiol level on the day of human chorionic gonadotropin administration, total follicles present on the day of human chorionic gonadotropin administration, follicles greater than 14 mm, oocytes retrieved, mature eggs, fertilization rate, and embryos transferred. Outcomes were stratified by age, including women less than 36 years old, 36 to 39 years old, and more than 39 years old.

RESULTS: There was no significant difference between follitropin beta and follitropin alfa in either the primary or secondary outcomes, although the pregnancy rate was significantly decreased with advancing age.

CONCLUSION: Success rates are similar, when stratified by age, in women undergoing IVF with either follitropin beta or follitropin alfa. (Am J Obstet Gynecol 2003;189:342-7.)

Key words: Recombinant follicle-stimulating hormone, follitropin alfa, follitropin beta, gonadotropin, in vitro fertilization

Follicle-stimulating hormone (FSH) is the primary hormone responsible for follicular recruitment and development. Follitropin beta (Follistim, Organon, West Orange, NJ) and follitropin alfa (Gonal F, Serono, Norwell, Mass) are recombinant FSHs (rFSH), both administered subcutaneously. They are synthesized by transfecting Chinese hamster ovarian cells with a plasmid that encodes for FSH, with subsequent protein production and glycosylation. Prior studies have shown an individual variability in response to rFSH. This has been found to be mainly related to ovarian sensitivity to FSH rather than to a difference in pharmacokinetics.1

Follitropin beta and follitropin alfa share identical amino acid sequences but differ from each other and human FSH as a result of their carbohydrate side chains. It has been shown that the clinical efficacy of FSH principally may be related to the proportion and amount of acidic isoforms and to the degree of its molecular complexity.2 Follitropin alfa has a lower pH than follitropin beta. Some studies have shown that this enhances receptor affinity, delays elimination time, and is a better inducer of folliculogenesis.3-5 Information about the possible clinical superiority of one preparation over the other is still under debate. Although prior studies have compared follitropin beta and follitropin alfa in pregnancy and delivery and found no statistical difference, no studies have compared the two drugs in different age groups.5,8

It is well known that fertility is negatively correlated with increasing age, as a result of both ovarian and endometrial factors.9 The implantation rate as a function of age has been reported to be constant up to age 35 years. Thereafter, a decline in implantation starts, with lower oocyte quality as a major factor for lower implantation rates, although uterine factors may contribute.10 Because many women of varying ages undergo assisted reproduction, it would be of benefit to tailor medical regimens more specifically and, therefore, minimize the time and financial investments patients expend on in vitro fertilization (IVF). Through this study, we investigated whether there is a difference in pregnancy rates between follitropin beta and follitropin alfa in women of varying ages.
Material and methods
A retrospective review of 365 IVF cycles at the University of Florida/Shands Hospital, 233 with follitropin alfa (Gonal F) and 132 with follitropin beta (Follistim), was performed with use of a comprehensive IVF computer database. All patients gave signed informed consent before participating in the IVF program at this tertiary care hospital. In addition to their infertility workup, which led to the selection of IVF as their best infertility therapy, all patients had, within a year of their treatment cycles, an assessment of the uterine cavity, semen analysis, and a clomiphene citrate challenge test. Exclusion criteria for IVF included abnormal ovarian reserve, age >42 years, or a uterine cavity abnormality before repair.

All cycles involved midluteal down-regulation with a gonadotropin-releasing hormone agonist (leuprolide acetate) for at least 10 days starting on cycle day 18 of a cycle in which an oral contraceptive was used. Luteal down-regulation was confirmed by ultrasonography and serum estradiol levels. The choice of the rFSH preparation was indiscriminate and made by the nursing staff. The normal starting dose of gonadotropin for ovarian stimulation was 300 IU for 2 days, followed by 225 IU daily for 2 days, unless otherwise indicated by previous gonadotropin response or patient age. One of the ampules of rFSH was replaced daily with FSH/luteinizing hormone (LH). After the first 4 days of stimulation, the dose of FSH was titrated on the basis of patient’s response. Follicular development and endometrial growth were monitored by vaginal ultrasonography in combination with blood samples for estradiol levels.

When an adequate stimulation was achieved (ie, a controlled rise in serum estradiol and two leading follicles with mean diameters of at least 18 mm), 10,000 IU of human chorionic gonadotropin (hCG) was given to effect final maturation of the follicles. Approximately 36 hours later, ovum retrieval was performed by transvaginal ultrasound-guided follicle aspiration. The nuclear maturity of oocytes was assessed according to established criteria. Fertilization by microdrop insemination or intracytoplasmic sperm injection (ICSI) was performed by standard procedures. ICSI was used if there was a sperm count of $<1 \times 10^6$ or previous poor fertilization had occurred in another IVF cycle. Embryos were cultured and then transferred approximately 72 hours later. The number of embryos transferred depended on the condition of the embryos and patient age, but two embryos were generally transferred in patients less than 35 years old and three embryos were transferred in patients greater than 35 years old. Luteal phase support was given with 50 to 100 mg of progesterone in oil intramuscularly daily, depending on the patient’s age. Clinical pregnancy was defined as sonographic visualization of at least one intrauterine fetus with cardiac activity.

The primary end point of this study was a clinical pregnancy. Secondary outcomes included number of days of stimulation, number of ampules per patient, serum estradiol level on the day of hCG administration, total number of follicles present on the day of hCG administration, number of follicles that measured greater than 14 mm on the day of hCG administration, the number of eggs retrieved, the number of mature eggs, the fertilization rate, and the number of embryos transferred. These outcomes were stratified with women less than or equal to 35 years of age, ages 36 to 39 years, and women 40 years of age or older.

Raw data were abstracted from the IVF database formatted in Microsoft Access. The data were then analyzed with use of SAS statistical software (SAS Institute, Cary, NC). A P value of < .05 was considered significant for all testing. Similarity of treatment groups on age, diagnosis, and pregnancy history was assessed with a two-sample $t$ test or $\chi^2$ where appropriate. Difference in secondary outcomes between the treatment groups was stratified on age group and tested with the Cochran-Mantel-Haenszel $\chi^2$ test or analysis of variance as appropriate to the measure. The primary end point of clinical pregnancy was stratified on age group and tested for differences in proportion with Cochran-Mantel-Haenszel $\chi^2$ test. The Fisher exact test was needed for one of the strata (age >40 years). Additionally, a logistic regression model was developed with clinical pregnancy as response. Predictor variables included age, diagnosis, insemination procedure, and treatment group (FSH type).

Results
Overall, the mean patient age of those receiving follitropin alfa was 33.75 years and the mean age of those receiving follitropin beta was 34.07 years (not significant) and there was no significant difference in the proportion of patients in each age group (<36, 36-39, >39 years) receiving follitropin beta versus follitropin alfa (Table I). The mean patient gravidity was 1.09 in the follitropin alfa group and 0.94 in the follitropin beta group (not significant). The mean number of spontaneous abortions before IVF participation was 0.39 in those receiving follitropin alfa and 0.26 in those receiving follitropin beta (not significant). There were mildly significant ($P = .02$) differences in the distribution of primary diagnosis between treatment groups (Table II), which were not considered to be clinically significant because nationally no difference in pregnancy rates have been seen with varying diagnosis (Society for Assisted Reproductive Technology/ Centers for Disease Control and Prevention 2000 data). In addition, although the follitropin alfa group had a higher percentage of male infertility, the fertilization rates after appropriate treatment (ICSI) was identical in both groups, so the pregnancy rate should not be affected in each group.
In patients less than or equal to 35 years old there was no difference between groups in the number of vials used per cycle or in the estradiol level on the day of hCG administration. A mean of 22.3 vials of follitropin alfa were used per cycle compared with 25.5 vials of follitropin beta. The mean estradiol levels on the day of hCG administration were 1801 (SD 1007) pg/mL for follitropin alfa and 1677 (SD 863) pg/mL for follitropin beta.

In patients 36 to 39 years old there was no difference in the number of vials used per cycle or in the estradiol level on the day of hCG administration. A mean of 34 vials of follitropin alfa and 33.2 vials of follitropin beta were used per cycle. The mean estradiol levels on the day of hCG administration were 1418 (SD 1014) pg/mL for follitropin alfa and 1445 (SD 877) pg/mL for follitropin beta.

In patients equal to or greater than 40 years old no significant difference was seen in the number of vials used for stimulation. A mean of 42.6 vials of follitropin alfa and 47.3 vials of follitropin beta were used per cycle. The mean estradiol levels on the day of hCG administration were not statistically different at 1004 (SD 716) pg/mL for follitropin alfa and 864 (SD 665) pg/mL for follitropin beta.

Overall, a statistically significant decrease in the estradiol level occurred on the day of hCG administration, whereas the number of vials used increased with increasing age ($P < .0001$).

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Additional secondary results can be seen in Table III. There was no statistical difference overall or when stratified for age or when evaluating the mean number of retrieved oocytes, the mean number of mature oocytes, the mean number of immature oocytes, the mean number of fertilized oocytes, and the mean number of embryos transferred. There were an increased number of ICSI inseminations in the follitropin alfa group, but this did not affect the fertilization rate in the groups. However, with both drugs there was a significant decrease in follicle number with increasing age ($P < .0001$).

Overall, there was no difference in pregnancy rate as evidenced by cardiac activity on ultrasound between the groups receiving follitropin beta and follitropin alfa. Table IV depicts clinical pregnancy success rates by treatment and age. Treatment group equivalence with respect to outcome was confirmed with age group-stratified (Cochran-Mantel-Haenszel) $\chi^2 (P = .121)$ and multiple logistic regression ($P = .240$). The 95% CI on the odds ratio for pregnancy (follitropin beta/follitropin alfa) was 0.82 to 2.81. Logistic regression also revealed that age was the only significant ($P < .0001$) predictor of clinical pregnancy. Regardless of the drug used, diagnosis, or insemination procedure, the odds of pregnancy were reduced by 10% per year of advancing age (Figure).

**Comment**

Recombinant FSH preparations have been marketed in Europe since 1996 and shortly thereafter in North America. Previously, gonadotropins were derived only from the urine of postmenopausal women (hMG). These hMG preparations contained an equal amount of FSH.
and LH. Because it became evident that even in gonadotropin down-regulated cycles exogenous LH was usually not necessary and could potentially be detrimental, purified urinary FSH preparations were developed. In addition to a more purified form of FSH, these products also reduced but did not eliminate urinary proteins. Allergic reactions, although uncommon, occurred as a result of these contaminating proteins. Expectations of limited raw material, a need for more consistent batch-to-batch bioavailability, and future concerns about infectious contamination led to the drive to develop alternate sources for gonadotropins.

Recombinant technology could reproducibly produce simple proteins, but gonadotropin structure was much more difficult to replicate. Each gonadotropin is composed of an α chain and a β chain that is transcribed separately and subsequently combined. Glycosylation of the protein backbone also occurs in the pituitary cell before release. These steps of producing recombinant α and β chains with subsequent glycosylation could only be accomplished in mammalian cells, thus the use of Chinese hamster ovary cells. The two available recombinant FSH drugs on the market are identical with respect to their amino acid structure to endogenous FSH, but they have slightly different glycosylation. This difference results in a more acidic preparation for follitropin alfa. This study sought to answer whether this difference is clinically significant in various-aged women undergoing IVF.

Past studies have indicated that both of the recombinant FSH preparations currently on the market are equally suitable for use in ovarian stimulation and have no significant clinical difference regarding number of follicles stimulated, number of oocytes retrieved and fertilized, or in the pregnancy rate in patients less than 40 years old.

We have reported in this study that not only are the preparations equal in their ability to stimulate IVF patients when examined as a whole, but that when age is considered there is no difference in clinical outcome between treatment groups. When patients were stratified by age, there was no difference in number of vials used, days of stimulation, peak estradiol level, total number of follicles, follicles >14 mm, number of mature oocytes retrieved, fertilization rates by either insemination or ICSI, or the rates of viable clinical pregnancy as defined by ultrasonographically demonstrated cardiac activity.

Although pregnancy rates, number of follicles stimulated, and peak estradiol levels were no different within each age group, we showed a declining response of older women to gonadotropin stimulation. Despite an increased amount of FSH administered to older women, the number of follicles stimulated, peak estradiol levels, and ultimately the pregnancy rate was reduced in the older age groups. This finding was not unexpected because the effect of advancing reproductive age in women is well known. However, in this study the age effect was even seen in women less than 35 years old, with a reduction of pregnancy of about 10% per year of advancing age when the data were analyzed by logistic regression.

Overall, there was no difference in pregnancy rate or any other secondary end point of ovarian stimulation in women undergoing IVF after luteal down-regulation when follitropin beta versus follitropin alfa was used for follicular development at any age, but, regardless of agent used, the odds of pregnancy drop by 10% per year of advancing age.

REFERENCES

large numbers of quality oocytes are available, resulting in large numbers of quality embryos, some of these are available for freezing to be used for frozen embryo transfer at a later time without the need for additional gonadotropin stimulation. Although pregnancy rates with frozen embryos are generally only approximately one third of those with fresh embryos, when one adds that additional pregnancy likelihood for each resultant frozen embryo transfer, the total fecundability of one stimulated cycle is realized.

A unique characteristic of this study is that comparisons were made by age of the female patient. Within the categories established there were no differences in any parameters assessed. The American Society of Reproductive Medicine recently initiated a campaign to encourage the prevention of infertility. This was done as a public service and took the form of posters placed on buses in four major US cities. The four life choices chosen for emphasis were (1) decreasing smoking, (2) maintaining appropriate weight, (3) preventing sexually transmitted diseases, and (4) encouraging conception at the earliest reasonable time.

A couple of years ago I had the privilege of reviewing the paper Dr Williams' presented for induction into our society. It dealt with the various factors contributing to success rate in donor insemination. The single most important factor Dr Williams pointed to in success of donor insemination was to the age of the woman. This seems to be a recurring theme in recent times, long ago understood, but accentuated now by the very good data that we have accumulated from activity in the assisted reproductive technologies. It may well turn out to be that in the use of gonadotropins, whether urinary products or the newer recombinant DNA ones, the most important variables are those in the patient rather than those in the drug, and that of all patient variables advancing age seems to be the greatest limiting factor to overcoming infertility.

In short, for those who do have to turn to the complex and arduous journey through IVF to reach “fertility nirvana,” at present it doesn’t seem to matter whether you go in a Ford or a Chevrolet—but you’d better start out early.

Dr Williams, did you look at differences in body weight or body mass index as it related to success rates? Although there was no statistically significant difference in pregnancy rates by age between these two drugs, what was the power of the study? Do you have any predictions on the future utility of the current availability of gonadotropins being able to be prescribed by mass or weight rather than by perhaps less-predictable international units reflecting bioavailability?

REFERENCES


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Discussion

Dr Barry Verkauf, Tampa, Fla. The history of gonadotropin preparations to stimulate ovarian activity begins in the late 1930s. These gonadotropins were of animal origin, extracted from pig pituitaries or from pregnant mare serum. During the 1950s, researchers turned to extracting human gonadotropins from pituitaries of cadavers and from human menopausal urine; the latter remained its principal source for almost five decades. The first urinary-derived hMG, Pergonal, was impure, and even in recent hMG preparations only 1% to 2% of the protein present is active gonadotropins.

As Dr Williams mentioned, concerns relative to availability of sufficient urine to meet product needs, the variability in batches of gonadotropins made from urine, process complexity, risk of sensitization from protein impurities, potential mediation of infection, and perhaps other undisclosed concerns drove the search for production of highly purified urinary FSH and then recombinant products for clinical use. For some time it has been touted that the lack of LH in these highly purified recombinant products is not deleterious. Recently this has been questioned. Numerous studies of a variety of sizes, quality, and research formats have been performed over the past several years comparing the effectiveness of these various gonadotropin products. Because they can be given subcutaneously, the newer recombinant ones are clearly more convenient, but there does not seem to be much difference in terms of resulting pregnancy rates.

In this paper Dr Williams has narrowed the focus by looking at two specific isoforms of essentially the same molecule, each produced by different manufacturers by recombinant technology to see if there were differences in clinical efficacy. This is a retrospective cohort study, not randomized, but with indiscriminate assignment of drug. In all parameters surveyed, there were no significant differences between the two products. The principal focus, pregnancy rates, were similar; secondary characteristics assessed such as a number of total follicles present, number of oocytes retrieved, number of mature eggs, and fertilization rate did not differ either. These secondary issues are important because of the concept of total cycle fecundability associated with an IVF cycle.