Comparison of Highly Purified FSH (Metrodin-High Purity) with Pergonal for IVF Superovulation

J. S. BAGRATEE,¹ G. LOCKWOOD,² A. LÓPEZ BERNAL,² D. H. BARLOW,² and W. L. LEDGER^{2,3}

Submitted: August 19, 1997
Accepted: September 22, 1997

Purpose: The use of highly purified follicle-stimulating hormone (Metrodin-HP) was compared with that of a preparation containing follicle-stimulating hormone and luteinizing hormone (Pergonal) for production of superovulation in an IVF program.

Methods: We used the Oxford Fertility Unit database to identify patients undergoing their first cycle of IVF, using either Metrodin-HP or Pergonal. Patients were treated with a standardized drug protocol and were stratified by age and cause of infertility. Ovarian stimulation with either Metrodin-HP (Serono Laboratories) or human menopausal gonadotropin (hMG; Pergonal; Serono Laboratories) after pituitary desensitization commenced in the midluteal phase of the preceding cycle. Monitoring was performed by ultrasound and serum estradiol measurement prior to transvaginal oocyte recovery, followed by IVF and transfer of no more than three embryos.

Results: For Metrodin-HP versus Pergonal, the rates of egg retrieval (98 vs 94%), fertilization (89 vs 92%), clinical pregnancy (32.9 vs 23.4%), miscarriage (4.1 vs 4.5%), live birth (26 vs 18.5%), and ovarian hyperstimulation syndrome (5.5% vs 5.9%) were similar in both groups. The apparent increase in clinical pregnancy and live birth with Metrodin-HP did not reach statistical significance. The dosages of gonadotropins used were comparable. Estradiol levels measured on day 8 of stimulation were significantly lower in the Metrodin-HP group than in the Pergonal group, but the difference did not reach statistical significance on the day of hCG administration. Significantly more follicles (greater than 12 mm) were obtained in the Metrodin-HP group, but the numbers of eggs recovered and fertilized were similar in the two groups.

Conclusions: These findings demonstrate that highly purified FSH (Metrodin-HP) is as effective and successful as

hMG (Pergonal) for ovarian stimulation in a standard IVF regimen. Exogenous luteinizing hormone (LH) is not required for satisfactory ovarian stimulation in IVF. Measurement of estradiol may be less helpful in the monitoring of Metrodin-HP cycles, but the level reached on the day of hCG administration can still be used to predict, and hence avoid, ovarian hyperstimulation syndrome.

KEY WORDS: gonadotropins; hyperstimulation; Metrodin-HP; ovarian stimulation; Pergonal.

INTRODUCTION

Gonadotropin preparations in current clinical use have been derived from the urine of postmenopausal women for more than 20 years. All contain both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The first preparation of human menopausal gonadotropin (hMG) was produced with equal amounts of FSH and LH, initially for the purpose of stimulating folliculogenesis in patients with amenorrhea due to a hypothalamic or pituitary disorder. Concerns about the possibly deleterious effects of the LH content of such preparations led to the development of more highly purified preparations of FSH (u-FSH; Metrodin; 75 IU FSH:<1 IU LH), which induce normal follicular development in women with gonadotropin deficiency (1) and have been used extensively with agonists of GnRH to stimulate multifollicular development for IVF, resulting in successful pregnancy (2). Gonadotropin therapy following pituitary desensitization with GnRH agonists is now a widely accepted protocol for ovarian stimulation prior to IVF and is associated with lower cycle cancellation rates and improved pregnancy rates compared to the use of gonadotropins alone (3).

As more highly purified preparations of FSH have become available for clinical use, anxieties have been expressed that the combination of highly purified FSH with GnRH analogue-mediated pituitary desensitization could result in a suboptimal circulating concentra-

¹ University of Natal and King Edward VIII Hospital, Durban, South Africa.

² Nuffield Department of Obstetrics & Gynaecology, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom.

³ To whom correspondence should be addressed.

tion of LH, with an adverse effect on the eventual yield of oocytes, or their quality. However, the requirement of the ovary for LH during folliculogenesis seems small, with basal levels of LH being sufficient to stimulate maximal thecal steroidogenesis in women who have not undergone pituitary downregulation (4). Indeed, in certain circumstances reduction of circulating levels of LH may be beneficial, because high levels of LH in the follicular phase may be associated with lower conception rates and higher rates of spontaneous abortion (5,6).

Because additional exogenous LH is not required for ovarian steroidogenesis, and high follicular-phase LH may be associated with a poor reproductive outcome, it seems clinically appropriate to use a gonadotropin preparation containing only FSH activity. A highly purified FSH (u-FSH-HP; Metrodin-HP; Serono Laboratories) has been pharmaceutically developed by immunoextraction of FSH from human postmenopausal urine. It has a specific activity of 9000 IU FSH/mg protein, and as greater than 95% of the protein content is FSH, it can be administered subcutaneously, with greater patient convenience. This route of administration results in FSH pharmacokinetics similar to those observed after intramuscular injection of highly purified u-FSH (7).

The purpose of this study was to compare the reproductive performance of patients using Metrodin-HP and hMG for ovarian stimulation in IVF cycles.

MATERIALS AND METHODS

The Oxford IVF database is a prospectively collected database of all IVF cycles carried out in the Oxford IVF unit and contains extensive clinical and laboratory details of the IVF cycle. In this study, we obtained data about all patients who underwent their first IVF cycle during a period of 18 months from December 1993.

Patients were allocated to receive either hMG (Pergonal) or u-FSH-HP (Metrodin-High Purity). Allocation to treatment was nonrandomized. Because all couples in this study were entering their first cycle of IVF, there were no firm data on which to base the choice of gonadotropin preparation. Patients who had previously undergone intrauterine insemination (IUI) with Metrodin-HP stimulation invariably chose this preparation. This group, by definition, had at least one patent fallopian tube and unexplained or moderate male-factor infertility. There was also a tendency for women with polycystic ovary syndrome (PCOS) to be

treated with Metrodin-HP, although this was not a specific requirement. Details of the distribution of patients between the two groups are presented in Table I.

All IVF cycles used a long protocol of pituitary downregulation using nafarelin (Synarel, Syntex Ltd.), beginning in the midluteal phase of the cycle. Pituitary desensitization was confirmed 17–24 days later with a measurement of plasma estradiol of less than 30 pg/ml (less than 110 pM). Once desensitization had been achieved, stimulation with Pergonal or Metrodin-HP was commenced at a daily dosage determined for the individual patient on the basis of age, weight, and early follicular phase gonadotropin concentrations. IVF unit staff were not blinded to the preparation used, because women using Metrodin-HP were encouraged to self-inject. Ovarian stimulation was monitored by daily ultrasound measurement of follicular diameters from the eighth day of gonadotropin administration. Serum estradiol assay was performed on day 8 and on the day of human chorionic gonadotropin (hCG) administration. Criteria for hCG administration were the presence of three follicles 16 mm or greater in diameter or five follicles 15 mm or greater in diameter. If serum estradiol concentrations exceeded 2000 pg/ml (7320 pM) and there were 15 or more follicles of 12 mm or greater diameter, further stimulation was withheld and hCG was not administered, to avoid the risk of severe OHSS (8).

Once the criteria for hCG administration were reached, analogue and gonadotropin administration was discontinued and an i.m. injection of 10,000 IU hCG (Profasi, Serono Laboratories; or Pregnyl, Organon Laboratories) was given.

Transvaginal oocyte recovery was performed 35 hr after hCG administration. Analgesia and sedation were

Table I. Patient Age and Causes of Infertility

	Metrodin-HP (n = 73)	hMG (n = 222)	P
Age (yr) ^a	33.4 ± 4.3 (24–42)	33.3 ± 4.5 (23–43)	NS ^b
Cause ^c			
Tubal	24 (33)	102 (46)	NS
Endometriosis	15 (21)	52 (23)	NS
Male factor	13 (18)	61 (27)	NS
PCOS	7 (10)	7 (3)	0.054
Other ovulatory problems	22 (30)	86 (39)	NS
Unexplained	7 (10)	16 (7)	NS

^a Range given in parentheses.

^b Not significant.

^c Percentage given in parentheses.

achieved using i.v. pethidine and diazepam. Oocytes were incubated for 5–6 hr prior to insemination with freshly prepared semen or thawed cryopreserved semen, where the use of donor spermatozoa was indicated. Each oocyte was inseminated with 400,000 spermatozoa in 2 ml of medium, and the oocytes were checked 12–18 hr later for the presence of two pronuclei.

On day 2, all embryos were assessed and the best three were selected for transfer on the basis of morphology, cleavage rate, quality of oocyte, and follicular size. All routine culture procedures were performed in Tyrode's T6 medium, supplemented when possible with 10% heat-inactivated serum from the patient. A maximum of three embryos was transferred to the uterus transcervically 50–60 hr after oocyte retrieval. Luteal-phase support was provided by hCG injections for patients having low or average (1000 or less pg/ml) estradiol concentrations on the day of hCG or by progesterone pessaries for those with estradiol levels above this threshold.

A pregnancy test was carried out using a monoclonal antibody-based urine test 2 weeks after embryo transfer. Patients with a positive test were scanned 4 weeks later to confirm the viability of the pregnancy and detect multiple sacs.

Statistical Analysis

Continuous data were summarized using means and SD, and both groups were compared using two-tailed Student's *t* test or χ^2 test as appropriate.

RESULTS

Table I presents the patient characteristics of those who received Pergonal or Metrodin-HP. There was no significant difference between the two groups with regard to mean age and cause of infertility.

Table II lists the characteristics of the stimulation regimen of the patients in the two groups. Both groups were similar with regard to the duration of analogue administration and the dosage and duration of both Pergonal and Metrodin-HP administration. There was no difference in the cancellation rate between the two gonadotropins prior to ovum pickup. One case (1.4%) in the Metrodin-HP group was canceled because of an excessively high estradiol level, suggesting a risk of ovarian hyperstimulation, whereas seven (3.2%) Pergonal cycles were canceled for this reason. More than

Table II. Stimulation Regimen

	Met-HP	hMG	P
Days of GnRH-a ^a	37.3 ± 10.2 (27–115)	37.4 ± 8.9 (27–81)	NS ^b
Days on gonadotropins ^a	8.9 ± 1.6 (7–12)	9.3 ± 1.5 (7–12)	0.053
Total ampoules used	27.7 ± 11.5	25.5 ± 10.9	NS
Cycles abandoned before OPU ^{c,d}	1 (1.4)	13 (5.9)	NS
No. of cycles for OPU ^d	72 (98)	209 (94)	NS

^a Range given in parentheses.

^b Not significant.

^c Ovum pickup.

^d Percentage given in parentheses.

90% of the patients treated with either gonadotropin reached oocyte recovery.

Table III shows the ovarian response and fertilization characteristics of patients treated with Pergonal or Metrodin-HP. Both groups commenced with similar basal estradiol levels, but day 8 estradiol levels were significantly lower in patients treated with Metrodin-HP. However, by the day of hCG administration, estradiol levels were similar in both groups. Significantly more follicles developed in patients treated with Metrodin-HP, but the number of eggs recovered and the fertilization rate were similar in both treatment groups.

Table IV outlines the outcome for IVF-ET. There were no significant differences with regard to clinical pregnancies, miscarriages, live births, and OHSS.

Table III. Ovarian Response and Fertilization

	Metrodin-HP (n = 73)	hMG (n = 222)	P
Basal estradiol (time of down-reg) (pg/ml) ^a	14.9 ± 6.2 (3–28)	13.3 ± 7.4 (1–30)	NS ^b
Day 8 estradiol (pg/ml) ^a	562 ± 420 (32–1902)	734 ± 446 (18–2396)	0.004
Day of hCG estradiol (pg/ml) ^a	1284 ± 695 (217–3750)	1402 ± 698 (176–4178)	NS
No. of follicles ^a	14.8 ± 6.5 (3–32)	13 ± 6.0 (2–33)	0.032
Diameter of leading follicle (mm) ^a	20.7 ± 2.1 (17–25)	20.8 ± 2.4 (17–24)	NS
No. of eggs recovered ^a	8.5 ± 4.0 (2–20)	7.5 ± 4.1 (0–25)	NS
No. of eggs fertilized ^a	6.1 ± 5.2 (0–17)	5.1 ± 4.4 (0–18)	NS
No. of embryos transferred ^a	2.05 ± 1.1 (0–3)	1.8 ± 1.6 (0–3)	NS
No. of cycles failed fertilization	8 (11%)	17 (8%)	NS
Fertilization rate	89%	92%	NS

^a Mean ± SE (range).

^b Not significant.

Table IV. Outcomes

	Metrodin-HP (n = 73)	hMG (n = 222)	P
Clinical pregnancies	24 (32.9) ^a	52 (23.4)	NS ^b
Miscarriages (less than 19 weeks)	3 (4.1)	10 (4.5)	NS
Ectopics	2	1	NS
Live births	19 (26)	41 (18.5)	NS
Singletons	14 (74)	23 (56.1)	NS
Twins	5 (26)	15 (36.6)	NS
Triplets	0	3 (7.3)	NS
OHSS (mild)	4 (5.5)	13 (5.9)	NS

^a Percentage given in parentheses.^b Not significant.

More patients in the Metrodin-HP group (26%) had live births compared to the Pergonal group (18.5%), but this was not statistically significant ($P = 0.22$).

DISCUSSION

This study of a prospectively collected data set shows that highly purified FSH (Metrodin-HP) is at least as effective as hMG (Pergonal) in an IVF ovulation induction regimen. The IVF performance endpoints, including cancellation rate, duration and dose of gonadotropin, oocyte parameters, fertilization rate, pregnancy rate, and live birth rate, were all similar in the two groups. Thus the substantial reduction in circulating levels of LH seen after pituitary down-regulation and stimulation with highly purified FSH does not appear to have an adverse effect on folliculogenesis and oocyte maturation. This finding is reassuring, given the impending arrival of recombinant FSH preparations that are devoid of any LH activity.

We observed a significant reduction in the amount of estradiol secreted on day 8 of stimulation in the Metrodin-HP group, and the amount of estradiol secreted per follicle also tended to be lower on the day of egg collection after Metrodin-HP. Our study was nonrandomized, so this result must be treated with caution. However, if confirmed in a prospective, randomized study, this finding could be of clinical significance because many IVF units use estradiol levels measured on day 8 of stimulation to predict and hence avoid OHSS. The criteria used for prediction of OHSS may have to be altered in Metrodin-HP or recombinant FSH-stimulated cycles, and it may be necessary to use other markers of follicular activity for prediction of OHSS.

Metrodin-HP has previously been compared with other gonadotropin preparations by Wikland *et al.* (9, 10) and Hull *et al.* (11). The study by Wikland *et al.* (9) obtained a 16.4% live birth rate using hMG and a calculated live birth rate of 31.3% using Metrodin-HP (not all patients had delivered by the completion of the study). Metrodin-HP alone gave a significantly higher pregnancy rate than a combination Metrodin-HP/Pergonal regime in a subsequent study (35 vs 18%) (10), whereas Hull *et al.* (11) found comparable clinical pregnancy rates for Metrodin and Metrodin-HP of 29 and 25% per cycle, respectively. Our results suggest a higher pregnancy and live birth rate after Metrodin-HP, but this trend was not significant. However, none of the comparative studies suggest any reduction in pregnancy rate after Metrodin-HP treatment. Many patients in our programs of ovulation induction, intra-uterine insemination, and IVF favor the ease of administration offered by Metrodin-HP, with subcutaneous administration and auto injection allowing treatment by the patient herself at home. The present study, coupled with those from elsewhere, allows Metrodin-HP users to be reassured that the new treatment regimen is at least as successful as the traditional use of intramuscular menopausal gonadotropins.

This study confirms the effectiveness of highly purified FSH for stimulation of follicle growth in IVF. A prospective, randomized trial is now needed to ascertain whether the perceived benefits of Metrodin-HP (ease of use and possibly a higher live birth rate) outweigh the cost disadvantage of using this gonadotropin rather than hMG.

REFERENCES

1. Couzinet B, Lestrat N, Brailly S, Forest M, Schaison G: Stimulation of ovarian follicular maturation with pure follicle-stimulating hormone in women with gonadotrophin deficiency. *J Clin Endocrinol Metab* 1988;66:552-556
2. Shaw RW, Ndkwe G, Imoedemhe DA, Bernard AG, Burford G: Twin pregnancy after pituitary desensitization with LHRH agonist and pure FSH. *Lancet* 1985;2:506
3. Hughes EG, Ferdorkow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA: The routine use of gonadotrophin-releasing hormone agonists prior to in vitro fertilization and gamete intra fallopian transfer. A meta-analysis of randomised controlled trials. *Fertil Steril* 1992;58:888-896
4. Bergh C, Olsson JH, Selleskog U, Hillensjo T: Steroid production in cultured thecal cells obtained from human ovarian follicles. *Hum Reprod* 1993;8:519-524
5. Homburg R, Armar NA, Eshel A, Adams J, Jacobs HS: Influence of serum luteinizing hormone concentrations on ovulation, conception and early pregnancy loss in polycystic ovary syndrome. *BMJ* 1988;297:1024-1026

6. Regan L, Owen EJ, Jacobs HS: Hypersecretion of luteinising hormone, infertility and miscarriage. *Lancet* 1990;336:1141–1144
7. Le Cottonec JY, Porchet HC, Beltrami V, Howles C: Comparative pharmacokinetics of two urinary human follicle stimulating hormone preparations in healthy female and male volunteers. *Hum Reprod* 1993;8:1604–1611
8. Forman RG, Frydman R, Egan D, Ross C, Barlow DH: Severe ovarian hyperstimulation syndrome using agonists of gonadotrophin-releasing hormone for in vitro fertilization. A European series and a proposal for prevention. *Fertil Steril* 1990;53:502–509
9. Wikland M, Borg J, Hamberger L, Salander P: Simplification of IVF: Minimal monitoring and the use of subcutaneous highly purified FSH administration for ovulation induction. *Hum Reprod* 1994;9:1430–1436
10. Wikland M, Nilsson L, Bergh C, Hamberger L: Results from in vitro fertilisation and intracytoplasmic sperm injection with purified gonadotrophins (Metrodin HP). *Hormone Res* 1995;43:238–240
11. Hull MGR, Joels LA, Prosser CJ, Ashcroft SA: Experience using preparations of follicle stimulating hormone alone to stimulate the ovaries for assisted conception after pituitary desensitization and simplified management of treatment. *Hormone Res* 1995;43:230–237