CLINICAL ASSISTED REPRODUCTION

Intracytoplasmic Sperm Injection After Follicle Stimulation with Highly Purified Human Follicle-Stimulating Hormone Compared with Human Menopausal Gonadotropin

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Purpose: Our purpose was to compare oocyte nuclear maturation and embryo quality after pituitary down-regulation and ovarian stimulation with highly purified follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG).

Methods: Fifty-five patients 37 years of age or younger who were undergoing in vitro fertilization (IVF)—intracytoplasmic sperm injection (ICSI) were evaluated retrospectively. In all cases, male factor was the only indication for treatment, with no female-related factors identified. Following pituitary down-regulation, patients were stimulated with hMG ($n = 20$) or highly purified FSH ($n = 35$). Main outcome measures included ovarian response to stimulation, oocyte maturity, and ICSI fertilization results. Secondary outcome measures included pregnancy rates and outcome.

Results: The ovarian response to stimulation was similar for the two groups, as were the percentage of metaphase II oocytes, fertilization and cleavage rates, and number and quality of transferred and cryopreserved embryos. Cycle outcome was comparable.

Conclusions: In normogonadotropic subjects, monocomponent therapy with highly purified FSH is as effective as hMG in stimulating ovarian follicular development, synchronization of oocyte maturation, and IVF-ICSI outcome. Our find-

ings support the conclusion that the luteinizing hormone component in the stimulation protocol is unnecessary.

KEY WORDS: GnRH-a; hMG; ICSI; oocyte maturity; pure FSH.

INTRODUCTION

Recently, the trend has been simplification of assisted reproductive technology (ART) programs (1,2). One factor in the simplification of treatment cycles has been the use of monogonadotropin therapy for controlled ovarian hyperstimulation (COH). Several groups (2–5) have reported apparently normal follicle and oocyte development, and excellent in vitro fertilization (IVF) outcome with the administration of follicle-stimulating hormone (FSH) alone during gonadotropin-releasing hormone agonist (GnRH-a) down-regulation protocols. These observations, together with the additional benefit of patient self-administration of highly purified FSH (hpFSH) by the subcutaneous route, have prompted us to incorporate hpFSH into all our ART programs, including the intracytoplasmic sperm injection (ICSI) program.

An important part of the ICSI procedure involves the removal of the oocyte–corona complex. This stripping of the vestments of the oocyte allows a precise determination of the stage of meiotic progression of the oocyte, and therefore, the nuclear maturity of the oocyte can be determined accurately. Using this approach, our group has recently reported that a greater percentage of mature oocytes results from ovarian stimulation with the long GnRH-a protocol compared

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with the short GnRH-a protocol (6). Furthermore, the fertilization and cleavage rates with ICSI were superior after the long GnRH-a stimulation protocol for COH (6). Despite the widespread use of pure FSH preparations in COH protocols, very few studies so far have focused on the nuclear maturity of denuded oocytes and compared the results with respect to the luteinizing hormone content of the gonadotropin preparation used in conjunction with the long GnRH-a protocol. The objectives of the present study were to determine whether oocyte maturity differs when human menopausal gonadotropin (hMG) or hpFSH is used for COH in the long GnRH-a protocol and whether the results of ICSI therapy are different between the two forms of gonadotropin preparations.

MATERIALS AND METHODS

Patients

This was a retrospective study including 55 couples with a diagnosis of male-factor infertility who underwent IVF-ICSI at the Toronto Centre for Advanced Reproductive Technology between September 1995 and December 1995. During this period, hpFSH was initially introduced into our clinical practice and, since then, has gradually replaced hMG for COH. This transition period has been used for the present analysis. Each female patient was to have met all the following criteria: (a) premenopausal and aged 18 to 37 years, (b) regular menstrual cycles of 21 to 35 days, (c) the presence of both ovaries, (d) normal baseline vaginal ultrasound scan, (e) no more than three previous IVF cycles, (f) no ART treatment or treatment with clomiphene citrate or gonadotropins for at least one full menstrual cycle, and (g) a normal uterine cavity. Infertility was attributable to male factor with no female factors identified, and potentially solvable by ICSI. Only ejaculated fresh semen was used for the ICSI procedure.

IVF-ICSI Procedure

All patients received leuprolide acetate (LA; Lupron, Abbott Pharmaceuticals, Montreal, Quebec, Canada), 1 mg subcutaneously daily, beginning on day 21 of the previous cycle up until cycle day 3, when pituitary down-regulation was evaluated by determination of serum estradiol concentration and transvaginal sonography (TVS) of the ovaries. If the serum estradiol level was less than 200 pM and no follicles greater

than 20 mm in diameter were noted on TVS, LA was decreased to 0.5 mg/day and 2 or 3 ampoules of gonadotropin was administered daily for 5 days, after which the dose was individualized according to ovarian response.

Patients received one of the following gonadotropin preparations according to the attending physician's preference and drug availability: hMG [Humegon, 75 IU (Organon, Toronto, Ontario, Canada), or Pergonal, 75 IU (Serono, Oakville, Ontario, Canada)] or hpFSH (Fertinorm, 75 IU; Serono). Human chorionic gonadotropin (10,000 IU; Profasi; Serono) was administered intramuscularly when at least two follicles were 18 mm or greater in diameter with serum estradiol levels within the attending physician's acceptable range for the number of mature follicles present.

Oocyte retrieval was performed 36 hr after the hCG administration by transvaginal ultrasound-guided needle aspiration under local anesthesia. Sperm preparation was performed using discontinuous Percoll (Pharmacia, Uppsala, Sweden) gradient centrifugation. Our technique for the ICSI procedure has been described in detail elsewhere (6). The oocytes were assessed for maturity just before the injection according to the criteria described by Veeck (7). The maturation stage was recorded as follows: prophase I, germinal vesicle visible, no polar body; metaphase I, no germinal vesicle visible, no polar body visible; metaphase II, no germinal vesicle visible, first polar body visible; and postmature, polar body visible, irregular degeneration of cytoplasm. Intracytoplasmic sperm injection was performed only on metaphase-II oocytes.

Approximately 18 hr after injection, the oocytes were checked for signs of fertilization (two pronuclei and two polar bodies). At 48 hr, embryos that had cleaved to the two-cell stage or further were identified and graded according to the criteria of Veeck (8) based on blastomere symmetry and volume of fragmentation. Up to three embryos were transferred to the uterus in 30 μ l of human tubal fluid containing 0.5% human serum albumin using a 3.5 French Tomcat catheter (Sherwood Medical, St. Louis, MO). Cleaving embryos in excess of three were cryopreserved.

Progesterone suppositories (Apothecary Shop, Markham, Ontario, Canada), 50 mg four times daily, administered by the vaginal route, were used for luteal-phase support starting on the day of embryo transfer. Luteal support was continued up to 14 days in the case of a negative pregnancy test or for at least the first 3 weeks of pregnancy if a patient conceived. A clinical pregnancy was defined as an ultrasound-confirmed

gestational sac within the uterus (which excludes ectopic and biochemical pregnancies). Each pregnancy was followed until its termination.

Statistics

The statistical package Sigmastat (Jandel Corporation, San Raphael, CA) was used for data analysis. Clinical characteristics of the two groups were analyzed using the unpaired Student's *t* test or the Mann-Whitney rank sum test. All other analyses were performed using chi-square analysis. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Cycle characteristics of the patients in the two groups are described in Table I. There were no cancellations due to poor ovarian response, lack of fertilization, or cleavage in any of the groups. The women who received hMG or hpFSH were similar in age, number of previous IVF cycles, number of gonadotropin ampoules used, duration of stimulation, serum estradiol concentrations on the day of hCG administration, the number of oocytes retrieved, and estradiol per oocyte ratio. Endometrial thickness was significantly increased following stimulation with hMG (12.0 ± 0.55 mm) compared to stimulation with hpFSH (10.5 ± 0.43 mm) (*P* = 0.041).

Oocyte maturity and results of the ICSI procedure are described in Table II. After stripping of the cumulus and assessment of nuclear maturity, there was no difference in the mean number and relative proportion of mature (metaphase-II) oocytes collected between hpFSH- and hMG-treated groups. The mean number of normally fertilized oocytes (two pronuclei) and the fertilization rate were also similar, about 65% in both

groups. No difference was found between the groups in the mean number and proportion of abnormally fertilized oocytes (more than two pronuclei), in embryo cleavage, or in the number of good-quality (grade-1 and -2) embryos obtained.

The outcome of embryo transfer is shown in Table III. All cycles in both treatment groups resulted in embryo transfer. There was no difference in the number of embryos replaced or cryopreserved between the groups. The clinical pregnancy rate per oocyte retrieval was 45.7% in the hpFSH group, compared with 35% in the hMG group (nonsignificant). One ectopic pregnancy occurred in the hpFSH group. Implantation, miscarriage, and live birth rates were all comparable between the two study groups.

DISCUSSION

Our study provides novel data about oocyte maturity and outcome of IVF-ICSI cycles comparing COH by hMG or hpFSH in the long protocol of GnRH-a administration. Our results suggest that hpFSH is as effective as hMG in stimulating ovarian follicular development and synchronization of oocyte maturation. Since monocomponent therapy with FSH induces adequate follicular development and favorable ICSI outcome, our findings support the conclusion that the use of luteinizing hormone in the usual ART stimulation protocol is unnecessary.

The ICSI procedure allowed us to assess nuclear maturity of the denuded oocyte in a more precise manner than conventional IVF. Ovarian response to COH and oocyte maturity are more directly related to the stimulation protocol used, as opposed to fertilization, cleavage, and implantation rates, where semen quality, laboratory conditions, uterine receptivity, and many yet undefined factors are also involved. To minimize

Table I. Clinical and Cycle Characteristics of Patients Stimulated with hpFSH and hMG^a

	hpFSH	hMG	<i>P</i>
<i>n</i>	35	20	
Age (years)	32.8 ± 0.6	33.2 ± 0.6	NS
Cycle number	1.31 ± 0.1	1.5 ± 0.2	NS
No. of gonadotropin amps used	30.4 ± 2.17	28.8 ± 1.2	NS
hCG day	13.1 ± 0.3	12.8 ± 0.4	NS
Estradiol (pg/mL) on hCG day	1805 ± 291	1840 ± 310	NS
No. of oocytes retrieved	13.6 ± 1.5	12.7 ± 1.7	NS
Estradiol:oocyte (pg/mL per oocyte)	143 ± 74	158 ± 31	NS
Endometrial thickness (mm)	10.5 ± 0.4	12.0 ± 0.5	0.0405

^a Values are means ± standard deviation. NS, not significant.

Table II. Oocyte Maturity and Results of the ICSI Procedure in Women Receiving hpFSH or hMG^a

	hpFSH	hMG	P
No. of oocytes in MII	10.1 ± 0.9	10.4 ± 1.4	NS
Percentage oocytes in MII	78.8 ± 2.6	83.0 ± 2.8	NS
0 pn	1.06 ± 1.2	1.6 ± 0.4	NS
2 pn	6.94 ± 0.7	6.6 ± 1.0	NS
>2 pn	0.86 ± 0.3	0.4 ± 0.2	NS
Normal fertilization rate (2 pn)(%)	68.9 ± 3.0	64.1 ± 4.1	NS
Cleaved zygotes	6.3 ± 0.6	6.4 ± 1.0	NS
Cleavage rate (%)	87.8 ± 3.2	88.2 ± 3.0	NS
G1 embryos	1.9 ± 0.4	1.3 ± 0.3	NS
G2 embryos	2.7 ± 0.4	2.8 ± 0.5	NS
G1 + G2 embryos	4.5 ± 0.5	4.1 ± 0.6	NS

^a Values are means ± standard deviation. MII, metaphase-II; NS, not significant; pn, pronuclei; G1, grade-1 embryos; G2, grade-2 embryos.

the variability in the effects of the aforementioned factors, we chose a relatively young female patient population, without identified infertility factors, and who underwent ICSI, in which consistently high fertilization and implantation rates can be achieved even when semen quality is poor. The specific criteria that we chose for inclusion of patients into our study undoubtedly restricted the size of the study group. Nevertheless, treating young female subjects who are free from any endocrine or anatomical abnormalities provides the rare opportunity to assess the clinical parameters involved with the IVF-ICSI procedure under almost-"optimal" conditions. Although this study was not randomized, we have used a relatively short period of 4 months for our analysis, to avoid the potential influence of time variation on laboratory protocols and conditions.

In a recent prospective study, Imthurn *et al.* (9), using a similar approach, compared oocyte maturity between hpFSH and hMG stimulation in IVF-ICSI using the short GnRH-a protocol. The proportion of mature oocytes (metaphase-II) and morphologically normal appearing oocytes was found to be significantly increased in the hpFSH group, thus leading to the conclusion that hpFSH synchronizes oocyte maturation better than comparable stimulation with hMG

when the short GnRH agonist protocol is being used. However, the use of the short protocol has not gained widespread acceptance, and the long protocol is presently the most frequently used. Compared with the short protocol, the longer regimen of GnRH-a administration has been found superior in terms of greater follicular recruitment, better oocyte recovery and quality, and higher fertilization and pregnancy rates (10). Our own experience has shown a significantly higher proportion of mature oocytes following pituitary down-regulation with the long protocol compared with the short protocol (81 vs. 26%, respectively; $P < 0.001$), and superior fertilization and cleavage rates after the long GnRH-a protocol (6).

Recently, Mercan *et al.* (11), in a retrospective study, compared the efficacy of a pure gonadotropin preparation (urinary hFSH) with a combination of urinary hFSH/hMG in the long GnRH-a protocol in patients undergoing conventional IVF-ET. All stimulation parameters, number of oocytes retrieved, proportion of mature oocytes (as determined indirectly by the appearance of the oocyte-corona-cumulus complex), and fertilization rates were similar in both groups. The clinical pregnancy rate per transfer was significantly higher in the hFSH-only group compared with the group receiving both hFSH and hMG.

Table III. Results of Embryo Transfer in Women Receiving hpFSH or hMG^a

	hpFSH	hMG	P
No. of embryos transferred frozen	2.8 ± 0.1	2.7 ± 0.2	NS
	2.6 ± 0.5	2.1 ± 0.7	NS
Clinical PR	45.7% (16/35)	35% (7/20)	NS
Implantation rate	21.42% (21/98)	16.7% (9/54)	NS
Miscarriage rate	12.5% (2/16)	14.3% (1/7)	NS
Live birth rate per oocyte retrieval	40% (14/35)	30% (6/20)	NS

^a Values are means ± standard deviation. NS, not significant; PR, pregnancy rate.

Several studies have suggested that the combination of pituitary down-regulation and hpFSH results in reduced concentrations of serum estradiol (12–14) and a longer stimulation phase (14), which may be attributed to profound down-regulation and low luteinizing hormone concentrations (14). A significantly lower fertilization rate with hpFSH compared to hMG was noted in an oocyte donation program (15). The actual duration and concentration of luteinizing hormone exposure required during follicular development in the human have not been established. In rats, the degree of endogenous luteinizing hormone activity required for normal estradiol production is known to be small because administered amounts that are not detectable in the circulation may be sufficient (16). Previous studies in the rat testis (17) and granulosa cells (18) suggest that less than 1% of luteinizing hormone receptors need to be occupied to elicit maximal steroid synthesis and secretion (the “spare receptor” hypothesis [17]).

Our results and those of other recent studies demonstrate equivalent serum estradiol concentrations to those achieved with the use of hMG with either hpFSH (present study; 19) or recombinant hFSH (20), suggesting that even with the long GnRH-a protocol, sufficient androgen substrate is present for maximal estradiol production. The hpFSH used in the present study has practically no luteinizing hormone activity, therefore, closely resembling recombinant hFSH. We have also included in our analysis the estradiol: oocyte ratio, which reflects the estradiol secretion per growing follicle (21). This ratio was derived from experience in hypogonadotropic hypogonadal women, where the amplitude of estradiol secretion per follicle is related directly to the dose of luteinizing hormone administered (21). The estradiol:oocyte ratio was comparable between our hpFSH and our hMG patients. This finding further confirms that following GnRH-a down-regulation, sufficient residual endogenous luteinizing hormone, or at least androgen substrate, was present for estrogen production during FSH-stimulated follicular development.

The only parameter where a difference was found between the two preparations was endometrial thickness, which was significantly increased in the hMG group compared with the hpFSH group (12.0 ± 0.55 vs. 10.5 ± 0.43 mm, respectively; $P = 0.04$). A similar trend was noted by Fried *et al.* (12), and it may be that luteinizing hormone or a product derived from its action may be important for endometrial growth. However, since endometrial thickness with both preparations was well within the range currently considered favorable for implantation and no differences were

noted in implantation or pregnancy rates, this difference is probably of no clinical significance.

In summary, by direct assessment of oocyte nuclear maturity, we found a comparable percentage of mature oocytes (metaphase-II) retrieved after COH with either hpFSH or hMG in the long GnRH-a protocol. Intracytoplasmic sperm injection performed on these oocytes resulted in similar fertilization, cleavage, implantation, and clinical pregnancy rates. Pregnancy outcome was also comparable for the two gonadotropin preparations. The present results suggest that, in combination with the long GnRH-a protocol, monocomponent therapy with FSH is sufficient for adequate follicular growth and development, and oocyte maturity and quality are not compromised in the absence of exogenous luteinizing hormone. Our observations are reassuring, and of major clinical relevance as we enter the era of recombinant DNA technology, where recombinant FSH preparations, without any luteinizing hormone activity, will be utilized for ART.

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