A prospective, randomized comparison of ovulation induction using highly purified follicle-stimulating hormone alone and with recombinant human luteinizing hormone in in-vitro fertilization

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The commercial availability of highly purified, s.c. administered urinary follicle stimulating hormone (FSH) preparations for ovarian stimulation marked the beginning of a new era in the treatment of infertility. As these new formulations contain essentially no luteinizing hormone (LH), supplemental LH may be needed for optimal folliculogenesis. It was the aim of this pilot study to compare fertilization rates, embryo morphology, implantation rates and pregnancy outcomes prospectively in two age-matched patient groups: women who received highly purified FSH (FSH-HP) (n = 17), and women who received FSH-HP plus recombinant human LH (rhLH, n = 14) throughout ovarian stimulation. All patients received mid-luteal pituitary down-regulation with s.c. gonadotrophin-releasing hormone (GnRHa) (leuprolide). Mean implantation rates were 26.9 and 11.9% in the FSH-HP only and FSH-HP + rhLH groups respectively. The mean clinical pregnancy/initiated cycle rate was 64.7 and 35.7% for the FSH-HP only and FSH-HP + rhLH patients respectively. FSH-HP patients and FSH-HP + rhLH patients achieved clinical pregnancy/transfer rates of 68.8 and 45.5% respectively. One patient in the FSH-HP + rhLH group had a spontaneous abortion; no pregnancy losses occurred in the FSH-HP only group. There were more cancellations for poor ovarian response among FSH-HP + rhLH patients (n = 3) than among FSH-HP patients (n = 1). The trend toward better pregnancy outcomes among patients who received FSH-HP without supplemental rhLH did not reach statistical significance. It is postulated that appropriate endogenous LH concentrations exist despite luteal GnRHa pituitary suppression, thereby obviating the need for supplemental LH administration.

Key words: FSH/IVF/ovulation induction/recombinant human luteinizing hormone

Introduction
The pharmacology of ovarian stimulation has been strongly influenced by the two-cell, two-gonadotrophin theory, and follicular stimulation protocols historically have included both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in an attempt to mimic normal physiology. The importance of theca compartment activity and, in particular, the quantity of LH necessary to facilitate optimal oocyte development have long been matters of keen interest (Ascoli et al., 1975; Pierce and Parsons, 1981; Talmadge et al., 1984; Baezinger et al., 1992; Porchet et al., 1995; Sills et al., 1998). Nevertheless, considerable variation exists in techniques of gonadotrophin administration (Abbasi et al., 1987; Duijker et al., 1993; Jennings et al., 1996). Differing views regarding the role of LH in ovarian stimulation contribute substantially to the divergence of treatment approaches for ovulation induction.

Since clinical conditions featuring absolute loss of endogenous LH and FSH are extremely rare, the pharmacological effects of these peptides have proven difficult to study. The recent commercial availability of highly purified FSH (FSH-HP) has offered an agent of substantially improved purity upon which important clinical observations may be made. Moreover, the synthesis of LH and FSH by recombinant-DNA technology (Härd et al., 1990; Hull et al., 1994) has facilitated a still more precise in-vivo assessment of the two-cell, two-gonadotrophin hypothesis.

Comparisons between patients receiving stimulation protocols with relatively high LH content and relatively low LH content have been reported previously (Edelstein et al., 1990; Daya et al., 1995; Fried et al., 1996; Mercan et al., 1997; Sills et al., 1998). It was the purpose of this prospective, randomized study to compare cycle characteristics in women undergoing ovulation induction for in-vitro fertilization (IVF) using highly purified urinary FSH both with and without supplemental LH derived from recombinant DNA technology (rhLH), following luteal pituitary suppression with gonadotrophin-releasing hormone agonist (GnRHa).

Materials and methods
Patient selection
Patients were enrolled from February to November 1996 after study approval was obtained from the Committee on Human Rights in Research at The New York Hospital (Protocol #0596–333). Informed consent was obtained from 30 healthy IVF patients between age 30 and 41 years, with a body mass index (BMI) between 18 and 35. Patients with a history of endocrinopathy or other illness, more than three prior gonadotrophin treatments for IVF, or use of any gonadotrophins within the preceding 6 months were excluded.

Before treatment all patients were interviewed and examined, and the infertility duration and aetiology was ascertained. Using random number tables, study patients were randomized into one of two treatment groups. Group 1 received only FSH-HP (Fertinex®, Serono

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Laboratories, Norwall, MA, USA), administered s.c. Group 2 received FSH-HP plus recombinant human luteinizing hormone (rhLH, Lhadi®, Serono), administered as separate s.c. injections. The FSH-HP contained essentially no LH (≤0.1 IU LH/1000 IU FSH).

The supplemental LH used in this study was synthesized by transfection of a Chinese hamster ovary cell line with a plasmid containing the two subunit DNA sequences encoding for human LH. Using recombinant DNA techniques, a consistent LH preparation of ultra-high purity was produced; it may be considered to contain essentially no FSH or other undesired constituent proteins (Keene et al., 1989; Härde et al., 1990; Mannaaerts et al., 1991). Recombinant human LH administered at doses of 75 IU has been shown to be equivalent to 75 IU of LH contained in human menopausal gonadotrophin (HMG), and has been well tolerated in doses up to 40 000 IU (Le Cotonnet et al., 1998).

Ovarian stimulation

Pituitary down-regulation was achieved by administration of leuprolide acetate (1 or 0.5 mg/day, s.c.) commencing 8 days after ovulation in the cycle preceding gonadotrophin treatment. Serum samples for oestradiol, FSH, and LH concentration determinations were obtained by venepuncture from all study patients between 0700 and 0930 on menstrual cycle day 3. The GnRHa dose was reduced by one-half on the day gonadotrophin therapy began for all patients, and maintained at that dose until the day of human chorionic gonadotrophin (HCG) administration (Damario et al., 1995).

For all study patients, controlled ovulation induction was achieved with 2–6 ampoules of FSH-HP, beginning on cycle day 3. For patients receiving FSH-HP with supplemental LH, one ampoule (75 IU) of rhLH was administered s.c. each day. Daily FSH-HP dose was adjusted in response to follicular growth and serum oestradiol concentrations. In general, a ‘step-down’ decremental pattern was followed for FSH-HP, as previously described (Damario et al., 1995). In this experimental protocol, the dose of rhLH remained constant throughout stimulation regardless of the FSH-HP dose. Up to 10 000 IU HCG (Profasi; Serono, West Orange, NJ, USA) was given i.m. when at least two follicles were ≥17 mm diameter.

Oocyte retrieval

Transvaginal ultrasound-guided needle aspiration of oocytes was performed 34–35 h after HCG administration. Propofol (Zeneca USA, Wilmington, DE, USA; 1 mg/kg) with fentanyl citrate (Astra Pharmaceuticals, Westborough, MA, USA; 100 μg) was given i.v. for analgesia in all cases. Immediately following retrieval, oocytes were washed in HEPES buffered human tubal fluid (HTF) medium prepared in our laboratory and placed into droplets of HTF + 10% matenal serum under oil (Squibb, Princeton, NJ, USA). Cells were incubated in a humidified 5% CO₂ atmosphere at 37°C.

Oocytes were graded for maturity using cumulus expansion criteria (Veeck, 1988). After 5 h, processed spermatozoa (total concentration = 1.5 × 10⁶/ml) were added to droplets of medium with each oocyte. In cases of male factor infertility (defined as spermatozoa concentration <20 × 10⁶/ml, motility <50%, and/or ‘strict’ normal forms morphology <15%), intracytoplasmic sperm injection (ICSI) was performed as reported previously (Palermo et al., 1996). Fertilization was considered successful after noting the presence of two pronuclei at 12–18 h. Prezygotes were then transferred to droplets of fresh media with 15% maternal serum for continued culture.

Embryo transfer

Up to four embryos were transferred at 72 h following oocyte retrieval. One patient in each group (both age ≥40 years) had five embryos transferred. Embryo morphology immediately prior to transfer was scored according to standard criteria (Veeck, 1991). Embryos were suspended in 20–30 μl 75% maternal serum with HTF contained within a Wallace (Marlow Surgical Technology, Willoughby, OH, USA) or TomCat (Sherwood Medical, St Louis, MO, USA) catheter, and delivered approximately 1 cm inferior to the uterine fundus. Non-transferred embryos suitable for freezing were cryopreserved if requested by the patient.

Luteal support

All patients received i.m. progesterone (Schein Pharmaceutical, Florham Park, NJ, USA; 50 or 100 mg/day progesterone in oil) following fertilization. Progesterone was maintained at this dose either until a negative pregnancy test was obtained (βHCG <5 IU by venepuncture, mean volume 7 ml) 2 weeks post-transfer, until clinical pregnancy was documented (defined as fetal cardiac activity seen via sonogram at cycle day 49), or until early pregnancy failure or demise was diagnosed.

Measured variables and statistical analysis

The following parameters were measured in this investigation: baseline serum oestradiol, LH, and FSH concentrations on menstrual cycle day 3, length of stimulation, serum oestradiol concentration on the day of HCG administration, total ampoules of gonadotrophin required for stimulation, total number of oocytes retrieved, and proportion of normally fertilized oocytes. Selected embryo morphological features, implantation and clinical pregnancy rates were also compared for both study groups.

Statistical analysis of values measured in both treatment cycles was by Student’s t-test or Fisher’s exact test, as appropriate, performed by computerized data program (Statsoft, Tulsa, OK, USA). A P < 0.05 was considered significant for all comparisons. Power analysis was performed by nQuery Advisor 2.0 (Statistical Solutions, Cork, Ireland).

Results

In this investigation, a total of 31 IVF cycles were undertaken by 30 patients. Study participants were randomized to receive either FSH-HP alone (n = 17) or FSH-HP with rhLH (n = 14). One patient re-entered the study after failing to conceive in the initial treatment using FSH-HP only (she was assigned to the opposite group for her second cycle).

Infertility aetiologies for the FSH-HP + rhLH treatment group included male factor (n = 9, 64%), tubal disease (n = 3, 18%), and idiopathic infertility (n = 2, 18%). For the FSH-HP only group, infertility diagnoses included male factor (n = 8, 47%), and tubal disease (n = 5, 29%); endometriosis and anovulation accounted for the remaining four patients. Patient age, BMI, and duration of infertility were similar for both treatment groups (Table I). Among patients randomized to receive FSH-HP, 11/17 (65%) had undergone prior gonadotrophin stimulation. Only one patient (7%) in the FSH-HP + rhLH group had any previous gonadotrophin treatment. While serum oestradiol and FSH obtained on cycle day 3 were not significantly different in the two treatment groups, patients randomized to the FSH-HP + rhLH group had higher serum LH concentrations on cycle day 3 (P < 0.05). The number of patients cancelled for poor follicular response (defined as serum oestradiol <100 pg/ml by the 5th day of gonadotrophin stimulation) was one (5%) in the FSH-HP group, and three (21.4%) in the FSH-HP + rhLH group.
No difference in mean stimulation length was observed between FSH-HP + rhLH and FSH-HP only groups. Follicular growth and development patterns were not significantly different in the two stimulation protocols. The average number of oocytes retrieved was also similar for the two groups. The mean cumulative amount of gonadotrophin used during ovulation induction was not significantly different between the two groups. Additionally, oestradiol fluctuations near the time of HCG administration were similar in magnitude and trend for both groups (Table II).

Rates of normal fertilization were similar in the FSH-HP only and the supplemental LH treatment groups. ICSI was performed in 50 and 64% of FSH-HP and FSH-HP + rhLH patients respectively (not significantly different). Embryo fragmentation and granularity observed before transfer were similar for both treatment protocols, as was the average cell number among transferred embryos. For study patients, assisted embryo hatching was performed with equal frequency irrespective of stimulation protocol. The mean embryo morphology score at transfer was identical in both treatment groups.

All patients tolerated their stimulation protocol well, and there were no cancellations due to adverse medication effects. There were no ectopic pregnancies in this study population.

The rate of implantation was not significantly different in the two groups, with 26.9 and 11.9% implantation achieved in the FSH-HP and FSH-HP + rhLH groups respectively (Table III). There was a trend toward improved clinical pregnancy/initiated cycle and clinical pregnancy/transfer results for the FSH-HP only group (Table III). However, because of limited sampling, these differences between the study groups did not reach statistical significance. One miscarriage occurred in the FSH-HP + rhLH group; no spontaneous abortions occurred in the FSH-HP group.

Discussion

Although the two-cell, two-gonadotrophin model for ovarian steroidogenesis is a fundamental tenet in reproductive endocrinology (Short, 1962; Armstrong et al., 1970), infertility treatments based on this concept have resulted in differing ovulation stimulation strategies. The present investigation documented serum baseline LH activity after pituitary suppression, but before gonadotrophin treatment. To describe the role of supplementary LH in the setting of luteal down-regulation with GnRHa, IVF patients undergoing ovulation induction using FSH-HP both with and without supplemental rhLH were monitored in a prospective, randomized manner.

This pilot study showed that oocyte yield, fertilization, and certain embryo morphological features are not substantially different between patients who received FSH-HP alone and those who received supplemental LH throughout follicular recruitment. The mean implantation rate and clinical pregnancy rates were apparently higher among patients receiving FSH-HP alone when compared to the rhLH group, although these differences were not statistically significant (P = 0.41 and

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**Table I. Pre-treatment characteristics of patients receiving FSH-HP with rhLH or FSH-HP alone**

<table>
<thead>
<tr>
<th></th>
<th>FSH-HP only (n = 17)</th>
<th>FSH-HP + rhLH (n = 14)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.4 ± 3b</td>
<td>36.7 ± 3</td>
<td>0.30</td>
</tr>
<tr>
<td>BMI</td>
<td>24.3 ± 3</td>
<td>23.9 ± 6</td>
<td>0.80</td>
</tr>
<tr>
<td>Infertility duration (months)</td>
<td>19.1 ± 5</td>
<td>20.1 ± 5</td>
<td>0.58</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oestradiol (pg/ml)c</td>
<td>26.8 ± 17</td>
<td>25.6 ± 10</td>
<td></td>
</tr>
<tr>
<td>FSH (mIU/ml)c</td>
<td>7 ± 2</td>
<td>6 ± 2</td>
<td>0.40</td>
</tr>
<tr>
<td>LH (mIU/ml)c</td>
<td>9 ± 4</td>
<td>16 ± 7</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Table II. Selected cycle features among patients treated with FSH-HP + rhLH or FSH-HP only. There were no statistically significant differences between the two groups**

<table>
<thead>
<tr>
<th></th>
<th>FSH-HP onlya</th>
<th>FSH-HP + rhLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampoules used (total)</td>
<td>29.8 ± 11</td>
<td>35.8 ± 10</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>12.8 ± 2</td>
<td>12.5 ± 1</td>
</tr>
<tr>
<td>Day of oestradiolbb</td>
<td>8.5 ± 1</td>
<td>8.0 ± 1</td>
</tr>
<tr>
<td>&gt;200 pg/ml</td>
<td>10.8 ± 1</td>
<td>10.3 ± 2</td>
</tr>
<tr>
<td>&gt;500 pg/ml</td>
<td>11.8 ± 2</td>
<td>11.1 ± 1</td>
</tr>
<tr>
<td>&gt;1000 pg/ml</td>
<td>977 ± 403</td>
<td>1085 ± 408</td>
</tr>
<tr>
<td>n – 1</td>
<td>1426 ± 692</td>
<td>1568 ± 629</td>
</tr>
<tr>
<td>n + 1</td>
<td>1665 ± 717</td>
<td>2054 ± 819</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10.7 ± 5</td>
<td>14.5 ± 8</td>
</tr>
<tr>
<td>Immature</td>
<td>1.4 ± 2</td>
<td>2.3 ± 3</td>
</tr>
<tr>
<td>MII</td>
<td>9.3 ± 4</td>
<td>11.8 ± 7</td>
</tr>
<tr>
<td>Post-mature</td>
<td>0</td>
<td>0.8 ± 3</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>75.5 ± 15</td>
<td>70.7 ± 21</td>
</tr>
<tr>
<td>Fragmentation rate</td>
<td>12.1 ± 10</td>
<td>11.9 ± 8</td>
</tr>
<tr>
<td>No. unen whole blastomers</td>
<td>0.9 ± 1</td>
<td>0.8 ± 1</td>
</tr>
<tr>
<td>Granular oocytes</td>
<td>0.56 ± 1</td>
<td>1.8 ± 2</td>
</tr>
<tr>
<td>No. embryos transferred</td>
<td>3.6 ± 1</td>
<td>3.8 ± 1</td>
</tr>
<tr>
<td>Embryo gradeab</td>
<td>2.2 ± 1</td>
<td>2.2 ± 1</td>
</tr>
<tr>
<td>Average cell no. at embryo transfer</td>
<td>7.7 ± 1</td>
<td>7.3 ± 1</td>
</tr>
</tbody>
</table>

**Table III. Reproductive outcomes in study patients as a function of stimulation protocol (FSH-HP + rhLH versus FSH-HP only). There were no statistically significant differences between the two groups.**

<table>
<thead>
<tr>
<th></th>
<th>FSH-HP only (%) (n = 17)</th>
<th>FSH-HP + rhLH (%) (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical pregnancy/initiated cycle</td>
<td>64.7</td>
<td>35.7</td>
</tr>
<tr>
<td>Clinical pregnancy/retrievalb</td>
<td>68.8</td>
<td>45.5</td>
</tr>
<tr>
<td>Clinical pregnancy/transferc</td>
<td>68.8</td>
<td>45.5</td>
</tr>
<tr>
<td>Ongoing pregnancy/initiated cycle</td>
<td>58.8</td>
<td>28.6</td>
</tr>
<tr>
<td>Mean implantation rated</td>
<td>26.9</td>
<td>11.9</td>
</tr>
</tbody>
</table>

FSH-HP = Fertinex; rhLH = recombinant human luteinizing hormone.  
*aDefined as fetal heartbeat seen on day 49 transvaginal ultrasonogram.  
*bOocyte aspiration.  
*cEmbyro transfer.  
*No. of fetal heartbeats/no. embryos transferred.
0.10 respectively). Such differences in implantation and clinical pregnancy rates in these patients should be interpreted with caution, for while these data may suggest a trend toward better outcomes following FSH-HP only treatment, a two-sided analysis established the detection of a significant difference ($P < 0.05$) in a study with a power of 80% would require a sample of 278 patients.

LH is a heterodimeric pituitary glycoprotein, with important roles in both ovarian steroid synthesis and ovulation (Channing et al., 1966; Armstrong et al., 1970). While normal ovulation is impossible without LH (Shoham et al., 1993), the preparations currently used in clinical infertility treatment have a broad range of LH content. The stereochemistry of LH is complex. The molecule’s tertiary structure and electrochemical charge are maintained by considerable glycosylation with sialic-acid residues, determining metabolic clearance and antigenic properties of LH isoforms. Experimental evidence has shown that complete desialation of LH and related glycoproteins results in substantial reduction of bioactivity (Ross et al., 1972), and can transform some gonadotrophin agonists into antagonists (Dunkel et al., 1993). In addition to these structural features of LH, polymorphisms of LH have been identified both during the female reproductive cycle (Wide and Bakos, 1993) and throughout life (Strollo et al., 1981). Even in selected anovulatory patients, circulating LH seems to have some different physiochemical characteristics when compared to endogenous LH assayed in fertile controls (Ding and Huhtaniemi, 1991). While valence and antigenic characteristics of LH appear to fluctuate over time, there is little doubt concerning the finding that less than 1% of follicular LH receptors need be bound to LH in order to facilitate normal steroidogenesis (Catt and Dufau, 1977; Doerr, 1979).

The up-regulation of LH-receptor (Ireland and Richards, 1978) and progesterone-receptor expression (Iwai et al., 1990; Parke and Mayo, 1991) within peri-ovulatory follicles are among the numerous molecular events triggered by LH. However, elevations of serum LH concentrations during the follicular phase have been associated with impaired reproductive outcomes by several investigators (Sagie et al., 1988; Regan et al., 1990; Chappel and Howles, 1991; Zelinski-Wooten et al., 1993). Among maturing follicles in the later follicular phase, the appearance of granulosa cell LH-receptors may facilitate a decreased reliance on FSH-dependent development and potentiate equivalent responses from either FSH or LH for continued follicular growth (Zeleznik and Hillier, 1984).

Earlier investigations showed that when recombinant FSH was administered alone in the setting of hypogonadotrophic hypogonadism, follicular growth was appropriate, although oestradiol production was low (Schoot et al., 1992). Both androgen and oestradiol concentrations were reduced in follicular fluid following such treatment, and blastocyst implantation was unsuccessful (Kousta et al., 1995). However, when the hypogonadotrophic hypogonadal patient received recombinant FSH supplemented with LH, follicular fluid androgen and oestradiol concentrations were much higher, and pregnancy could be established (Balasch et al., 1995).

How oestradiol might influence or reflect human oocyte health has not been fully elucidated, although preliminary research has suggested a potential role as a growth factor. Diminished serum oestradiol concentrations most probably represent impaired ovarian steroidogenesis after GnRHa down-regulation, and assessment of oestradiol response patterns have long been considered important markers for IVF cycle success (Liu et al., 1991; Davis and Rosenwaks, 1992; Damario et al., 1995). Nevertheless, oocytes have been recovered and successfully fertilized in the setting of extremely low oestradiol concentrations (i.e., 17α-hydroxylase deficiency) (Rabinovici et al., 1989). Among study patients included here, steroidogenesis was apparently unaffected since oestradiol concentrations were not significantly reduced when FSH-HP was given without supplemental LH. Standard oestradiol response curves may not be applicable when FSH-HP is used for ovulation induction, however (Miller et al., 1998).

Pharmacologically-induced hypo-oestrogenic states provide both a view to the oestrogenic contribution to reproduction, and approximate (through pituitary down-regulation) a hypogonadotrophic setting permitting assessment of treatment outcome following therapy with known gonadotrophins. Experimental results using GnRH antagonists, first in primates (Karnitis et al., 1994; Zelinski-Wooten et al., 1997) and later in humans (Mannaerts, 1997), further refined specific roles for FSH and LH by creating transient but profound arrest of endogenous gonadotrophin release.

The fact that pre-treatment serum LH concentrations differed in the two groups is notable, since those patients subsequently receiving supplemental rhLH already demonstrated a higher resting serum LH tone. It may be that subtle differences in reproductive outcome evidenced here are related to significant differences in baseline LH concentrations which could not be anticipated by randomization.

In summary, data from this pilot study support the concept that LH is needed only in trace amounts for follicular recruitment and development (Daya et al., 1995). The results of this study show that when a highly purified urinary FSH preparation is used after pituitary down-regulation with luteal GnRHa, the presence of extra (recombinant) LH throughout ovulation induction does not materially alter cycle performance. While such rhLH co-therapy appears to confer no benefit for implantation and clinical pregnancy rates, additional data are required to quantify the significance of this preliminary finding. Should clinical use of GnRH-antagonists (and rhFSH) become more prevalent, the resulting near complete loss of LH in the reproductive hormonal milieu may offer a compelling role for rhLH in selected patients. Other potentially important roles for rhLH might include its use as a substitute for HCG in oocyte maturation and/or ovulation. These applications represent topics of current research.

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