The effect of recombinant human luteinizing hormone on oocyte/embryo quality and treatment outcome in down-regulated women undergoing in vitro fertilization

O efeito do hormônio luteinizante humano recombinante sobre a qualidade oocitária/embrionária e o resultado do tratamento em pacientes submetidas à fertilização in vitro

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ABSTRACT

Objective: To compare oocyte/embryo quality and treatment outcome in patients undergoing IVF/ICSI using recombinant luteinizing hormone (rhLH) or human menopausal gonadotrophin (hMG) as a supplement to recombinant follicle-stimulating hormone (rhFSH) during ovarian stimulation. Methods: After pituitary desensitization, sixty women undergoing their first IVF treatment cycle were randomized to receive a fixed daily dose of either rhFSH (225 IU/d) plus hMG (75 IU/d) (group A, n=30) or rhLH (75 IU/d) (group B, n=30). The number/quality of metaphase II (MII) oocytes, zygote/embryo quality, and clinical pregnancy/implantation rates were compared between the two groups. Results: The mean number of normal/abnormal MII oocytes was 4.5±3.10/2.8±3.13 in group A and 2.4±1.47/4.0±3.70 in group B, showing a significant difference between groups (p=0.006; p=0.01; respectively). The fertilization rate was similar in both groups, yet the number of zygotes judged normal was significantly higher (p=0.02) in group A (2.4±2.40) as compared to group B (1.2±1.76). The mean number and quality score of embryos at transfer were similar in both groups. Overall clinical pregnancy/implantation rates were 46.4%/25.3% in group A and 38.1%/17.1% in group B. The trend toward better pregnancy outcomes among patients in group A did not reach statistical significance. Conclusions: Our study suggests that the addition of recombinant luteinizing hormone instead of human menopausal gonadotrophin to recombinant follicle-stimulating hormone throughout ovulation induction in down-regulated women undergoing IVF does not improve ovarian response and has a negative impact on oocyte/zygote quality. The result is a trend toward poorer treatment outcome.

Keywords: Embryo implantation; In vitro fertilization; Oocytes; Pregnancy; Luteinizing hormone; Zygote

RESUMO

Objetivo: Comparar a qualidade oocitária/embrionária e o resultado do tratamento de pacientes submetidas a fertilização in vitro (FIVETE)/ICSI, utilizando hormônio luteinizante recombinante (rhLH) ou gonadotrofina de mulher menopausada (hMG) como suplemento do hormônio foliculo-estimulante recombinante (rhFSH) para indução da ovulação. Métodos: Após a inibição hipofisária, 60 mulheres submetidas a seu primeiro ciclo de tratamento de fertilização in vitro foram randomizadas para receber uma dose diária fixa de rhFSH (225 UI/d) associada a hMG (75 UI/d) (grupo A, n=30) ou a rhLH (75 UI/d) (grupo B, n=30). O número/qualidade dos oócitos em metáfase II (MII), a qualidade dos zigotos/embriões e as taxas de gestação clínica e de implantação foram comparados entre os grupos. Resultados: O número médio de oócitos MII normais/anormais foi de 4,5±3,10/2,8±3,13 no grupo A e de 2,4±1,47/4,0±3,70 no grupo B, que mostrava uma diferença significativa entre os grupos (p=0,006, p=0,01; respectivamente). A taxa de fertilização foi similar nos dois grupos. Contudo, o número de zigotos considerados normais foi significativamente maior (p=0,02) no grupo A (2,4±2,40) do que no grupo B (1,2±1,76). O número e a qualidade embrionária médios no momento da transferência de embriões foram similares nos dois grupos. As taxas de gestação clínica e de implantação foram 46,4%/25,3% no grupo A e 38,1%/17,1% no grupo B. A tendência observada de melhores resultados em termos de gestação nas pacientes do grupo A não atingiu significância estatística. Conclusões: Este estudo sugere que a adição de rhLH em vez de hMG ao rhFSH durante a estimulação da ovulação, após a supressão hipofisária em pacientes submetidas à FIVETE, não melhora a resposta ovariana e tem um impacto negativo sobre a qualidade dos oócitos/zigotos. Os resultados sugerem uma tendência a resultados piores neste grupo de pacientes.

Descritores: Implantação do embrião; Fertilização in vitro; Oócitos; Gravidez; Hormônio luteinizante; Zigoto
INTRODUCTION

The last decade has witnessed not only a remarkable advancement in understanding hormonal control of human folliculogenesis, but also the development of recombinant DNA biotechnology. This progress has provided new products used in controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF), free from the potential problems associated with human source material and with good clinical efficacy.

The rationale behind COH has been linked to the two-cell, two-gonadotrophin theory, in which follicle-stimulating hormone (FSH) and luteinizing hormone (LH) play separate but complementary roles in the complex regulation of folliculogenesis and ovulation in the natural cycle. In this process, the theca interna layer of the developing follicles responds to LH and stimulates the theca interna complex regulation of folliculogenesis and ovulation. In the natural cycle. In this process, the theca interna layer of the developing follicles responds to LH and in the natural cycle. In this process, the theca interna layer of the developing follicles responds to LH and in the natural cycle. In this process, the theca interna layer of the developing follicles responds to LH and in the natural cycle. In this process, the theca interna layer of the developing follicles responds to LH and. 

Granulosa cells become responsive to FSH and are capable of converting the theca cell-derived androgen substrate to estrogens through induction of aromatase enzyme activity. Therefore, estrogens play an essential role in: a) improving follicle and oocyte maturity; b) priming the hypothalamic-hypophyseal unit in preparation for the preovulatory gonadotrophin surge; and c) inducing the endometrial changes necessary for embryo implantation. In the late stage of follicular development, FSH is responsible for the induction of LH receptors in granulosa cells, which become receptive to LH stimulation. Thus, LH exerts its influence on both theca and granulosa cells, completely replacing FSH action in the stimulation of the aromatase system activity. Ultimately, the interaction between LH and the preovulatory follicle disturbs the theca cell/cumulus oophorus contact and induces meiotic maturation of the oocyte, determines follicular rupture and causes granulosa cell luteinization.

Ovarian paracrines, including theca-derived androgens and granulosa-derived insulin-like growth factors (IGFs) and inhibins, produced in response to FSH and LH, mediate a short-loop feedback signaling between granulosa and theca cells and are involved in estrogen secretion during preovulatory folliculogenesis. The presence of minute amounts of LH allows FSH to activate the paracrine signaling between granulosa and theca cells (mediated by IGFs and inhibins), which supports thecal androgen synthesis and thus could explain how treatment with FSH alone is capable of stimulating ovarian estrogen synthesis in many clinical situations.

For years, human menopausal gonadotrophin (hMG) containing variable FSH/LH ratios has been successfully used for ovulation induction in IVF patients, ensuring adequate estradiol production and endometrial maturation for embryo implantation. In the 1980’s, purification techniques led to the development of urinary FSH (uFSH) preparations (with < 0.1% LH contamination), which became a therapeutic option for ovarian stimulation. The ultimate step in FSH purification was achieved when recombinant human FSH (rhFSH) was introduced in the mid-1990’s. Recent clinical trials have shown that the administration of rhFSH alone in IVF cycles is as effective in terms of number/quality of oocytes retrieved and embryos obtained and clinical pregnancy/implantation rates as uFSH or hMG, supporting the concept that co-administration of LH is not required for ovulation induction. However, the role of LH during folliculogenesis and in ovarian stimulation is still unclear, contributing to the discrepancy of treatment protocols for ovulation induction in IVF. Experimental and clinical studies introduced the concept of ‘threshold’ and ‘ceiling’ levels of LH during folliculogenesis. Profound suppression of peripheral LH, as observed after downregulation of the gonadotrophin-releasing hormone agonist (GnRH-a), impairs adequate ovarian steroidogenesis, resulting in a reduced number of oocytes and poor fertilization rates. On the other hand, exposure of follicles to high concentrations of LH can lead to atresia or premature luteinization, and thus the oocyte/embryonic development and reproductive outcome may be compromised.

The recent availability of human LH synthesized by recombinant DNA technology, along with rhFSH and GnRH-a, has provided the necessary tools to evaluate the individual roles of FSH and LH during ovulation induction. Studies comparing the outcome of IVF cycles in women undergoing ovarian stimulation using FSH (uFSH, rhFSH) with and without supplemental recombinant human luteinizing hormone (rhLH) after GnRH-a downregulation, showed no difference between groups when ovarian response, fertilization rates and embryo number/quality at transfer were compared. However, there was a trend toward poorer clinical outcome (clinical pregnancy/cycle and implantation rate) for the FSH + rhLH group. The authors concluded that the addition of rhLH to FSH, from the first day of COH in downregulated women, could compromise treatment outcome.

OBJECTIVE

The purpose of this randomized study was to compare oocyte/embryo quality and treatment outcome in patients undergoing IVF/ICSI using recombinant...
human luteinizing hormone (rhLH) or human menopausal gonadotrophin (hMG) as a supplement to recombinant human follicle-stimulating hormone (rhFSH) during controlled ovarian hyperstimulation.

METHODS
Patients
Sixty age-matched couples referred to Clinic DIAISON, from May 2002 through January 2003, for their first IVF cycle, due to male factor infertility (sperm count \(<5 \times 10^6/ml\) ), were included in the present prospective study. Approval from the Clinic’s Ethics Committee and signed informed consent term to participate in the study were obtained. Before treatment, the couples underwent a standard protocol of infertility evaluation including at least two semen analyses (World Health Organization criteria, 1992)\(^{(15)}\), basal FSH and estradiol (E2) concentration measurements on cycle day 3, and hysterosalpingography or hysterosonography. Inclusion criteria for women were: age \(<45\) years, day 3 FSH\(<15\) mIU/ml, day 3 E2 \(<60\) pg/ml, and a normal uterine cavity. Patients presenting poor ovarian response, patients who were azoospermic on the day of oocyte retrieval, and patients that failed to achieve fertilization or transfer of at least one embryo were excluded from this study \((n=11)\).

Stimulation protocol
All patients underwent standard downregulation with daily subcutaneous administrations of 1 mg of GnRH-a (leuprolide acetate; Serono, Sao Paulo, Brazil), starting on day 21 of a spontaneous menstrual cycle. After confirmation of pituitary suppression by both transvaginal scanning and serum estradiol concentration \(<50\) pg/ml, the GnRH-a dose was reduced to 0.5 mg/day and maintained until the day of human chorionic gonadotrophin (hCG) administration. The patients were randomized into one of two treatment groups according to a computer-generated list of random numbers, which were placed in sealed envelopes. Group assignment was done on the first day of gonadotrophin injection (cycle day 3). Group A \((n=30)\) received a fixed daily dose of 225 IU of recombinant human follicle-stimulating hormone (rhFSH) (Serono, Sao Paulo, Brazil), administered subcutaneously (SC) plus 75 IU of human menopausal gonadotrophin (hMG) (Serono, Sao Paulo, Brazil), administered intramuscularly (IM). Group B \((n=30)\) received a fixed daily dose of 225 IU of recombinant human follicle-stimulating hormone (rhFSH) (Serono, Sao Paulo, Brazil), administered SC, plus 75 IU of recombinant human luteinizing hormone (rhLH) (Serono, Sao Paulo, Brazil), administered SC, plus 75 IU of human follicle-stimulating hormone (rhFSH) (Serono, Sao Paulo, Brazil), administered SC, plus 75 IU of human follicle-stimulating hormone (rhFSH) (Serono, Sao Paulo, Brazil), administered SC, plus 75 IU of human follicle-stimulating hormone (rhFSH) (Serono, Sao Paulo, Brazil), administered SC.

Comparison of demographic characteristic, stimulation data, sonographic and endrocime parameters between patients receiving rhFSH+hMG (group A) or rhFSH+rhLH (group B)

Table 1. Comparison of demographic characteristic, stimulation data, sonographic and endocrine parameters between patients receiving rhFSH+hMG (group A) or rhFSH+rhLH (group B)

<table>
<thead>
<tr>
<th>Variable</th>
<th>rhFSH+rhLH ((n=28)) mean (±SD)</th>
<th>rhFSH+rhLH ((n=21)) mean (±SD)</th>
<th>(P) value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.4±3.5</td>
<td>37.4±3.6</td>
<td>1.000</td>
</tr>
<tr>
<td>Infertility duration (years)</td>
<td>4.2±3.1</td>
<td>3.5±2.6</td>
<td>0.113</td>
</tr>
<tr>
<td>BMI</td>
<td>22.2±3.2</td>
<td>21.6±2.3</td>
<td>0.471</td>
</tr>
<tr>
<td>Stimulation (days)</td>
<td>10.9±1.5</td>
<td>11.0±1.7</td>
<td>0.837</td>
</tr>
<tr>
<td>Gonadotrophin ampoules (total)</td>
<td>43.4±6.6</td>
<td>44.0±6.8</td>
<td>0.757</td>
</tr>
<tr>
<td>Follicles (\geq10) mm(^a)</td>
<td>11.5±5.2</td>
<td>11.1±4.6</td>
<td>0.781</td>
</tr>
<tr>
<td>Endometrial thickness (mm(^b))</td>
<td>10.8±2.3</td>
<td>11.1±1.8</td>
<td>0.627</td>
</tr>
<tr>
<td>Estradiol (pg/ml(^b))</td>
<td>1477±1099</td>
<td>1488±1179</td>
<td>0.953</td>
</tr>
<tr>
<td>Progesterone (ng/ml(^b))</td>
<td>0.8±0.4</td>
<td>0.7±0.3</td>
<td>0.342</td>
</tr>
<tr>
<td>LH (mIU/ml(^b))</td>
<td>2.4±1.4</td>
<td>2.1±1.1</td>
<td>0.409</td>
</tr>
<tr>
<td>FSH (mIU/ml(^b))</td>
<td>17.0±5.5</td>
<td>16.3±4.4</td>
<td>0.638</td>
</tr>
</tbody>
</table>

SD, standard deviation.

Student’s t-test and ANOVA.

\(^a\) On the day of hCG administration.

No statistically significant difference was found between found the two groups.

Oocyte retrieval
Transvaginal ultrasound-guided oocyte retrieval was scheduled 35 to 36 hours after hCG administration. Anesthesia was induced (2.5 mg/kg, intravenously (IV), bolus injection) and maintained (0.4 to 0.8 mg/kg, IV, every five minutes) with propofol (AstraZeneca, Caponago, Italy) in all cycles\(^{(16)}\). All women received 200 mg doxycycline and 15 mg methylprednisolone daily for five days, starting on the day of oocyte retrieval. The retrieved oocyte-corona-cumulus complexes were immediately washed in modified human tubal fluid (mHTF; Irvine Scientific, Santa Ana, CA, USA), placed into droplets of human tubal fluid (HTF; Irvine Scientific, Santa Ana, CA, USA) supplemented with 10% synthetic serum substitute (SSS; Irvine Scientific, Santa Ana, CA, USA) under oil (OVOILä-100; Vitrolife, Gothenburg, Sweden) and incubated in a humidified 5.2% CO\(_2\) atmosphere at 37°C for three to five hours before insemination.
Assessment of oocyte morphology

Immediately before intracytoplasmic sperm injection (ICSI), the oocytes were exposed to 80 IU/ml hyaluronidase (type VIII H3757; Sigma, St Louis, MO, USA) in mHTF for <30 seconds and mechanically denuded of their surrounding cumulus and corona radiata cells by aspirating several times through a 135-mm pipette (Stripper; Mid-Atlantic Diagnostics, Marlton, NJ, USA) in a hyaluronidase-free medium (mHTF + 20% SSS). Prior to microinjection, each denuded oocyte was photographed using an imaging and database software (InfoproWeb; Anflatech, Sao Paulo, Brazil). The oocytes were classified based on their nuclear maturity as metaphase II (MII), metaphase I (MI), and prophase I (PI), and their cytoplasmic morphology assessed according to Van Blerkom (1994)(17). Examination was done on the heated stage (37°C) of an inverted microscope at X200 magnification (Eclipse TE2000-U with Hoffman modulation contrast; Nikon, Melville, NY, USA) by the same embryologist in all cases. The oocytes were classified as: 1) normal - clear homogeneous appearance throughout the whole ooplasm; 2) dark/granular - coarse, dense, dark ooplasm; 3) clustered - clustering of organelles and vesicles in the central portion of the ooplasm, resulting in a crater-like appearance; 4) SER - a single massive accumulation of saccules of the smooth-surfaced endoplasmic reticulum of pronuclear dimension; 5) vesiculated - highly irregular and coarse-looking ooplasm; 6) necrotic - region of intracellular necrosis indicated by small, membrane-and-coarse-looking ooplasm; 7) polarized - well-defined portion of the cortical ooplasm depleted of organelles; and 8) vacuolated - fluid-filled vacuoles, varying in size and number within the ooplasm. Based on the frequency of aneuploidy in each pattern of dysmorphism(17), the abnormal oocytes were further divided into two groups: (i) major cytoplasmic abnormalities (dark/granular, clustered, SER); (ii) minor cytoplasmic abnormalities (vesiculated, necrotic, polarized, vacuolated).

ICSI procedure and embryo culture

Only MII oocytes were used for ICSI. Each MII oocyte was placed in a separate 5 ml droplet of culture medium (mHTF + 20% SSS) under oil in a Petri dish (Falcon 1006; Becton Dickinson, Franklin Lakes, NJ, USA). Prepared spermatozoa were added to a 5 ml droplet of 10% polyvinylpyrrolidone (PVP Solution; Irvine Scientific, Santa Ana, CA, USA) in the center of the Petri dish. A single motile spermatozoon was immobilized by mechanical damage of the tail membrane with the microinjection pipette and then injected into the center of a MII oocyte, while positioning the polar body at 12 o’clock as previously described by Palermo et al(19).

After micromanipulation, oocytes were washed, checked for signs of degeneration, and cultured individually in 100 ml of culture medium (HTF + 15% SSS) under oil in Falcon 3002 dishes, to allow separate evaluation of embryo development. After fertilization assessment, all oocytes were transferred into new Falcon 3002 dishes and cultured separately in 100 ml droplets of culture medium (HTF + 15% SSS) until transfer. All cultures were maintained at 37°C in a humidified atmosphere of 5.2% CO₂ in air.

Zygote and embryo grading

Fertilization assessment was carried out 18-20 hours after ICSI. Oocytes presenting two pronuclei (2PN) and two polar bodies were considered normally fertilized. Zygote scoring was based on the size and location of pronuclei and the number, size and distribution patterns of the nucleolar precursor bodies (NPB) in the nuclei, as described by Tesarik et al(19). Zygotes were considered normal (pattern 0) when the pronuclei presented equal sizes, were centrally located and contained at least three NPB in each pronucleus, the difference in the number of NPB between the two pronuclei did not exceed three, and the NPB were aligned at the point of contact of the two pronuclei. Zygotes showing abnormal pronuclear patterns (unequal sizes, displacement to the periphery of the cell, lack of apposition, inequality in size, number and/or alignment of NPB) were assigned to a single group (non-pattern 0). All cleaving embryos were evaluated, on days 2 (42-45h) and 3 (68-72h) after ICSI, for: a) number, regularity and symmetry of blastomeres; b) fragmentation (score) – 1: £ 10% of anucleated fragments, 2: 11-30% of anucleated fragments, 3: >30% of anucleated fragments; and c) presence (at least one blastomere exhibiting >1 nucleus) or absence of multinucleated blastomeres (MNB). The embryo quality score was based on the criteria described by Veeck(20). Immediately before transfer, the selected embryos were scored according to quality and fragmentation, and the embryo development rate (the sum of the number of blastomeres of each embryo transferred divided by the number of embryos transferred per patient) was calculated. The selected embryos were hatched using acid Tyrode’s solution and returned to the incubator until transfer. All laboratory procedures and evaluations were performed by one of the authors (PMP).

Embryo transfer and luteal support

Embryo transfers were performed with a Wallace catheter (SIMS Portex; Hythe, Kent, UK) under
transabdominal ultrasound guidance, 72 hours after oocyte retrieval. All catheters were loaded with a maximum of four embryos in 20-30 ml of culture medium (mHTF + 50% SSS). In each patient, the catheter was gently inserted through the cervical canal and internal os, with its tip placed approximately 1.0 cm from the uterine fundus. The embryos were gently injected into the uterine cavity. The catheter was removed one minute after the injection of the embryos and checked under a microscope for retained embryos, blood and/or mucus. Patients remained in bed for one hour after transfer. Supernumerary good-quality embryos were frozen at cleavage stage, using a slow freezing–thawing protocol with 1.5 mol/l 1,2-propanediol and 0.1 mol/l sucrose as cryoprotectants, in a programmable cryomachine (Kryo 10 Series III, Planer Products Ltd, Sunbury-on-Thames, UK) with manual seeding at −7°C.

Luteal phase support was initiated on the day of oocyte retrieval in the form of intravaginal natural micronized progesterone (Besins Iscovesco, Paris, France) at a dose of 800 mg daily, and maintained at this dose until a serum pregnancy test was done two weeks after embryo transfer. If it was positive (bhCG > 25 mIU/ml), transvaginal sonography was performed three weeks later to confirm the clinical pregnancy, evidenced by fetal heart activity, and to establish the number of gestational sacs. Implantation rate was defined as the number of fetal hearts in activity, divided by the number of embryos transferred. Micronized progesterone supplementation was provided for another six weeks in those achieving pregnancy.

Measured variables and statistical analysis

Primary end points in this investigation were the total number and quality of MII oocytes retrieved, the zygote and embryo quality, and the clinical pregnancy and implantation rates. Secondary end points were duration of stimulation (days), levels of serum E2, P, FSH and LH, number of follicles ≥ 10 mm diameter, endometrial thickness on the day of hCG administration, 2PN fertilization rate, and embryo cleavage rate.

The data were analyzed using NCSS 2001 software (Number Cruncher Statistical System; Kaysville, UT, USA). Statistical evaluation was performed with the unpaired Student’s t-test, one-way analysis of variance (ANOVA), chi-square test, and Fisher’s exact t-test where appropriate; a p-value < 0.05 was considered to be significant.

RESULTS

Of the 60 randomized patients, 49 (group A, n=28; group B, n=21) completed the IVF cycle and had at least one embryo transferred in this study. Oocyte retrieval was not performed in one group A and three group B patients, due to poor ovarian response. One patient in group B was azoospermic on the day of oocyte retrieval (patient’s frozen backup semen was used to perform ICSI). Complete fertilization failure was observed in one group A and three group B patients. Two patients in group B had no embryo transfer because of poor embryo quality after fertilization.

The mean number of oocytes and their maturation status at retrieval were similar in both groups. However, the assessment of the cytoplasmic organization revealed a significantly higher incidence of dysmorphic oocytes in group B when compared to group A (62.2% vs. 38.1%, respectively; p = 0.014). Further analysis of the abnormal cytoplasmic organization, grouping oocytes according to the pattern of dysmorphism and incidence of aneuploidy, showed that the incidence of major cytoplasmic abnormalities (dark/granular, clustered, SER) was similar in both groups. Yet, the incidence of minor cytoplasmic abnormalities (vesiculated, necrotic, polarized, vacuolated) was significantly higher (p= 0.000) in group B (28.1%) than in group A (9.4%) (table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>rhFSH+hMG (n=28)</th>
<th>rhFSH+HLH (n=21)</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocytes retrieved</td>
<td>8.6±4.8</td>
<td>8.6±5.6</td>
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</tr>
<tr>
<td>Oocyte maturity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MII</td>
<td>7.2±4.4</td>
<td>6.4±3.4</td>
<td>0.493</td>
</tr>
<tr>
<td>Immature</td>
<td>0.5±0.9</td>
<td>0.5±1.1</td>
<td>0.728</td>
</tr>
<tr>
<td>Post-mature</td>
<td>0.1±0.6</td>
<td>0.5±1.2</td>
<td>0.133</td>
</tr>
<tr>
<td>Cytoplasmic organization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>4.5±3.1</td>
<td>2.4±1.5</td>
<td>0.006</td>
</tr>
<tr>
<td>Major abnormalities</td>
<td>2.1±3.0</td>
<td>2.2±3.1</td>
<td>0.923</td>
</tr>
<tr>
<td>Minor abnormalities</td>
<td>0.7±1.7</td>
<td>1.8±1.7</td>
<td>0.000</td>
</tr>
</tbody>
</table>

SD, standard deviation.

* Calculated by ANOVA

The frequency of the different cytoplasmic organization patterns in MII oocytes after stimulation with rhFSH+hMG (group A) or rhFSH+HLH (group B) is presented in table 3. The incidence of MII oocytes displaying a morphologically normal ooplasm was significantly higher in group A (125/202) than in group B (51/135) (p = 0.017). While there was no difference between groups regarding dark/granular, clustered and SER patterns (major cytoplasmic abnormalities), a significantly higher incidence of vesiculated (8.1% vs. 1.0%; group B and A, respectively; p = 0.004) and polarized (15.6% vs. 0.5%, group B and A, respectively; p = 0.000) patterns was observed when the two groups were compared.
A total of 195 and 135 MII oocytes were injected in groups A and B, respectively. The percentage of degenerated oocytes after ICSI was similar in both groups. The overall normal fertilization rate (2PN) was 73.8% (144/195) in group A and 75.5% (102/135) in group B, revealing no difference between the two groups. The rate of abnormal fertilization, defined as the presence of one or >2 pronuclei per zygote, was not statistically significant when the two groups were compared. The morphological assessment of the zygotes revealed a significantly higher incidence of the pattern 0 in group A (50.7%) compared to group B (26.5%) (p < 0.02). No significant difference between groups was found for the cleavage rates on day 2 and day 3, the number of 4-cell and 8-cell embryos on days 2 and 3, respectively, or the incidence of embryos with at least one multinucleated blastomere (table 4).

Treatement outcome is presented in table 5. The mean number of embryos transferred per cycle was similar for the two treatment groups. The analysis of embryo quality, based on embryo and fragmentation scores and on the embryo development rate, showed no difference between groups. The clinical and ongoing pregnancy rates did not differ significantly between the two groups, 46.4% (13/28) and 39.3% (11/28) for group A and 38.1% (8/21) and 33.3% (7/21) for group B. The miscarriage rate was similar in both groups (7.1% vs. 4.8%; group A and B, respectively). There was a tendency towards higher embryo implantation rates in group A (25.3%) compared to group B (17.1%), but the difference did not reach statistical significance.

**DISCUSSION**

The data presented in this paper suggest that the addition of recombinant luteinizing hormone instead of human menopausal gonadotrophin to recombinant follicle-stimulating hormone throughout ovulation induction in downregulated normogonadotrophic women undergoing IVF, not only does not improve ovarian response, but has a negative impact on oocyte and zygote quality, reflected by a higher incidence of oocyte dysmorphism and poor-prognosis zygote pattern. This fact could be associated to a trend toward poorer treatment outcome among patients receiving rhFSH+rhLH when compared to the rhFSH+hMG group, since the mean clinical pregnancy (38.1% vs. 46.4%, respectively) and implantation rates (17.1% vs. 25.3%, respectively) were apparently lower in the former group, even though these differences did not reach statistical significance (p = 0.79 and p = 0.40, respectively).

In this study, the comparison between patients receiving either rhLH or hMG as a supplement to rhFSH throughout controlled ovarian hyperstimulation showed no difference in terms of duration of stimulation, number of gonadotrophin ampoules administered, hormonal and sonographic profile on the day of hCG administration, oocyte yield, fertilization and embryo quality on days 2 and 3. The data are in agreement with two previous reports in which patients treated with highly purified FSH (FSH-HP)(11) or rhFSH(12) alone were compared to those receiving supplemental rhLH throughout the follicular phase. Sills et al. also found no difference in pregnancy (68.8% vs. 45.5%; FSH-HP only and FSH-HP+rhLH, respectively) and implantation (26.9% vs. 11.9%; FSH-HP only and FSH-HP+rhLH, respectively).
rates when the groups were compared, but showed a trend toward better treatment outcome for the FSH-HP only group. In the study of Balasch et al.\(^{(12)}\), the number of pregnancies was too small to investigate the implantation rates, and due to poor results obtained in the group treated with rhFSH + rhLH (n=15; no pregnancies) it was discontinued. Recently, Griesinger et al.\(^{(13)}\) assessed the effect of using rhFSH alone or a combination of rhFSH and rhLH, starting on cycle day 2, on ovarian stimulation parameters and treatment outcome in a fixed GnRH-antagonist multiple-dose protocol. The authors found no positive trend in favor of the addition of LH to the stimulation protocol on treatment outcome parameters, showing that there was no difference between groups in terms of stimulation length (11.4 vs. 12.0 days; rhFSH only and rhFSH+rhLH, respectively), clinical pregnancy (23.0% vs. 16.0%; rhFSH only and rhFSH+rhLH, respectively) and implantation rates (13.8% vs. 8.1%; rhFSH only and rhFSH+rhLH, respectively).

Although FSH and LH are both required for normal folliculogenesis and estrogen production, the role of LH during folliculogenesis in ovulation induction in patients undergoing IVF is still debatable. In 1994, Hillier\(^{(2)}\) introduced the concept of a specific range for LH requirement in ovarian follicular development, in which optimal folliculogenesis is dependent on a minimal exposure to LH (threshold effect) and has a finite requirement for its exposure (ceiling effect), beyond which follicular growth is arrested. Based on this concept, different authors showed that either too low\(^{(21,22)}\) or too high\(^{(23-24)}\) LH levels during ovulation induction compromised reproductive performance. Clinical evidence indicates that elevated LH concentrations during folliculogenesis and the periovulatory period not only have a detrimental effect on oocyte quality, subsequent fertilization, cleavage and embryo quality, but also on the treatment outcome, reflected by lower pregnancy and higher miscarriage rates\(^{(25-26)}\).

Before fertilization and normal embryo development, the oocyte must acquire a variety of properties (nuclear and cytoplasmic) at specific stages of folliculogenesis that define its own developmental potential. Oocyte maturation involves nuclear changes, from the first meiotic prophase to the metaphase of the second meiotic division, as well as cytoplasmic modifications, including the development of the capacity to decondense sperm chromatin and increase the levels of glutathione\(^{(27)}\), the expression of a histone exchange activity\(^{(28)}\), the cortical granule release\(^{(29)}\), and the ability to generate and sustain calcium oscillations\(^{(30)}\), acquired through maturation and late growth phases of oogenesis and which may be critical determinants of the oocyte developmental competency. LH plays a fundamental role in androgen production by theca cells from the earliest stages of follicular growth, and the LH receptor is also expressed in cumulus cells during follicular development, suggesting that LH might exert an effect throughout the oocyte’s growth phase. Excess androgen production secondary to LH has been associated with cell death of both oocytes and granulosa cells, as well as follicular growth arrest, as evidenced in both animal and clinical studies\(^{(31-32)}\). Thus, specific changes in the follicular microenvironment during folliculogenesis, due to an imbalance in the LH/FSH ratio, could have deleterious effects on oocyte nuclear and/or cytoplasmic maturation, consequently leading to preimplantational embryo development failure.

The observation of a higher incidence of abnormal cytoplasmic organization patterns in the group treated with rhFSH and rhLH in the present study could be related to the trend toward poorer treatment outcome in this group, since the acquisition of cytoplasmic competence during follicular development is as important as nuclear maturation for the normal embryonic developmental potential. This could also be laboratory evidence of the LH ‘ceiling’ effect, suggesting that overexposure of sensitive follicles to LH during folliculogenesis could result in abnormal cytoplasmic maturation. On the other hand, since progesterone and LH levels remained low on the day of hCG administration in both groups, premature luteinization could not have been responsible for the poor oocyte quality evidenced in the rhFSH+rhLH group.

Recently, the identification of cytoplasmic abnormalities at the time of ICSI was considered a useful indicator of subsequent fertilization, embryo quality and ongoing pregnancy rates of in-vivo matured MII oocytes. Kahraman et al.\(^{(33)}\) observed that poor ongoing pregnancy rates were achieved in couples with centrally located granular cytoplasm, even if fertilization, embryo quality and total pregnancy rates were normal. The significantly lower fertilization rate of oocytes treated by conventional IVF, but not by ICSI (37.7% and 65.3%, respectively), in the group treated with rhFSH+rhLH compared to that in the group treated with rhFSH only (75.0% and 74.1%; IVF and ICSI, respectively), observed by Balasch et al.\(^{(12)}\) could be explained by this finding. On the other hand, the observation of normal fertilization rates in our study (79.0% and 86.6%; rhFSH+hMG and rhFSH+rhLH, respectively), despite the higher incidence of oocyte dysmorphism in the group treated with rhFSH+rhLH, could be attributed to ICSI, since this method overcomes almost all barriers to fertilization.
morphological defects of the oocyte\textsuperscript{(33,34)}, and is in accordance with the fertilization rates observed in previous studies\textsuperscript{(11-12)}.

Van Blerkom et al.\textsuperscript{(35)} showed that the interaction between the spermatozoon and the ooplasm occurs in a very specific and highly polarized way. Subtle defects in cellular and nuclear organization could lead to early developmental failure. Payne et al.\textsuperscript{(36)} demonstrated that normal fertilization follows a defined course of events, including circular waves of granulation within the ooplasm (organelle migration), the extrusion of the second polar body, the formation and apposition of male and female pronuclei in a central location, and alignment of the nucleoli at the pronuclear interface. Based on these physiological events, zygotes were scored according to the pronuclear alignment, number, size and polarity of the nucleoli, and cytoplasmic heterogeneity (cortical halo). Various studies positively correlated the scoring system with embryo quality on day 3, blastocyst formation and quality on day 5, and implantation and delivery rates\textsuperscript{(19,37-38)}.

This study reports a significantly higher incidence of pattern 0 zygotes (‘good quality’) in the group treated with rhFSH+hMG (50.7%) when compared to that in the rhFSH+rhLH group (26.5%) ($p < 0.02$). However, this difference was not apparent while evaluating embryo quality and development rate on days 2 and 3, since the incidence of 4-cell / 8-cell embryos, and of embryos with multinucleated blastomeres was similar in both groups. These findings are in contrast with those of other studies that showed a positive correlation between ‘good quality’ zygotes and cleavage stage embryos\textsuperscript{(37,39)}. On the other hand, the higher incidence of ‘good quality’ zygotes in the group treated with rhFSH+hMG could also explain the trend toward better treatment outcome, reflected by the group’s higher implantation rate. It is tempting to speculate that abnormal cytoplasmic organization could compromise the defined sequence of events that lead to normal pronuclear and nucleoli alignment and thus impair the developmental competence of the zygote.

Tésarik and Mendoza\textsuperscript{(25)} observed that the addition of exogenous LH (hMG) to rFSH in the ovarian stimulation protocol applied to young normogonadotrophic women with high basal LH levels ($\geq 1$ IU/L) damaged the oocyte and embryo quality and decreased the implantation rate. The number of zygotes of poor morphology was higher and the number of cleaving embryos of excellent morphology was lower in this group of patients as compared to the group of patients treated with rhFSH alone. The authors supposed that the strong stimulation of theca interna cells by the action of endogenous and exogenous LH in the mid-follicular phase could lead to excess androgen production, causing imbalance in the intrafollicular androgen-to-estrogen ratio and thus impairing cytoplasmic maturation and early post-fertilization development of human oocytes. However, other studies failed to demonstrate a negative effect of LH (hMG) and rhFSH on oocyte and zygote/embryo quality\textsuperscript{(40-41)}.

The data presented here suggest that the use of rhLH as a supplement to rhFSH during controlled ovarian hyperstimulation may be detrimental to oocyte and zygote quality. The absence of a similar effect in the hMG + rhFSH treatment group could be attributed to the fact that the LH-like activity of hMG preparations is due to the fact that they are composed of LH and hCG, the immunoreactivity of hCG amounting to as much as 25% of the total LH-like activity\textsuperscript{(42)}, and that hCG could have a function of its own in follicular development and maturation, as previously observed\textsuperscript{(43)}. The significantly poorer oocyte and zygote quality observed in the group treated with rhLH, despite the similar serum LH levels on the day of hCG administration as in the group treated with hMG, concurs with a previous observation that the threshold dose of 75 IU rhLH does lead to a very significant pharmacodynamic effect, without measurable variations in serum LH levels\textsuperscript{(44)}.

**Conclusions**

In conclusion, the present study suggests that the addition of rhLH instead of hMG to rhFSH throughout ovulation induction in downregulated women undergoing IVF does not improve ovarian response and has a negative impact on oocyte/zygote quality. The embryo quality (fragmentation score, symmetry, blastomere multinucleation) and development rate (mean number of blastomeres at transfer) on the day of embryo transfer failed to identify a difference between treatment groups (despite a trend toward poorer treatment outcome observed in the rhLH group). Therefore, a close examination of oocyte and zygote morphology could represent a powerful, non-invasive tool for monitoring the effects of the different drugs used for ovulation induction in IVF on cycle performance (mainly pregnancy and implantation rates). Optimal LH ranges and the stage of the follicular phase at which rhLH should be administered to modulate and improve folliculogenesis in ovulation induction remain to be elucidated. The addition of rhLH in the late follicular phase of downregulated patients stimulated with rhFSH was recently shown to
benefit treatment outcome in women above 35 years of age\(^{(45-48)}\) and is part of an ongoing clinical research at our IVF Unit.

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The effect of recombinant human luteinizing hormone on oocyte/embryo quality and treatment outcome in down-regulated women undergoing in vitro fertilization


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