Human cumulus-free oocyte maturational profile and in vitro developmental potential after stimulation with recombinant versus urinary FSH

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BACKGROUND: This study compares the influence of recombinant (r)FSH and urinary (u)FSH stimulation on oocyte and embryo quality in patients undergoing ICSI. METHODS AND RESULTS: Denuded oocyte maturity in ICSI patients was graded on a scale from metaphase II (MII) to prophase for nuclear maturation of oocytes. The relationships of cumulus-free oocyte maturational profiles with in vitro outcome parameters were evaluated. In the study population with an unknown distribution of FSH receptor polymorphisms, the ovarian response to rFSH stimulation was significantly different from that of uFSH stimulation, including lower number of oocytes retrieved/oocytes in MII, higher fertilization rates and higher good quality embryo rates. In the study population with a similar distribution of FSH receptor polymorphisms, the ovarian responses to rFSH were lower numbers of oocytes in MII, higher fertilization rates and higher good quality embryo rates, but the total number of oocytes retrieved was not influenced, in comparison with ovarian stimulation with uFSH. CONCLUSIONS: rFSH stimulation appears to influence oocyte quality and subsequent embryo quality in comparison with uFSH stimulation. FSH receptor polymorphisms seem to be an intrinsic factor influencing the ovarian response to FSH stimulation.

Key words: embryo quality/FSH/ICSI/oocyte quality/ovarian stimulation

Introduction

Multiple factors are important in influencing the pregnancy rate of patients undergoing assisted reproductive technology procedures. These factors include the aetiology of infertility, patient age, type of ovarian stimulation and follicular phase estradiol (E2) levels (Jones et al., 1983; Edwards et al., 1984; Wood et al., 1985). These factors affect the number and quality of oocytes that develop and, subsequently, the number of embryos transferred. The number and quality of embryos transferred appears to be the most important factor influencing the pregnancy rate. For this purpose, several investigators also tried to predict the ovarian response and assisted reproductive technology outcome before or during controlled ovarian stimulation (Gosden et al., 1985; Loumaye et al., 1990; Winslow et al., 1991; Fanchin et al., 1994; Licciardi et al., 1995; Scott et al., 1995; Hansen et al., 1996; Huang et al., 2001). However, these predictions are still not 100% reliable.

Ovarian stimulation is used in the majority of assisted reproduction units in order to improve the success rate by increasing the number of mature oocytes and, thus, the number of embryos to be replaced. Pharmaceutical preparations of human gonadotrophins play an important role in the treatment of human infertility, and have been used widely to stimulate follicular development in infertile women (Diamond et al., 1986; Nevus et al., 1987; Chang et al., 1993; Hwang et al., 1993; Strickler et al., 1995; Surrey et al., 2000). During the 1970s, urinary (u)hMG was the only gonadotrophin used in infertility treatment, but since the 1980s, a variety of subproducts of uhMG have been produced with the intention of eliminating most or all of the LH content (Zafeiriou et al., 2000). During the mid-1990s, recombinant (r)FSH was produced in vitro from hamster ovarian cell cultures, and this step was considered a landmark in the production of gonadotrophins (Out et al., 1997). The introduction of recombinant human FSH (rFSH) appears to be a milestone in the development of drugs for the treatment of infertility. The clinical efficacy and safety of these preparations have been established. However, most urine-derived FSH preparations have limited biochemical purity, ranging from 1 to 3%. The urinary proteins may have negative effects on follicular recruitment and development (Giudice et al., 1994). rFSH is produced by a Chinese hamster ovary (CHO) cell line, transfected with the genes encoding for the two FSH subunits. This results in an almost totally pure FSH preparation.
However, uFSH has substantial amounts of impurities, such as urinary proteins and LH. The use of rFSH offers advantages over urine-derived FSH, because the manufacturing process of a recombinant DNA technology product can be better controlled and an improved batch-to-batch consistency can be achieved. Furthermore, this new technology provides the opportunity to produce a highly purified product, which may result in a higher clinical tolerance and a decreased risk of unwanted reactions with this preparation. A series of clinical trials were collected to compare the efficiencies between rFSH and uFSH stimulation in assisted reproductive technology programmes using meta-analysis (Daya et al., 2002; Al-Inany et al., 2003). Most of these trials focused on clinical parameters such as number of mature oocytes obtained, dosage/duration of gonadotropin used, fertilization and pregnancy rates. Fertilization and pregnancy rates are not sensitive indicators of oocyte quality because they can be affected by other factors such as semen quality and uterine receptivity. 

Prior to ICSI, oocytes must be separated from their surrounding cumulus/corona cells within a few hours after retrieval, and this allows a more precise determination of oocyte morphology (Rattanachaiyanont et al., 1999; Ng et al., 2001) and creates a good model to study oocyte maturational profiles in detail and developmental potential in vitro (Huang et al., 1999, 2002).

Given the limited information available concerning oocyte and embryo quality after simulation with either rFSH or uFSH, the present study was designed to analyse the maturational profiles and in vitro developmental potentials of cumulus-free oocytes in ICSI patients.

Materials and methods

Study population

We analysed 286 consecutive cycles among 248 infertile couples who underwent ICSI in our assisted reproductive technology programme between January 1, 1998 and December 31, 2001. During this period, rFSH was initially introduced into our clinic and, since 1999, has gradually replaced uFSH for controlled ovarian stimulation. All couples had male factor infertility as the major infertility factor. Those patients with cycles stimulated using the short protocol of GnRH agonist (luprolide acetate; Takeda, Japan), in combination with the use of hMG (Pergonal; Serono, Switzerland) or for treatment by IVF, were excluded from the analysis. Patients who had only one ovary were also excluded. Females who smoked were excluded from this study, and all women recruited had basal FSH concentrations <15 IU/l.

Controlled ovarian stimulation

During this time, rFSH (Gonal-F, 75 IU/ampoule; Serono, Switzerland) or uFSH (Metrodin, 75 IU/ampoule; Serono, Italy) was used for the controlled ovarian stimulation at the physician’s preference. Ovarian stimulation and oocyte retrieval were performed as described in previous studies (Chang et al., 1993; Lan et al., 2002). Briefly, all patients underwent a long stimulation protocol using a GnRH agonist therapy followed by the administration of uFSH or rFSH. All patients received leuprolide acetate (LA) (Lupron; Abbott Laboratories, USA), 1 mg (age <35 years) or 0.5 mg (age ≥35 years), s.c. daily, beginning on day 21 of the previous cycle until cycle day 3 of the next cycle; pituitary down-regulation was then evaluated by determination of serum estradiol (E2) concentration and transvaginal sonography (TVS) of the ovaries. If the serum E2 level was <35 pg/ml and no follicles >10 mm in diameter were noted on TVS, LA was decreased to a half dose and continued until the day of hCG. (Pregnyl; N.V. Organon, The Netherlands) administration. If the pituitary was not suppressed, LA was continued at the same dose and the serum E2 level was rechecked every 3 days until suppression was achieved. Patients received fixed starting doses (age <35 years: 225 IU/day; age ≥35 years: 300 IU/day) of either rFSH or uFSH, after pituitary suppression with LA. Gonadotrophin was administered daily for 5 days, after which the dose was individualized according to ovarian follicular growth. Starting on the fifth day of stimulation, the patients were monitored every 2–3 days with TVS and serum E2 measurements. hCG was administered (10 000 IU) i.m. when TVS revealed at least one follicle with a mean diameter of ≥17 mm and/or when serum estradiol levels were >400 pg/ml. Oocytes were retrieved by transvaginal aspiration under ultrasound guidance 35–37 h subsequent to the injection of hCG.

Following oocyte retrieval, the oocytes were exposed to type VIII hyaluronidase (80 IU/ml; Sigma Chemical Co., USA) for a period of 5–6 s. The oocytes were then cleaned from the surrounding cumulus cells by aspiration through a series of pipettes with decreasing inner diameters (commencing at 220 µm, and progressing through 200, 180 and finally 160 µm in diameter), in 100 µl droplets of human tubal fluid medium. The oocyte maturity was defined as prophase, metaphase I (MI) or metaphase II (MII) oocyte. The denuded oocytes were classified into two groups according to the developmental stage for analysis of developmental potential. Group I consisted of oocytes that were in MI at the removal of cumulus/corona cells, while group II consisted of oocytes that were in prophase or MI. The denuded oocytes of both groups were cultured in M2 culture medium (Medicult, Denmark) for 3–8 h. The group I oocytes were injected with sperm 3–4 h after retrieval. Group II oocytes were examined every 2 h, and ICSI was performed immediately after appearance of the first polar body (MII stage), which occurred between 4 and 8 h after oocyte retrieval. Oocytes that did not develop to MII within 8 h of incubation were discarded.

ICSI procedure and embryo culture

The procedure of sperm injection was based upon the protocol described by Palermo et al. (1992), although the preparation of sperm droplets was modified for this study. To prepare the sperm droplets, 1 µl of prepared sperm suspension was added to 2–4 µl of M2 medium (Medicult) containing no polylvinylylpyrroloidone, as described previously (Tsai et al., 2000). Subsequent to the completion of the ICSI procedure, the oocytes were cultured as for standard IVF (Huang et al., 2002). The oocytes were assessed for the presence of pronuclei subsequent to a period of 16–18 h of incubation. Fertilization of oocytes was considered successful when two clear pronuclei were observed. If only one pronucleus was observed, a second evaluation was carried out 4 h later in order to determine whether the pronuclear status had changed.

The degree of fertilization success, embryonic cleavage and the resultant morphological embryo quality (Veeck, 1998) were assessed after ~48 h of culture, and embryos were transferred from day 3 to day 5 or 6 subsequent to oocyte retrieval. Daily luteal phase supplementation of micronized progesterone (Utrogestan; Piette International Laboratories, Belgium) 800 mg intravaginally was begun on the day of oocyte retrieval, and 5000 IU hCG was administered on day 6 after oocyte retrieval in all patients.

Clinical pregnancy was determined by identifying a gestational sac at a gestational age of 7 weeks by means of TVS. Micronized progesterone was given to pregnant patients for a period of 4 weeks.
Statistical analysis

Statistical evaluation was performed using one-way analysis of variance, the Mantel–Haenszel test, Fisher’s exact test and the χ²-test where appropriate. Differences were considered to be statistically significant at P < 0.05. This study was approved by the institutional review board of the hospital and written informed consent was obtained from the patients prior to the commencement of the study.

Results

Although no significant differences were found between the two groups with regard to demographic data, dosage or duration of gonadotrophin used and endometrial thickness, the mean number of ovarian follicles and oocytes retrieved in the rFSH group was significantly lower than in the uFSH group (Table I). Furthermore, the distribution of MII and MI oocytes at the removal of cumulus/corona cells in the rFSH group was significantly different from that in the uFSH group (MII oocytes: 60.4 versus 84.9%; MI oocytes: 33.3 versus 7.7%; P < 0.0001) (Figure 1). Nevertheless, the percentage of total denuded oocytes that reached stage MII following the 4–8 h culture and could be used for sperm injection was similar in the two groups (89.1 versus 89.9%). MII oocytes at the removal of cumulus/corona cells in the two groups almost could be used for sperm injection following the 4–8 h culture (100 versus 99.5%, P = 0.102). However, MI oocytes at the removal of cumulus/corona cells following the 4–8 h culture had a higher percentage reaching stage MII and getting the procedure of ICSI in the rFSH group than did those in the uFSH group (86.9 versus 64.9%, P < 0.0001).

The developmental competence of the oocytes in the two groups was compared in Figure 2. MII oocytes that extruded the 1st polar body at the removal of the cumulus/corona cells (group I oocytes) had better fertilization rates and embryo quality than in vitro-matured oocytes (group II oocytes) that extruded the 1st polar body following the removal of cumulus/corona cells and in vitro culture in the patients receiving either rFSH or uFSH stimulation (Figure 2A or 2B, P < 0.001). Although MII oocytes at the removal of cumulus/corona cells could be obtained from the rFSH group or uFSH group, the normal fertilization rates of these oocytes injected with sperm in the patients with rFSH stimulation (98.4%) was significantly higher than in patients who underwent uFSH stimulation (90.5%) (Figure 2C). The proportion of these fertilized oocytes that developed to good morphology embryos among patients with rFSH stimulation was still higher than in patients with uFSH stimulation (92.1 versus 81.2%, P < 0.001). Simultaneously, MII oocytes, maturing in vitro from MI, were also obtained from the rFSH and uFSH groups. However, the normal fertilization rates of these oocytes injected with sperm in the patients undergoing rFSH stimulation (81.9%) was significantly higher than in patients who underwent uFSH stimulation (69.1 versus 44.8%, P < 0.05).

The overall outcomes of treatment cycles among the two groups are shown in Table II. Although there were higher
numbers of oocytes retrieved and MII oocytes among the patients with rFSH stimulation, the proportion of these oocytes that fertilized and developed to day 2 embryos with good morphology in the rFSH group was higher than in the uFSH group (93 versus 88.7% for fertilization rate; 86 versus 79.8% for the good morphology embryo rate). The mean number of good morphology embryos transferred was similar to that in the uFSH group. The pregnancy rate per transfer in the rFSH group was higher, but not significantly higher, than in the uFSH group, probably due to the small sample size. Nevertheless, the implantation rate was significantly higher in the rFSH group (23 versus 7.8%, \( P = 0.019 \)). However, the maturational profiles of the oocytes were significantly different from that in the uFSH group (Figure 3). Moreover, the normal fertilization rates were significantly higher in the rFSH group than in the uFSH group (94.5 versus 82.9%, \( P = 0.006 \)) in Figure 4. The percentage of good morphology embryos in the rFSH group was significantly higher than in the uFSH group (79.4 versus 65.2%, \( P = 0.026 \)).

Figure 2. Developmental competence of oocytes in women receiving rFSH or uFSH stimulation. The denuded oocytes were classified into two groups according to the stage of nuclear maturation for analysis of developmental potential. Group I consisted of oocytes that were in metaphase II at the removal of cumulus/corona cells, while group II consisted of oocytes that were in metaphase I and cultured to show the appearance of first polar body, which occurred between 4 and 8 h after oocyte retrieval. Good embryos have grade 1 or 2 morphology on day 2. (A) Developmental competence of group I and group II oocytes in women receiving rFSH stimulation. (B) Developmental competence of group I and group II oocytes in women receiving uFSH stimulation. (C) Developmental competence of group I oocytes in women receiving rFSH or uFSH stimulation. (D) Developmental competence of group II oocytes in women receiving rFSH or uFSH stimulation. Data are presented as percentages. For all graphs, *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) compare the data between the women receiving rFSH and uFSH stimulation or between the group I and group II oocytes.
Discussion

Assisted reproductive technology is a complex and costly technique with stringent indications for treating infertility. Providing infertile couples with accurate information about their chances of pregnancy is a priority in all assisted reproductive technology programmes. Although many factors influence the success of assisted reproductive technology, there is a general consensus that the key role is played by ovarian response to FSH stimulation. A good response to stimulation results in a number of good quality oocytes collected at retrieval. The number of good quality embryos developed in vitro from the culture depends on the number of good quality oocytes collected at retrieval (Huang et al., 1999, 2002). At the very least, the number of good quality embryos transferred and uterine receptiveness determines the outcomes of assisted reproductive technology programmes. Patient characteristics (intrinsic factors) and ovarian stimulation protocol (extrinsic factors) seem to determine the ovarian response to stimulation. The patient characteristics include cause of infertility, age,

Table II. Outcomes of treatment cycles in women receiving rFSH or uFSH stimulation

<table>
<thead>
<tr>
<th>Gonadotrophin administered</th>
<th>rFSH (n = 151)</th>
<th>uFSH (n = 135)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mature oocytes injected</td>
<td>802</td>
<td>894</td>
<td></td>
</tr>
<tr>
<td>Normal fertilization rate (%)</td>
<td>746 (93)</td>
<td>793 (88.7)</td>
<td>0.002</td>
</tr>
<tr>
<td>Cleavage rate on day 2 (%)</td>
<td>687 (92.1)</td>
<td>762 (96.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>No. of good embryos on day 2 (%)</td>
<td>591 (86.0)</td>
<td>608 (79.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>2.8 ± 1.3</td>
<td>3.7 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of good embryos transferred</td>
<td>2.3 ± 1.1</td>
<td>3.2 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of transfer cycles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>77</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td>Day 4</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
<td>65</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate/cycle (%)</td>
<td>62 (41.1)</td>
<td>54 (40)</td>
<td>0.855</td>
</tr>
<tr>
<td>Pregnancy rate/transfer (%)</td>
<td>62 (42.5)</td>
<td>54 (40.9)</td>
<td>0.751</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>92 (21.9)</td>
<td>92 (18.6)</td>
<td>0.227</td>
</tr>
<tr>
<td>High-order multiple pregnancy rate (%)</td>
<td>6 (9.7)</td>
<td>10 (18.5)</td>
<td>0.160</td>
</tr>
</tbody>
</table>

Good quality embryos: grade 1 or 2 embryos.

Table III. Ovarian responses and treatment outcomes in the same patients receiving rFSH and uFSH stimulation respectively

<table>
<thead>
<tr>
<th>Gonadotrophin administered</th>
<th>rFSH (n = 18)</th>
<th>uFSH (n = 18)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles</td>
<td>19</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.2 ± 4.0</td>
<td>32.2 ± 4.4</td>
<td>0.454</td>
</tr>
<tr>
<td>Ampoules of 75 IU FSH</td>
<td>27.9 ± 5.6</td>
<td>30.9 ± 6.3</td>
<td>0.159</td>
</tr>
<tr>
<td>Units of FSH administered</td>
<td>2129 ± 452</td>
<td>2365 ± 486</td>
<td>0.158</td>
</tr>
<tr>
<td>Days of FSH treatment</td>
<td>8.7 ± 1.4</td>
<td>8.4 ± 0.9</td>
<td>0.499</td>
</tr>
<tr>
<td>Endometrial thickness on day of hCG (cm)</td>
<td>1.2 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>0.858</td>
</tr>
<tr>
<td>Estradiol (pg/ml) on hCG day</td>
<td>1641 ± 1135</td>
<td>1845 ± 1580</td>
<td>0.660</td>
</tr>
<tr>
<td>Follicles ≥16 mm in diameter on hCG day</td>
<td>3.9 ± 2.3</td>
<td>4.2 ± 3.2</td>
<td>0.807</td>
</tr>
<tr>
<td>Follicles ≥13 mm in diameter on hCG day</td>
<td>8.8 ± 4.4</td>
<td>8.2 ± 4.5</td>
<td>0.683</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>6.6 ± 3.7</td>
<td>7.0 ± 4.4</td>
<td>0.754</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>3.4 ± 1.5</td>
<td>3.6 ± 1.2</td>
<td>0.715</td>
</tr>
<tr>
<td>No. of good embryos transferred</td>
<td>2.5 ± 1.2</td>
<td>2.8 ± 1.1</td>
<td>0.463</td>
</tr>
<tr>
<td>Pregnancy rate/transfer (%)</td>
<td>8 (44.4)</td>
<td>4 (22.2)</td>
<td>0.157</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>14/61 (23)</td>
<td>5/64 (7.8)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Figure 3. Maturational profile of oocytes in the same patients receiving rFSH and uFSH stimulation respectively. Metaphase II oocytes extruded the first polar body at the removal of cumulus/corona cells and metaphase I oocytes did not show the first polar body and germinal vesicle at the removal of cumulus/corona cells. Data are presented as percentages. For all graphs, *P < 0.05, **P < 0.01, ***P < 0.001 compare the data between the women receiving rFSH and uFSH stimulation.
ovarian reserve, and possibly individual FSH–FSH receptor interactions (Sherins et al., 1995; Garrido et al., 2000; Perez Mayorga et al., 2000; Sudo et al., 2002). FSH is a key player in human reproduction (Nieschlag et al., 1999). This gonadotrophin exerts its trophic and stimulatory effects on gametogenesis by binding to a specific receptor located exclusively on the surface of Sertoli cells in the testis and granulosa cells in the ovary. The FSH receptor is a G-protein-coupled receptor and its main signal transduction mechanism involves activation of adenylyl cyclase and elevation of intracellular cyclic AMP (Simoni et al., 1997). It was soon evident that the FSH receptor and its promoter bear some very common single nucleotide polymorphisms (SNP), which seem to be important in determining the response to FSH stimulation (Perez Mayorga et al., 2000; Sudo et al., 2002). In any case, since individuals with different type of FSH receptor variants appear to respond differently to FSH stimulation in vivo, the variant may play some role in determining ovarian response to pharmacological stimulation with FSH (Perez Mayorga et al., 2000; Sudo et al., 2002). It has also been demonstrated that frequency distribution of SNP and allelic variants of the FSH receptors are different between Caucasians and Japanese (Perez Mayorga et al., 2000; Sudo et al., 2002). Another study extended this finding in a group of 231 women including subjects of Caucasian, Arabian, Asian and Latin American origin (J.Laven et al., unpublished data). Therefore it seems that ethnic background and individualized allelic variants of the FSH receptors should be considered when comparing the response of controlled ovarian stimulation in assisted reproductive technology treatment cycles.

How should we assess the assisted reproductive technology outcome following ovarian stimulation? In general, there are several clinical parameters to be used. Most of these trials focus on clinical parameters such as number of total oocytes/ mature oocytes obtained, good morphology embryos transferred, dosage/duration of gonadotrophin used, fertilization and pregnancy rates. However, oocyte and embryo parameters associated with the quality of the oocyte/early embryo are correlated with pregnancy outcome (Serhal et al., 1997; Xia, 1997; Ebner et al., 1999; Tesarik and Greco, 1999). Complete maturation of the oocyte determines oocyte quality. Complete maturation of oocytes should include both nuclear maturation and cytoplasmic maturation. For oocyte nuclear maturation, resumption and progression of meiosis to MII cannot be used as a sole determinant of an oocyte’s developmental competence (Leibfried-Rutledge et al., 1989). Extensive changes in protein synthesis and post-translational modifications in the cytoplasm take place simultaneously with nuclear maturation (Bachvarova et al., 1987). Both nuclear and cytoplasmic maturation play important roles in achieving successful fertilization and subsequent development (Eppig et al., 1994, 1996). Nuclear maturation of oocytes can be noted from the static morphological appearance. However, cytoplasmic maturation of oocytes needs to be assessed through the static morphological change (De Sutter et al., 1996; Garside et al., 1997; Serhal et al., 1997; Balaban et al., 1998; Ebner et al., 2000) and dynamic change of oocytes in vitro. Despite the selection of mature oocytes based on the appearance of the first polar body, which presumes nuclear maturation, no convenient measure of cytoplasmic maturity has been identified, apart from the ability of the oocyte to activate and support embryo development. Another consideration in devising a screening protocol for oocyte quality involves avoiding the potential rate-limiting step and minimizing the variability due to sperm capacitation and penetration. Thus, ICSI was selected as the best inseminating technique for quality evaluation studies (Huang et al., 1999, 2002; Ng et al., 2001; Nusser et al., 2001; Ebner et al., 2003; Rienzi et al., 2003; the present study). It provides an opportunity for an accurate evaluation of oocyte maturity after cumulus cell removal. The timing of meiotic progression of oocytes can be assessed more accurately, and the mechanisms of maturation and early embryo development can be studied in greater detail through these cumulus-free oocytes. For ICSI treatment, morphological assessment of oocytes and embryos remains the gold standard for determination of oocyte and embryo quality in clinical practice. The morphological assessment of oocytes includes the morphology of nuclear maturation (De Sutter et al., 1996; Serhal et al., 1997; Balaban et al., 1998), polar body morphology (Ebner et al., 2000), zona diameter (Garside et al., 1997) and cytoplasmic appearance (e.g. dark cytoplasm). However, even with this series of morphological assessments, it is still difficult to assess the degree of cytoplasmic maturation of oocytes. In clinical practice, the developmental potential of fertilized oocytes is a good model to assess the degree of cytoplasmic maturation. Other non-invasive tests such as early cleavage to the 2-cell stage (Shoukir et al., 1997; Lan et al., 2003), development of the blastocyst (Lan et al., 2003) and metabolic uptake of glucose and pyruvate (Devreker et al., 2000) may also be helpful in assessing the degree of cytoplasmic maturation of oocytes and embryo quality. In the present study, we continue to use ICSI as the inseminating technique for quality evaluation studies. Using this model, we demonstrated significant differences in oocyte profile and embryo quality based on whether rFSH or uFSH was used for.
ovarian stimulation regarding oocyte quality and subsequent embryo quality for the two different preparations of FSH.

There are two groups of patient characteristics included in the study. One group of the patient characteristics in Table I included general parameters in terms of age, ovarian reserve, aetiology of infertility and duration of infertility. However, the frequency distribution of FSH receptor allelic variants between rFSH and uFSH group was unknown. Another group of the patient characteristics in Table III had a similar frequency distribution of FSH receptor allelic variants because of the same-patient population receiving rFSH and uFSH stimulation respectively. The data in the rFSH and uFSH groups, which had unknown frequency distribution of FSH receptor allelic variants, were comparable with respect to dosage/duration of FSH used and endometrial thickness on hCG day. However, the estradiol levels, number of follicles on hCG day and number of total oocytes/MII oocytes retrieved were significantly higher in the uFSH group than in the rFSH group. On the contrary, MII oocytes at the removal of corona/cumulus cells which were collected from rFSH-stimulated patients, achieved better rates of fertilization and good morphology embryos than those collected from uFSH-stimulated patients. Similarly, MII oocytes matured from MII stage in vitro culture achieved better rates of injection, fertilization and good morphology embryo in rFSH-stimulated patients than those in uFSH-stimulated patients too. Simultaneously, MII oocytes at the removal of cumulus/corona cells collected from either rFSH or uFSH-stimulated patients had better fertilization rates and embryo morphologies than MII oocytes matured from metaphase I stage in vitro in the present and previous studies (Huang et al., 1999, 2002). Regarding the patient population with the similar frequency distribution of FSH receptor allelic variants in Table III, the ovarian responses were comparable between the rFSH and uFSH groups. However, the maturational profile and developmental competence of oocytes at the removal of cumulus/corona cells in the women receiving rFSH stimulation were significantly better than those in the women receiving uFSH stimulation. Moreover, the implantation rates were significantly higher in the rFSH group than in the uFSH group when the number of good morphology embryos transferred was similar. However, the pregnancy rates per transfer were similar, probably due to the small sample size. Because of small sample sizes in the patient population with the similar frequency distribution of FSH receptor allelic variants in Table III, definite conclusions concerning the differences of ovarian response between rFSH and uFSH cannot be drawn from this study population. However, the sample sizes are sufficient to compare the significant difference in the implantation rates between the rFSH and uFSH groups. It becomes increasingly clear that some LH activity is essential for optimal maturation and development of the follicle-oocyte unit during ovulation induction treatment (Lévy et al., 2000). The fewer follicles developed and lower oocyte yield in the rFSH is hard to explain. We speculate that the differences observed in our study may be due to the different LH or urinary protein content in the two FSH preparations. The urinary proteins may have negative effects on follicular recruitment and development (Giudice et al., 1994). Alternatively, oocyte cytoplasmic maturity might be influenced by the large amounts of urinary proteins, such as cytokines, growth factors, transferrins and other proteins that are found in uFSH. Poor cytoplasmic maturation of oocyte in the uFSH group resulted in poor fertilization and embryonic developmental competence compared with the rFSH group. However, the answer remains to be further elucidated.

There have been many studies comparing the effects of rFSH stimulation with urinary-derived gonadotrophins (hMG, FSH and FSH-HP) in assisted reproductive technology cycles since the mid-1990s. Previous studies have demonstrated that rFSH is more effective in inducing multifollicular development compared with highly purified uFSH (Bergh et al., 1997; Hoornans et al., 1999; Frydman et al., 2000). However, there are limited published studies in comparing the differences between rFSH and uFSH stimulations, which focused on evaluation of oocyte quality in the ICSI cycles as was the case in our study. It has been demonstrated that rFSH is as safe and effective as uFSH in stimulating ovarian follicular development in a multicentre and prospective study (Recombinant Human FSH Study Group, 1995). In this study, the patient population included patients from Belgium, France, Spain, Sweden, Switzerland and the UK, and only IVF cycles were recruited. There were comparable results in terms of patient characteristics and post-embryo transfer outcomes. Although these were not significantly different due to the sample size <70, the number of oocytes recovered or fertilized is less in the rFSH group than in the uFSH group. Ravhon et al. (2001) in a retrospective and single centre study demonstrated that the implantation and pregnancy rates were similar when either rFSH or uFSH is used. However, the uFSH group, compared with the rFSH group, had a significantly higher number of oocytes retrieved and fertilized, and higher units of FSH used. In this study, the patient study population was from the UK and the sample sizes were 1388. Similarly, our previous study (Lan et al., 2002) and present study have shown that rFSH stimulation in controlled ovarian stimulation decreased the number of MII oocytes in comparison with uFSH stimulation. Contrary to the above studies, Out et al. (1995) prospectively demonstrated a higher number of oocytes recovered and high quality embryos in rFSH stimulation after a long protocol of pituitary down-regulation than in uFSH-stimulated women undergoing IVF cycles (Out et al., 1995). However, the implantation and pregnancy rates were similar between the two groups. In this multicentre study, the infertile female subjects were recruited at 18 different IVF centres throughout Europe and the sample sizes were 981.

Because of the conflicting evidence as described above, it remains unclear whether the use of rFSH will achieve a better ovarian response and assisted reproductive technology outcome after ovarian stimulation. There are still some biases in these studies, which may include the ethnic origin, such as the frequency distribution of allelic variants of FSH receptors, possible minimal differences in the patient characteristics and the sample size. Nevertheless, in contrast to the above studies, we have shown the effects of different gonadotrophin preparations on oocyte and embryo quality. This is the first demonstration that there are different degrees of oocyte quality
and subsequent embryo quality between rFSH and uFSH stimulation. Oocyte quality is a unique characteristic of each individual stimulation cycle. Intrinsic oocyte differences are expected that may reflect discrepancies between nuclear and cytoplasmic maturation. Any activity that requires the creation and development of an embryo, in the context of infertility treatment, is dependent not only on oocyte quantity but also on the intrinsic ability of that oocyte to support development. Oocyte quality considerations are of unique concern in any procedures where efficiencies are low, because intrinsic differences between oocytes cannot be separated readily from extrinsic factors. Confounding factors that may participate in generating intrinsic oocyte variability include different preparations of gonadotrophins for stimulation, the degree of ovarian stimulation, and the stage of the oocyte. It is also clear that cytoplasmic maturation is critical to the production of viable embryos. Previously, others (Flood et al., 1990) tested the hypothesis that in vitro-matured monkey oocytes lacked essential cytoplasmic factors by conducting ooplasmic transplants from MII to germinal vesicle oocytes, rendering the latter developmentally competent. In the present study, a similar number of good morphology embryos for transfer in the same patient population with similar frequency distribution of FSH receptor variants receiving rFSH or uFSH stimulation led to significantly different implantation rates. This phenomenon can be explained by the better embryo quality with rFSH stimulation than with uFSH stimulation. Nevertheless, the differences in quality within embryos with good morphology seem to result from different degrees of cytoplasmic maturation of the oocytes. Furthermore, MII oocytes that extruded the first polar body on the removal of cumulus/corona cells had better fertilization rates and embryo morhologies than in vitro-matured oocytes that extruded the first polar body following the removal of cumulus/corona cells and in vivo culture in the present and previous studies (Huang et al., 1999, 2002). It is possible that during the final stages of oocyte maturation, it acquires cytoplasmic mechanisms that are necessary not only to support fertilization but also for embryo development. In other words, the quality of cytoplasmic maturation is the probable cause for differences in fertilization and embryo quality and influences the developmental competence of oocytes resulting from rFSH or uFSH stimulation. In the present study, we also saw this phenomenon. Furthermore, the degree of cytoplasmic maturation in MII oocytes (in vitro-matured or in vivo-matured oocytes) could also explain the differences in fertilization rates and subsequent embryo quality given the two preparations of FSH stimulation.

Although there were confounding results between the two types of ovarian FSH stimulation with regard to the number of oocytes and pregnancy/implantation rates in several studies, the numbers of MII oocytes at the removal of the cumulus/ corona cells or following the removal of cumulus/corona cells and in vitro culture were significantly different between rFSH and uFSH stimulation in the present study. The quality of MII oocytes from the rFSH stimulation was better than from the uFSH stimulation. The different degree of MII oocyte quality resulted not from nuclear maturation but from different degrees of cytoplasmic maturation. It is also suggested that FSH receptor polymorphism may be an intrinsic factor and that FSH stimulation is an extrinsic factor affecting the quantity and quality of oocytes. Because of the possible minimal differences in the patient characteristics and the differences of FSH–FSH receptor interaction from the published studies, definite conclusions concerning the efficacy of rFSH and uFSH cannot be drawn from the individual studies. However, this is the first evidence to demonstrate the differences in oocyte/embryo quality between rFSH and uFSH stimulation.

References


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In vitro cumulus-free oocyte maturity