Individual versus standard dose of rFSH in a mild stimulation protocol for intrauterine insemination: a randomized study

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BACKGROUND: Controlled ovarian stimulation (COS) and intrauterine insemination (IUI) are often used as the first-line treatment for subfertile couples. To minimize the variability in ovarian response in patients’ first treatment cycle, we recently developed a recombinant follicle-stimulating hormone (rFSH) dosage nomogram. The nomogram has now been tested.

METHODS: Multicentre randomized controlled trial (RCT) including 228 ovulatory patients scheduled for COS and IUI. Patients were randomized to ‘individual’ (50–100 IU rFSH/day, n = 113) or ‘standard’ (75 IU rFSH/day, n = 115) dose. ‘Individual’ dose was prescribed according to the nomogram, which was based on patients’ body weight and antral follicle count. The primary end-point was the proportion of patients with two to three follicles ≥14 mm (maximum two follicles ≥18 mm) on the day of hCG (leading follicle = 18 mm). Primary analysis was made by intention-to-treat.

RESULTS: In the ‘individual’ group, 79/113 (70%) of the patients developed two to three follicles versus 64/115 (56%) in the ‘standard’ group [absolute difference = 14.3 percentage points; 95% confidence interval (CI) 2–26, P = 0.03; absolute difference = 14.4; 95% CI 2–27, P = 0.02, when adjusting for centre]. Among patients with two to three follicles, the proportion of patients with two follicles was 46/79 (58%) in the ‘individual’ group versus 34/64 (53%) in the ‘standard’ group, P = 0.54. Ongoing pregnancy rate was 23/113 (20%) in the ‘individual’ group and 21/115 (18%) in the ‘standard’ group and the rate of multiple gestations was 1/113 (1%) versus 5/115 (4%), P = 0.21.

CONCLUSIONS: This RCT is the first to clinically test a dosage nomogram in ovulatory IUI patients’ first rFSH treatment cycle. Dosing according to the nomogram was superior to standard dosing.

Trial registration: ClinicalTrials.gov Identifier NCT00374634.

Key words: IUI / controlled ovarian stimulation / individual dosing / dosage nomogram / ovarian response

Introduction

Intrauterine insemination (IUI) is often used as a first-line treatment of subfertile couples (Karande et al., 1999; Goverde et al., 2000; National Collaborating Centre for Women’s and Children’s Health, 2004; Steures et al., 2005; Danish Fertility Society, 2006, 2007) and IUI is the most commonly used treatment for subfertility in Denmark (Danish Fertility Society, 2006, 2007). In ovulatory patients, the treatment is normally combined with controlled ovarian stimulation (COS).

As demonstrated in a recent meta-analysis, multifollicular growth is associated with a higher pregnancy rate compared with monofollicular growth (van Rumste et al., 2008), but the disadvantage of the stimulation is the risk of multiple pregnancy. Over the past decade, stimulation with recombinant follicle-stimulating hormone (rFSH) has been used for COS prior to IUI. The multiple-dose injection device (Puregon® Pen) allows for individual dosing strategies, including dose adjustments during the stimulation period, and compared with clomiphene citrate, anti-estrogenic side effects such as hot flushes and
headaches are less frequent. For an appropriate balance between treatment effectiveness and the risk of multiple pregnancy, the number of mature follicles in ovulatory patients should not exceed two to three.

Whereas the starting dose in the second treatment cycle is generally based on the ovarian response in the first cycle, the optimal dose in the first cycle is less obvious. Thus, information on predictive factors of the ovarian response to COS in the first treatment cycle is needed. Such factors can be used for development of dosing models as previously demonstrated in *vitro* fertilization (IVF) patients (Popovic-Todorovic et al., 2003a, b) and in anovulatory patients treated with FSH (Nyboe Andersen et al., 2008). After validation of the models, they can serve as evidence-based individualized treatment (Fauser et al., 2008).

We recently tested the following parameters as predictors of ovarian response to COS in ovulatory IUI patients: woman’s age, menstrual cycle length, smoking status, body weight, BMI, total antral follicle count (AFC), ovarian volume, ovarian stromal blood flow, basal FSH and estradiol (Freiesleben et al., 2008). Derived from two independent significant predictors, we developed an rFSH dosage nomogram for use in patients’ first treatment cycle. On the basis of the patient’s body weight (kg) and total AFC on cycle day (cd) 3, an individual rFSH starting dose of 50, 75 or 100 IU was proposed (Fig. 1).

The purpose of this study was to test if rFSH dosing according to the nomogram (Fig. 1) resulted in more patients with appropriate ovarian response in the first cycle, the optimal dose in the first cycle is less obvious. Thus, information on predictive factors of the ovarian response to COS in the first treatment cycle is needed.

The CONSORT (Consolidated Standards of Reporting Trials) diagram shows the flow of participants through each stage of the study (Fig. 2). A total of 234 patients were included to obtain 222 patients for the per protocol (PP) analysis. The patients were ‘standard’ patients who were scheduled for COS and IUI. The indications for treatment were: unexplained infertility, male factor infertility (minimum 2 million progressive motile spermatozoa after density gradient centrifugation) or minimal–mild endometriosis. Inclusion criteria were: (i) female age between 25 and 39 years (both included), (ii) spontaneous regular menstrual cycle within the range of 21–35 days (intra-individual variation maximum ± 3 days), (iii) two ovaries with no cysts (>20 mm), (iv) bilateral tubal patency, (v) first rFSH cycle and (vi) only minimal or mild disease in the case of endometriosis (ASRM, 1997). Patients treated with donor sperm were allowed to enter the study only if pregnancy had not occurred after insemination in minimum three spontaneous cycles. Exclusion criterion was: (i) patients with any disease where administration of the standard rFSH dose was not appropriate.

**Materials and Methods**

**Study design**

This randomized multicentre study was performed from September 2006 to September 2008 at four public fertility clinics in Denmark. Three centres worked on a 7-day schedule and one centre was closed on Sundays. The Danish National Committee on Biomedical Research Ethics approved the study (KF 02 310088). All patients participated after verbal and written informed consent. The study was conducted according to International Conference on Harmonisation (ICH) guidelines and Good Clinical Practice (GCP). Two independent external monitors from The GCP Unit, Copenhagen Region, and the GCP Unit in Århus, Denmark, monitored the study.

**Participants**

The CONSORT (Consolidated Standards of Reporting Trials) diagram shows the flow of participants through each stage of the study (Fig. 2). A total of 234 patients were included to obtain 222 patients for the per protocol (PP) analysis. The patients were ‘standard’ patients who were scheduled for COS and IUI. The indications for treatment were: unexplained infertility, male factor infertility (minimum 2 million progressive motile spermatozoa after density gradient centrifugation) or minimal–mild endometriosis. Inclusion criteria were: (i) female age between 25 and 39 years (both included), (ii) spontaneous regular menstrual cycle within the range of 21–35 days (intra-individual variation maximum ± 3 days), (iii) two ovaries with no cysts (>20 mm), (iv) bilateral tubal patency, (v) first rFSH cycle and (vi) only minimal or mild disease in the case of endometriosis (ASRM, 1997). Patients treated with donor sperm were allowed to enter the study only if pregnancy had not occurred after insemination in minimum three spontaneous cycles. Exclusion criterion was: (i) patients with any disease where administration of the standard rFSH dose was not appropriate.

**Treatment protocol**

The patients were requested to contact the fertility clinic on cd 1 and were examined at the clinic on cd 3 (range 2–4). Clinical data were recorded and two-dimensional transvaginal sonography was performed. The antral follicles (2–10 mm) were counted and ovarian volume was calculated from the measurements of the maximum longitudinal (D1), anterior–posterior (D2) and transverse (D3) diameters using the ellipse formula D1 × D2 × D3 × 0.523. Experienced doctors in the fertility clinics performed the ultrasound scans. Blood samples were drawn from the antecubial vein for assays of baseline FSH and estradiol levels. FSH and estradiol measurements were not used during the treatment, but exclusively for analytic purposes.

Participants were assigned to either ‘individual’ rFSH dose (50, 75 or 100 IU/day) or ‘standard’ rFSH dose (75 IU/day) (Puregon®, Organon a division of Schering-Plough). The individual dose was identified using the nomogram (Fig. 1) based on the patients’ weight (kg) and total AFC on cd 3. Patients who were at the dividing line between 50 and 75 IU were prescribed 50 IU/day, whereas those who were at the dividing line between 75 and 100 IU were prescribed 100 IU/day.

The rFSH starting dose was fixed the first 5 days of stimulation. After an ultrasound examination on Day 6 of stimulation, the dose was adjusted in both study arms according to the predefined criteria: (i) if >3 follicles were ≥10 mm, the dose was reduced, (ii) if all follicles were <10 mm and there was no growth of the endometrium, the dose was increased, (iii) in all other cases and in the case of follicular asynchrony (≥4 mm difference from the leading follicle to the second largest follicle), the dose was maintained.

After Day 6 of stimulation, patients underwent ultrasound examination minimum every 2–3 days, scheduled according to an expected growth of 2 mm/day of the follicles ≥12 mm. The gonadotrophin-releasing hormone (GnRH) antagonist ganierek 0.25 mg (Orgalutran®, Organon a division of Schering-Plough) was administered daily from the day the leading follicle reached a diameter of 14 mm to prevent premature luteinizing hormone rises and luteinization (Lambalk et al., 2006). Human
chorion gonadotrophin (hCG) (Pregnyl® 5000 IU, Organon a division of Schering-Plough) was administered the day the leading follicle reached a diameter of 18 mm. All injections were administered subcutaneously. Appropriate ovarian response was defined as two to three follicles \( \geq 14 \text{ mm} \) and maximum two follicles \( \geq 18 \text{ mm} \) on the day of hCG administration. Inappropriate ovarian response was defined as either one, or more than three follicles \( \geq 14 \text{ mm} \) or more than two follicles \( \geq 18 \text{ mm} \) on the day of hCG. To minimize the risk of multiple pregnancy, IUI was cancelled if more than three follicles were \( \geq 14 \text{ mm} \) or if more than two follicles were \( \geq 18 \text{ mm} \) on the day of hCG, and the patients were offered conversion of the treatment cycle to IVF.

A single IUI was carried out 38 h after hCG administration. Semen samples were prepared with density gradient centrifugation using Pure Sperm separation. IUI was done irrespective of the total motile sperm count on the day of IUI. No luteal phase supplementation was prescribed unless the treatment cycle was converted to IVF, in which case standard IVF procedures were used.

If menstrual bleeding was absent 2 weeks after IUI, a urine pregnancy test was done at home and if positive, pregnancy was confirmed by measurement of serum hCG. An ultrasound examination was done at 8 weeks of gestation, and the number of live fetuses was recorded. An ongoing pregnancy was defined as an intrauterine pregnancy with minimum one fetus with heart activity by transvaginal ultrasound at 8 weeks of gestation. End of study was defined as the day of hCG. All participants were followed up to record whether or not an ongoing pregnancy occurred.

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**Figure 2** CONSORT diagram showing the flow of participants through each stage of the trial.
Hormone assays
Fresh blood samples were analysed locally using immunoassays. Detection limits for FSH were \( \leq 1.0 \text{ IU/l} \) and intra- and inter-assay coefficients of variations \(<8\%\). For estradiol, detection limits were \( \leq 0.10 \text{ nmol/l} \) and intra- and inter-assay coefficients of variations \(<15\%\).

Study end-points
The primary end-point was the proportion of patients in each group with an appropriate ovarian response (two to three follicles \( \geq 14 \text{ mm} \) of which a maximum of two follicles were \( \geq 18 \text{ mm} \)) on the day of hCG. Secondary end-points were: (i) the proportion of patients with an inappropriate ovarian response (only one or too many follicles for IUI) and (ii) pregnancy rates.

Sample size calculation
In our previous study (Freiesleben et al., 2008), doses of 75 IU rFSH/day yielded two to three mature follicles in (86/159) 54% of the patients. The present study was designed to test whether individual rFSH dosing increased the proportion of patients with two to three mature follicles compared with standard rFSH dosing. It was considered highly clinical relevant if the proportion was increased by one-third to 72% (absolute difference = 18). A sample size of \(~\sim 220\) participants for the per-protocol (PP) analysis would allow us to detect this difference, with a two-sided significance level of 0.05 and 80% power.

Randomization
An independent statistician provided a randomization list generated by computerized block randomization. Participants were randomized to one of the two treatment groups ‘individual’ or ‘standard’ dose at a ratio of 1:1. In each centre, an equal number of patients were randomized to the two groups. Three of the participating centres included minimum 30 patients each, but for the flexibility of the study, 48 envelopes were prepared for each of the three centres. The fourth centre (Righospitalet, Copenhagen) included the rest of the patients. From the randomization list, an independent secretary prepared the numbered and opaque envelopes. Independent monitors from the GCP Unit at Copenhagen University sealed the envelopes, which were distributed to the four centres at initiation of the study. The envelopes were opened in consecutive order by one of the investigators after verbal and written informed consent, after the ultrasound examination on cd 3, and immediately before starting rFSH stimulation. The block sizes were not disclosed during the study. The monitors verified the order in which the patients were included and that all spare envelopes were unopened at the end of the study.

Statistical analysis
The primary statistical analysis was carried out according to intention-to-treat (ITT) principle (n = 228), which included all patients who fulfilled the inclusion criteria. Reassessment of entry criteria was applied identically in both study groups. Six patients were false inclusions and did not meet the inclusion criteria due to: polycystic ovary syndrome and metformin treatment (n = 1), not first rFSH cycle (n = 1), age criteria not fulfilled (n = 2) and moderate or severe endometriosis (n = 2). One patient in the individual dose group was mistakenly treated with 75 IU rFSH/day instead of 50 IU/day. She developed \( > 3 \) follicles and the treatment cycle was converted to IVF. This patient was included within the individual dose group for ITT analyses. In the PP analysis (n = 222), six patients were excluded due to the following protocol deviations: one patient who did not receive the allocated intervention and five patients who had protocol deviations upon rFSH dose adjustment according to the pre-defined criteria on Day 6 of stimulation. Two patients cancelled treatment before the day of hCG (on cd 15 and 17) and were analysed according to the last observed response (Hollis and Campbell, 1999).

Data are presented as mean or median according to data distribution. A two-sided \( P \)-value of \(<0.05\) was considered statistically significant. Differences in treatment outcome between the groups are presented in percentage points and the 95% confidence interval (CI) for the difference was calculated. Comparisons of continuous variables between the groups were made by the independent samples \( t \)-test or the Mann–Whitney U-test where appropriate. Comparisons of proportions between the groups were made by \( \chi^2 \) tests or Fisher’s exact test where appropriate. Additionally, the difference between the group proportions was calculated for each centre for the primary end-point. A \( \chi^2 \) test was used to test, if these were homogenous. To allow for possible confounding by centre, a joint estimate of the group difference was calculated as a weighted average of the centre-specific differences.

Statistical analysis of the data was performed with SPSS (Statistical Package for Social Science) software, version 15.0 (Chicago, IL, USA), and SAS, version 9.1.

Results
Patient characteristics
There were no statistically significant differences between treatment groups in any of the baseline (cd 3) parameters (Table I). Mean age (SD; standard deviation) of the patients in the study was 32.6 (3.5)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Individual rFSH dose (n = 113)</th>
<th>Standard rFSH dose (n = 115)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.6 ± 3.6</td>
<td>32.6 ± 3.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.0 ± 11.0</td>
<td>67.3 ± 12.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 ± 3.5</td>
<td>23.4 ± 3.8</td>
</tr>
<tr>
<td>Cycle length (days)</td>
<td>28.4 ± 1.4</td>
<td>28.6 ± 1.9</td>
</tr>
<tr>
<td>Smokers</td>
<td>15 (13)</td>
<td>14 (12)</td>
</tr>
<tr>
<td>Duration of infertility</td>
<td>2.1 ± 1.4</td>
<td>2.3 ± 1.4</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>75 (66)</td>
<td>66 (57)</td>
</tr>
<tr>
<td>Parous women¹</td>
<td>11 (10)</td>
<td>19 (17)</td>
</tr>
<tr>
<td>Infertility diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexplained</td>
<td>56 (50)</td>
<td>51 (44)</td>
</tr>
<tr>
<td>Male factor, partner’s sperm</td>
<td>48 (42)</td>
<td>48 (42)</td>
</tr>
<tr>
<td>Male factor, donor sperm</td>
<td>7 (6)</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Combined</td>
<td>1 (1)</td>
<td>0</td>
</tr>
<tr>
<td>Antral follicle count²</td>
<td>18 ± 8.6</td>
<td>17 ± 8.1</td>
</tr>
<tr>
<td>Ovarian volume³</td>
<td>10.3 ± 4.7</td>
<td>10.4 ± 4.6</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>7.3 ± 2.4</td>
<td>7.4 ± 2.3</td>
</tr>
<tr>
<td>Estradiol (nmol/l)</td>
<td>0.18 ± 0.07</td>
<td>0.18 ± 0.06</td>
</tr>
</tbody>
</table>

Values are mean ± SD or number (column percentage).

¹Labour at or after 22 weeks of gestation.
²Total count, left + right ovary.
³Total volume (ml), left + right ovary.
years, mean body weight (SD) was 67.1 (11.5) kg, mean duration of infertility (SD) was 2.5 (1.4) years, mean total AFC (left + right ovary) (SD) was 18 (8) and mean baseline (cd 3) FSH was 7.4 (2.3) IU/l. In the individual dose group (n = 113), 24 (21%) patients had an rFSH dose of 50 IU/day, 56 (50%) patients had 75 IU/day and 33 (29%) patients had 100 IU/day versus 115 patients in the standard dose group who all had 75 IU/day.

Primary end-points

The number of patients with appropriate ovarian response (two to three follicles) was 79/113 (70%) and 64/115 (56%) in the ‘individual’ and ‘standard’ dose groups, respectively (Table II); absolute difference between the groups was 14.3% (95% CI: 2–26, P = 0.03). If continuity correction is used, the values are: 95% CI: 1–27, P = 0.04. The absolute difference in each centre was: 19.8 (47/63 – 34/62) (95% CI: 3–35, P = 0.02) in Centre 1 (n = 125), 37.3 (12/17 – 6/18) (95% CI: 4–61, P = 0.03) in Centre 2 (n = 35), 14.3 (11/19 – 13/18) (95% CI: −16 to 41, P = 0.36) in Centre 3 (n = 37) and 0 (9/14 – 11/17) (95% CI: −30 to 32, P = 1.00) in Centre 4 (n = 31). As indicated by the overlapping CIs, these differences were not statistically different (P = 0.08, in a χ² test with three degrees of freedom). A weighted average of the differences yielded a value (absolute difference adjusted by centre) of 14.4 (95% CI 2–27, P = 0.02). Among the patients who had an appropriate ovarian response, the proportion of patients with two follicles was 46/79 (58%) in the individual dose group versus 34/64 (53%) in the standard dose group [absolute difference 5.1 (95% CI: −11 to 21, P = 0.54)] (Table II). In the PP analysis (n = 222), 77/110 patients (70%) in the individual dose group had an appropriate ovarian response versus 63/112 patients (56%) in the standard dose group [absolute difference 13.8 (95% CI: 1–26, P = 0.03)].

Secondary end-points

Outcomes according to treatment group are listed in Table II. The number of patients with only one follicle was 26/113 (23%) in the individual dose group and 38/115 (33%) in the standard dose group; absolute difference was 10.0 (95% CI: −2 to 21, P = 0.09). The number of patients who matured too many follicles for IUI was 8/113 (7%) in the individual dose group and 13/115 (11%) in the standard dose group; absolute difference was 4.2 (95% CI: −4 to 12, P = 0.27). The detailed distribution of follicles ≥14 mm on the day of hCG according to group as randomized and rFSH starting dose is shown in Fig. 3.

The ongoing pregnancy rate was 23/113 (20%) in the individual dose group and 21/115 (18%) in the standard dose group (P = 0.69), and the ongoing multiple pregnancy rate was 1/113 (1%) and 5/115 (4%), respectively. Among the patients who were inseminated, the ongoing pregnancy rate was 21/101 (21%) and 20/99 (20%) in the

Table II Outcome according to treatment group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Individual rFSH dose (n = 113)</th>
<th>Standard rFSH dose (n = 115)</th>
<th>P-value</th>
<th>Difference 1 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appropriate ovarian response</td>
<td>79 (70)</td>
<td>64 (56)</td>
<td>0.03</td>
<td>14.3 (2 to 26)</td>
</tr>
<tr>
<td>Proportion with two mature follicles</td>
<td>46/79 (58)</td>
<td>34/64 (53)</td>
<td>0.54</td>
<td>5.1 (−11 to 21)</td>
</tr>
<tr>
<td>One mature follicle</td>
<td>26 (23)</td>
<td>38 (33)</td>
<td>0.09</td>
<td>10.0 (−2 to 21)</td>
</tr>
<tr>
<td>Too many mature follicles for IUI</td>
<td>8 (7)</td>
<td>13 (11)</td>
<td>0.27</td>
<td>4.2 (−4 to 12)</td>
</tr>
<tr>
<td>Cycles with dose adjustment on Day 6</td>
<td>19 (17)</td>
<td>23 (20)</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Stimulation days</td>
<td>8.2 ± 2.1</td>
<td>8.4 ± 2.3</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Consumption of FSH (IU)</td>
<td>638 ± 249</td>
<td>651 ± 243</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Endometrium on day of hCG (mm)</td>
<td>8.7 ± 2.0</td>
<td>8.5 ± 1.7</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>IUI</td>
<td>101 (89)</td>
<td>99 (86)</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>8 (7)</td>
<td>11 (10)</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Overall positive hCG</td>
<td>27 (24)</td>
<td>23 (20)</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Overall ongoing pregnancies</td>
<td>23 (20)</td>
<td>21 (18)</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>Ongoing multiple gestations</td>
<td>1 (1)</td>
<td>5 (4)</td>
<td>0.21</td>
<td>3.5 (−2 to 10)</td>
</tr>
<tr>
<td>Positive hCG 2 weeks after IUI</td>
<td>24/101 (24)</td>
<td>21/99 (21)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Ongoing pregnancies after IUI</td>
<td>21/101 (21)</td>
<td>20/99 (20)</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Multiple gestations</td>
<td>1/21 (5)</td>
<td>5/20 (25)</td>
<td>0.09</td>
<td>20.2 (−2 to 42)</td>
</tr>
</tbody>
</table>

Values are mean ± SD or number (%).

aIndependent samples t-test.

bχ² test.

cFisher’s exact test.

dMore than three follicles ≥14 mm on the day of hCG of which maximum two follicles were ≥18 mm.

eTwo of the patients in the standard dose group who were cancelled due to too many follicles did not have IVF. They had four and five mature follicles, respectively.

fTwo weeks after IUI or embryo transfer.

ghHeart activity at 8 weeks of gestation.

iAll were twin pregnancies except from one triplet pregnancy in the individual dose group, which was reduced to twins.

jAbsolute difference between the groups (%).
individual and standard dose groups, respectively. The rate of multiple ongoing pregnancies after IUI was 1/21 (5%) in the individual dose group and 5/20 (25%) in the standard dose group; absolute difference 20.2 (95% CI: $-2$ to 42, $P = 0.09$). The only multiple pregnancy in the individual dose group was a triplet pregnancy after insemination with donor sperm, and the five multiple pregnancies in the standard dose group were twin pregnancies conceived after insemination with the partner’s sperm. In the 19 patients in whom the treatment cycle was converted to IVF, there were no statistically significant differences between the outcomes according to randomized group. The number of oocytes achieved upon follicle aspiration was 1–6 in both study groups. In total, 16 patients achieved embryo transfer; nine patients had elective single-embryo transfer (eSET), six patients had single-embryo transfer and one patient had double-embryo transfer. Nine patients had 1–5 embryos cryopreserved. In the fresh cycle, three patients had an ongoing pregnancy (all were singletons). In the individual dose group, 79 patients had an appropriate ovarian response. Seventy-five of these patients were inseminated. Of the 79 patients, the rate of patients with positive hCG was 19/79 (24%), and 17/79 (22%) had an ongoing pregnancy (including one multiple pregnancy).

Adverse reactions/events

No serious adverse reactions or events (e.g. ovarian hyper-stimulation syndrome) occurred in any of the groups.

Discussion

This randomized controlled trial (RCT) is the first to test a dosing model in ovulatory IUI patients’ first rFSH treatment cycle. The study demonstrated that significantly more patients in the individual dose group had an appropriate ovarian response compared with the standard dose group. The 95% CI for the difference indicated that the ‘true’ difference is somewhere between 2 and 26 percentage points in favour of individual dosing. Also, the CI includes the ‘assumed’ absolute difference (18). Concerning the secondary endpoints, the rate of multiple pregnancies was lower in the individual dose group, but the difference was not statistically significant at the 5% level. Fewer patients in the individual dose group were converted to IVF, and relatively more patients in each of the individual dose categories had an appropriate ovarian response (two to three follicles) compared with the standard dose group. In the 100 IU dose group, however, the rate of patients with >3 follicles was slightly higher (Fig. 3). Additionally, a post-hoc analysis within the standard dose group demonstrated that four patients with three follicles would have been prescribed 100 IU/day if treated with an individual dose. For further improvement of the nomogram, it is therefore possible that the dividing line between 75 and 100 IU should be placed further to the right, thereby increasing the threshold for prescribing 100 IU. The different (though not statistically different) proportions of patients with an appropriate ovarian response in each centre could be a coincidence, but it could also be due to different characteristics of the patients in the four centres.

It can be discussed whether or not participants found not to satisfy the entry criteria after randomization, so-called ‘false inclusions’, should be included in ITT analysis (Hollis and Campbell, 1999). Reascertainment of the entry criteria was applied identically in each group and monitored by external GCP Units, and ‘false inclusions’ were excluded. Regarding the primary end-point, a consequence analysis which included the six ‘false inclusions’ ($n = 234$) showed that 79/116 (68%) in the individual dose group versus 66/118 (56%) in the standard dose group had two to three mature follicles; absolute difference = 12.2 (95% CI: 0–24, $P = 0.06$). The difference is borderline significant, but the absolute difference and corresponding 95% CI for the difference is very similar to analysis excluding ‘false inclusions’ (absolute difference = 14.3, 95% CI: 2–26, $P = 0.03$) and to the PP analysis (absolute difference = 13.8, 95% CI: 1–26, $P = 0.03$). Also, the CI includes values that we would still consider to be clinically important.

Strengths and limitations

The strength of the study is the prospective randomized design including only first rFSH stimulation cycles, which minimizes the risk of selection bias. No participants were lost to follow-up. After randomization, the study was open without blinding. This design was accepted due to the objective (follicles and pregnancy) end-points and strict protocolized stimulation and treatment criteria. The patients administered the medicine as self-injections. To maximize compliance, all patients received careful verbal and written information on medicine self-administration including dosage, and a medicine account was kept for each patient. A limitation of the study is that the sample size was insufficient to detect statistical significant differences in pregnancy and multiple pregnancy rates between the groups.

Although there was no difference in the mean number of stimulation days between the groups (Table II), too early or delayed administration of hCG could bias the study. Two patients in the standard dose group had hCG 1 day too early (leading follicle $= 17$ mm). In one of the patients, the leading follicle was the only follicle $> 11$ mm. The other
patient also had one follicle of 15 mm and one follicle of 16 mm but no other follicles >11 mm. Three patients in each study group had hCG 1 day late. None of these patients had intermediate-size follicles that could influence on the primary end-point. Two of the three patients from the individual dose group matured more than two to three follicles versus none from the standard dose group. Therefore, when considering the expected growth of 2 mm/day of follicles ≥ 12 mm, we find it unlikely that these eight patients induced bias.

**Comparison with other studies**

An RCT tested a very mild COS GnRH-antagonist protocol before IUI (Ragni et al., 2004). Patients received 50 IU of rFSH/day (n = 32) or 50 IU of rFSH on alternate days (n = 34). The proportion of cycles with only one follicle ≥ 16 mm was 53.3% and the proportion of cycles with only one follicle ≥ 11 mm was 33.3% in the group that received daily rFSH, versus 78.8% and 63.6% in the ‘alternate days’ group. In our study, the proportion of cycles with only one follicle ≥ 14 mm was 23% in the individual dose group versus 33% in the standard dose group. The similar rate (33%) of monofollicular cycles in the group that received 50 IU/day in the study by Ragni et al. (2004) and our standard dose group (75 IU/day) may be explained by a lower BMI in Ragni’s study population. Another inequality between the two studies is that anovulatory patients with polycystic ovarian syndrome were included in the study by Ragni et al. (2004).

A different IUI study used an rFSH starting dose of 150 IU/day combined with GnRH antagonist in up to six cycles, and compared outcome of a weekend-free IUI protocol with results in standard IUI cycles, where IUI was performed 36–38 h after reaching optimal follicular growth (Matorras et al., 2006). The mean number of follicles ≥ 16 mm was ~5. The pregnancy rate per cycle was 16% in both groups and the total multiple pregnancy rate was 15/68 (22%) including two triplets and two quadruplets (Matorras et al., 2006). According to our definition of appropriate ovarian response, the dose of 150 IU/day is too high for our population. However, a dose of 150 IU/day may suit a small fraction of our patients, e.g. women with very high body weight (kg) and low AFC.

One argument against COS and IUI is the risk of multiple pregnancies. It has been suggested that IUI should be performed in the natural cycle only (Goverde et al., 2005). Also, expectant management for up to 6 months has been suggested in couples with unexplained subfertility and an intermediate prognosis of a spontaneous pregnancy (Steures et al., 2006). The disadvantage in these two regimens may be a longer time to pregnancy and a higher dropout rate.

Considering the low number of oocytes achieved in the patients whose treatment cycles were converted to IVF, the ongoing pregnancy rate and the number of cryopreserved embryos were reasonable. Furthermore, eSET was done in 9 of the 16 patients, who had embryo transfer. The results of the converted cycles correspond well to the results of a recent meta-analysis (Verberg et al., 2009). The meta-analysis showed that optimal embryo implantation rates were obtained with 5 oocytes retrieved following mild ovarian stimulation for IVF. The explanation for this could be that the relatively small number of oocytes obtained after mild stimulation represent the best oocytes of the cohort in a given cycle as suggested by Hohmann et al. (2003), and that endometrial receptivity is optimal at this number of oocytes.

In conclusion, this study demonstrated that individual dosing according to the nomogram (Fig. 1) resulted in a more appropriate ovarian response (two to three follicles) compared with a standard dose of 75 IU rFSH/day. The nomogram should only be applied in populations with characteristics similar to our study population, being treated in a GnRH-antagonist COS protocol. Importantly, the mean total AFC (2–10 mm) in the population should be ~18.

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