Outcome from consecutive assisted reproduction cycles in patients treated with recombinant follitropin alfa filled-by-bioassay and those treated with recombinant follitropin alfa filled-by-mass

Juan Balasch obtained his MD degree (1974) and the speciality degree in Obstetrics and Gynaecology (1977) at the Faculty of Medicine-Hospital Clinic, University of Barcelona in Spain. The PhD degree was granted to him at the same University in 1979. At present he is full professor in Obstetrics and Gynaecology and Head of the Fertility Unit at the Faculty of Medicine-Hospital Clinic, University of Barcelona. Professor Balasch is Past President of the Spanish Fertility Society and has more than 200 publications in international journals and books; in a series of studies he and his team developed a new hypothesis on the pathogenesis of the ovarian hyperstimulation syndrome. He serves as ad-hoc reviewer or is on the Editorial Board of different international journals dealing with fertility, gynaecological endocrinology and human reproduction. Professor Balasch’s current research interests include assisted reproduction, repeated abortion, implantation failure and ovarian (hyper)stimulation.

Juan Balasch, Francisco Fàbregues, Joana Peñarrubia, Montserrat Creus, Dolors Manau, Ester Vidal, Roser Casamitjana, Juan A Vanrell

1Institut Clinic of Gynecology, Obstetrics and Neonatology, and 2Hormonal Laboratory; Faculty of Medicine-University of Barcelona, Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain
3Correspondence: Institut Clinic of Gynecology, Obstetrics and Neonatology, Hospital Clinic; C/Casanova 143, 08036 Barcelona, Spain (Tel: +34 93 2275534) (Fax: +34 93 2275454) (e-mail: jbalasch@medicina.ub.es)

Abstract

Recent advances in manufacturing procedures for r-hFSH have resulted in a preparation (follitropin alfa) that is highly consistent in both isoform profile and glycan species distribution. As a result, follitropin alfa can be reliably quantified and vials can be filled by mass. This study compared the clinical results in a well-established assisted reproduction programme during the crossover from standard follitropin alfa filled-by-bioassay (FSH-bio) to follitropin alfa filled-by-mass (FSH-mass). The study included the last 125 patients treated with FSH-bio and the first 125 patients receiving FSH-mass for ovarian stimulation in their first assisted reproduction treatment cycle. Patient baseline characteristics were almost identical in the two groups. The duration of ovarian stimulation was significantly shorter in the FSH-mass group. The number of patients receiving the HCG injection and undergoing oocyte retrieval, follicular development and the serum concentration of oestradiol on the day of HCG injection were similar for the two treatment groups. The oocyte yield and the fertilization rates were similar in both groups of patients. However, embryo quality and implantation rates were significantly higher in the FSH-mass group. Accordingly, in spite of the mean number of embryos transferred being significantly lower in the FSH-mass group, there was a trend for higher clinical pregnancy rates in this group of patients. It is concluded that the new formulation of FSH-mass is more effective than the standard FSH-bio in terms of embryo quality, implantation rates, and number of days of stimulation.

Keywords: assisted reproduction, follitropin alfa, FSH, gonadotrophins, recombinant technology

Introduction

FSH plays a key role in inducing follicular development for assisted reproduction treatment and the amount of remaining endogenous LH after pituitary down-regulation is still sufficient to support FSH-induced follicular growth and oestrogen biosynthesis (Balasch et al., 2001, 2003; Peñarrubia et al., 2003). This seems very important after the advent of recombinant human FSH (r-hFSH), which is completely devoid of LH activity (Howles, 1996; Olijve et al., 1996). For
50 years, therapeutic preparations of gonadotrophins, including r-hFSH, have been quantified with a rat in-vivo bioassay (the Steelman–Pohley bioassay) in biological international units (IU). This assay has limited precision, requires large numbers of laboratory animals and involves cumbersome procedures for data generation and interpretation (Driebergen and Baer, 2003).

The manufacturing procedures employed for the r-hFSH, follitropin alfa, result in a preparation that is highly consistent in both isoform profile and glycans species distribution. As a result, follitropin alfa can be reliably quantified using an optimized size exclusion high-performance liquid chromatography method, and vials can be filled and released for clinical use in mass units (Driebergen and Baer, 2003). It has been recently stressed that preliminary clinical studies suggest that the filled-by-mass process results in a product that delivers a more consistent clinical response and is more effective than follitropin alfa vials filled by bioassay in women undergoing controlled ovarian stimulation (Driebergen and Baer, 2003; Hugues et al., 2003). However, available data on the subject in the literature are rather scanty and in fact, only one full paper showing that the new method for quantifying recombinant human FSH delivers an improved consistency in clinical outcome has been published (Hugues et al., 2003).

In Spain, follitropin alfa filled-by-mass was made commercially available in March 2003 and progressively replaced follitropin alfa filled-by-bioassay. Thus, the aim of this study was to compare the clinical results in a well established assisted reproduction programme where pituitary down-regulation is routinely used, during the transition period from stimulating patients with follitropin alfa filled-by-bioassay (r-hFSH-bio) to follitropin alfa filled-by-mass (r-hFSH-mass).

Materials and methods

Patient population

A total of 250 consecutive infertile women undergoing their first cycle of IVF or intracytoplasmic sperm injection (ICSI) treatment (thus avoiding possible bias from experience with previous cycles regarding ovarian response) were included in this study. Those 250 women included the last 125 patients treated with r-hFSH-bio and the first 125 patients receiving r-hFSH-mass for ovarian stimulation in their first assisted reproduction treatment cycle, and who fulfilled the inclusion criteria reported below. All IVF and ICSI cycles undergone between 17 February 2003 and 5 July 2003 at the Fertility Unit of the Hospital Clinic of Barcelona. Up to 5 April, r-hFSH-bio only was used (total 96 cycles); from then until 25 May, the two products were used (total 83 cycles, r-hFSH-bio = 29; r-hFSH-mass = 42), and from 26th May, r-hFSH-mass only was used (total 83 cycles).

All patients were infertile but otherwise healthy premenopausal women, had both ovaries and no previous ovarian surgery, and none had occult ovarian failure on the basis of their cycle day 3 FSH concentrations of <12 IU/l [standard International Reference Preparation (IRP) 78/549]. No patient had received any hormone therapy, including gonadotrophins, for at least 6 months preceding the study.

Patient indications for IVF/ICSI included the following main diagnosis: male factor infertility, unexplained infertility, endometriosis, and tubal infertility. The data set was analysed retrospectively.

Treatment cycle

All patients received standard ovarian stimulation with recombinant FSH under pituitary suppression with gonadotrophin-releasing hormone (GnRH) agonist according to a protocol previously reported (Balasch et al., 2001; Peñarrubia et al., 2003). In all women, pituitary desensitization was achieved by subcutaneous administration of triptorelin acetate (Decapeptyl 0.1 mg; Ipsen Pharma, Barcelona, Spain) (0.1 mg daily, which was reduced to 0.05 mg after ovarian arrest was confirmed) and was started in the mid-luteal phase of the previous cycle. Gonadotrophin stimulation of the ovaries was started when serum oestradiol concentrations declined to <50 pg/ml and a vaginal ultrasonographic scan showed an absence of follicles >10 mm diameter. On days 1 and 2 of ovarian stimulation, 450 and 300 IU/day of recombinant human FSH-bio or FSH-mass (the mass equivalent to 75 IU of recombinant FSH being 5.5 µg) (Gonal-f, Serono S.A., Madrid, Spain) respectively were administered subcutaneously. On days 3 and 4 of ovarian stimulation, 150 IU per day of FSH were administered to each patient. From day 5 onward, FSH was administered on an individual basis according to the ovarian response as assessed by sequential transvaginal ultrasonography and serum oestradiol measurements. The criteria for human chorionic gonadotrophin administration (HCG; 250 µg) (Ovitrelle; Serono S.A.) were the presence of two or more follicles >18 mm in diameter with ≥4 follicles measuring ≥14 mm in association with a consistent rise in serum oestradiol concentration. The cycle was cancelled when there were <3 follicles with diameter ≥14 mm after 8–9 days of gonadotrophin therapy or after 4–5 additional treatment days without attaining, or the imminent prospect of attaining, the criteria for HCG administration.

Oocyte aspiration was performed with vaginal ultrasonography 35–36 h after HCG administration. The maturation status of the oocytes and the embryo grading were recorded according to published criteria (Veeck, 1999). Embryos were classified as follows: grade 1, perfectly symmetrical with no fragmentation; grade 2, perfectly symmetrical with slight fragmentation (>20% fragmentation of the total embryonic volume); grade 3, uneven blastomeres with no fragmentation; grade 4, uneven blastomeres with gross fragmentation (>20% fragments). Embryo quality was established at day 2 of cleavage according to the number of blastomeres (fewer than 4, or 4 or more blastomeres) and embryo grading (grade 1–2 or 3–4) as previously reported (Isaza et al., 2002). Up to three embryos per patient (depending on the age of the patient, the indication for IVF/ICSI, and the number and quality of embryos available per replacement) were replaced under ultrasonographic guidance and the luteal phase was supported with vaginal micronized progesterone (600 mg/day), starting on the day following oocyte aspiration and continuing either up to menstruation, or if the patient became pregnant, for at least the first 3 weeks of pregnancy. Ovarian hyperstimulation syndrome (OHSS) was diagnosed according to the
classification proposed by Golan et al. (1989). Pregnancy was diagnosed by increasing serum concentrations of β-HCG after embryo transfer, and the subsequent demonstration of an intratuterine gestational sac by ultrasonography.

**Hormone assays and ultrasonography**

Hormones were measured using commercially available kits as reported previously (Balasch et al., 2001; Peñarrubia et al., 2003). Oestradiol concentrations in serum were estimated by a competitive immunoenzymatic assay (Immuno 1, Technicon; Bayer, Tarrytown, NY, USA). The sensitivity was 10 pg/ml and the interassay coefficient of variation (CV) was 5%. FSH and LH serum concentrations were measured by an immunoenzymatic assay with two monoclonal antibodies (Immuno 1, Technicon; Bayer) and data expressed in terms of IRP 78/549 and 68/40 respectively. The sensitivity of the assays was 0.1 IU/l for FSH and 0.3 IU/l for LH, and interassay CV were 2.7 and 3.1% respectively. Total β-HCG was measured by a solid-phase, two-site chemiluminescent enzyme immunometric assay standardized against the Third International Standard 75/537 (Immulite; Diagnostic Products Co., Los Angeles, CA, USA), with a detection limit of 2 IU/l. The inter-assay CV was 5.8%.

Ultrasound scans were performed using a 5 mHz vaginal transducer attached to an Aloka sector scanner (Model SSD-U). Aloka Co. Ltd, Tokyo, Japan).

**Transducer attached to an Aloka sector scanner (Model SSD-U)**

**Statistics**

Data were analysed by SPSS statistical software (SPSS Inc., Chicago, IL, USA). All continuous variables were tested for normality, Therefore, parametric testing was employed. The Mann–Whitney U-test, the chi-squared test and the Pearson bivariate method, as appropriate, were used to perform statistical comparisons. Data are presented as mean ± SD. \( P < 0.05 \) was considered statistically significant.

**Results**

The results are summarized in Tables 1–3. As shown in Table 1, the main demographic and baseline characteristics of the patients in groups r-hFSH-bio and r-hFSH-mass, including age, BMI, duration and cause of infertility, gonadotrophin measurement on cycle day 3, and number of patients undergoing ICSI, were almost identical. This supports the validity of the comparison process.

**Table 2** shows data regarding gonadotrophin treatment and ovarian response in the two study groups. The time to ovarian arrest and the total amount of recombinant FSH administered were similar in both treatment groups but the duration of ovarian stimulation was significantly shorter in the r-hFSH-mass group. The number of patients receiving the HCG injection and undergoing oocyte retrieval, and the total number of growing follicles, the serum concentration of oestradiol and the endometrial thickness on the day of HCG injection were similar for the two treatment groups.

As shown in Table 3, the oocyte yield, the number of metaphase II oocytes, and the fertilization rates were similar in both groups of patients. However, embryo quality as assessed on day 2 and implantation rates were significantly higher in the r-hFSH-mass group. Accordingly, in spite of the mean number of embryos transferred being significantly lower in the r-hFSH-mass group, there was a trend for higher clinical pregnancy rates in this group of patients, although differences did not reach statistical significance. There was no correlation between days of ovarian stimulation and embryo quality (data not shown). Miscarriages and severe OHSS rates were similar in the two treatment groups.

**Discussion**

Therapeutic preparations of human FSH are used broadly to induce multiple follicular maturation in assisted reproduction technologies. Prior to the advent of recombinant DNA technology, all therapeutic FSH preparations were extracted from post-menopausal urine, i.e. human menopausal gonadotrophin and urofollitropin. The rather crude manufacturing methods used during the mid-to-late twentieth century required the collection of vast quantities of urine and led to FSH preparations with varying degrees of protein contamination, although the most recent urinary FSH product, highly purified urinary FSH, contains >95% pure FSH (Giudice et al., 1994; Howles and Wikland, 1999).

The above notwithstanding, given the highly variable composition of urinary gonadotrophins, there were (and continue to be) significant batch-to-batch inconsistencies in terms of contamination, bioactivity, immunoreactivity, LH content and FSH isoform profile (Driebergen and Baer, 2003). As a result, the quantification of FSH presented a serious problem, given that its expression by mass was meaningless. Therefore, in order to quantify FSH content and standardize proprietary preparations it was necessary to use an in-vivo bioassay. Bioassays make use of internationally accepted standards (provided by the World Health Organization) that enable samples of unknown biopotency to be estimated. The test results on the bulk preparations allow filling of FSH vials/ampoules according to the desired FSH bioactivity measured in international units (IU). This activity if confirmed by a final bioassay on the finished product just prior to its release. The result has to be within a specification range of 80–125% of the labelled potency (Driebergen and Baer, 2003).

A number of FSH bioassays have been developed in this respect. However, the one required by regulatory agencies (European Pharmacopoeia) is the Steelman and Pohley rat in-vivo bioassay (Steelman and Pohley, 1953). This is based on the fact that immature female rats (21–22 days old), pretreated with HCG, are sensitive to exogenous FSH and that there is a linear relationship between administered FSH and ovarian weight. FSH is injected subcutaneously once daily for 3 days with an autopsy being performed after 72 h. Measuring rat ovarian weight, however, has inherent limitations, hence the European Pharmacopoeia defines an activity range within which an FSH batch is acceptable for clinical use. The practical consequence is that a labelled 75 IU FSH ampoule may theoretically contain anything between 48 and 117 IU FSH (finished product FSH activity, fiducial limits) (Hugues et al., 2003).

The manufacture of r-hFSH (follitropin alfa) using recombinant DNA technology now ensures a constant supply.
Table 1. Patient baseline characteristics in groups r-hFSH-bio and r-hFSH-mass.

<table>
<thead>
<tr>
<th>Variable</th>
<th>r-hFSH-bio group (n = 125)</th>
<th>r-hFSH-mass group (n = 125)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.6 ± 3.6</td>
<td>34.1 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 ± 4.3</td>
<td>23.1 ± 4.6</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>4.9 ± 1.6</td>
<td>5.0 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Infertility factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male factor (n, %)</td>
<td>68 (54.4)</td>
<td>66 (52.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Unexplained (n, %)</td>
<td>22 (17.6)</td>
<td>25 (20.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Endometriosis (n, %)</td>
<td>18 (14.4)</td>
<td>19 (15.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Tubal factor (n, %)</td>
<td>17 (13.6)</td>
<td>15 (12.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3 FSH (IU/l)</td>
<td>7.4 ± 1.7</td>
<td>7.3 ± 1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3 LH (IU/l)</td>
<td>5.8 ± 2.9</td>
<td>5.7 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Day 3 oestradiol (pg/ml)</td>
<td>43.8 ± 16.4</td>
<td>39.2 ± 18.9</td>
<td>NS</td>
</tr>
<tr>
<td>No. with ICSI (n, %)</td>
<td>82 (65.6)</td>
<td>84 (67.2)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD or n (%).

Table 2. Ovarian stimulation characteristics in groups r-hFSH-bio and r-hFSH-mass.

<table>
<thead>
<tr>
<th>Variable</th>
<th>r-hFSH-bio group (n = 125)</th>
<th>r-hFSH-mass group (n = 125)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time for ovarian arrest (days)</td>
<td>14.1 ± 2.2</td>
<td>14.9 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Days of ovarian stimulation</td>
<td>11.1 ± 2.7</td>
<td>10.1 ± 2.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Total IU of FSH</td>
<td>2581 ± 907</td>
<td>2447 ± 861</td>
<td>NS</td>
</tr>
<tr>
<td>Patients with HCG and ovum retrieval (n, %)^a</td>
<td>113 (90.4)</td>
<td>114 (91.2)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of follicles on HCG day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10–14 mm</td>
<td>4.1 ± 3.2</td>
<td>3.9 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>&gt;14 and &lt;18 mm</td>
<td>8.7 ± 2.9</td>
<td>9.1 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>≥18 mm</td>
<td>4.1 ± 2.2</td>
<td>4.5 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Oestradiol on HCG day (pg/ml)</td>
<td>2208 ± 1036</td>
<td>2090 ± 835</td>
<td>NS</td>
</tr>
<tr>
<td>Endometrial thickness on HCG day (mm)</td>
<td>11.0 ± 2.3</td>
<td>11.1 ± 2.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD or n (%).

^aThere were 12 (9.6%) and 11 (8.8%) cycles cancelled due to a poor ovarian response in groups r-hFSH-bio and r-hFSH-mass respectively.

of the most biochemically pure FSH preparation (13,750 IU FSH/mg protein) with high batch-to-batch consistency in isoform profile and glycan species distribution (Driebergen and Baer, 2003). The most significant advantage of this isoform and glycan species consistency is that it permits FSH to be reliably quantified in mass units using a specific physicochemical technique, size exclusion high-performance liquid chromatography (Driebergen and Baer, 2003).

Potential clinical implications of follitropin alfa filled-by-mass are the most important aspect for the practicing clinician. In this regard, in a recent clinical study (Hugues et al., 2003) four batches of follitropin alfa were each filled in vials by bioassay, as well as four batches by mass. When comparing the clinical response of these batches, those patients receiving the filled-by-mass preparation had a significantly more consistent overall response to FSH stimulation. Greater control of the dose of FSH delivered is therefore associated with an improved consistency of clinical response. This is important considering that it is well established that patients’ responses to ovarian stimulation treatment are variable. As previously stressed (Hugues et al., 2003), parameters contributing to this variability are numerous and include patient’s age, ovarian reserve, pretreatment with GnRH analogues and oral contraceptive pill, and polycystic ovaries. The levels of ovarian response directly and indirectly impact on the clinical pregnancy rates, since the latter is directly related to the number and quality of embryos obtained in vitro and to the number of embryos replaced, both of which are strong prognostic factors for conception (Templeton and Morris, 1998; Hugues et al., 2003).

Thus, adding additional variability in this context is unlikely to be beneficial. On the contrary, ensuring additional consistency in the quantity of recombinant human FSH delivered to the patient may result in better chances of achieving a pregnancy.
per cycle of stimulation. This is supported by the present study where patients treated with r-hFSH-mass had better embryo quality and thus, in the face of lower number of embryos replaced, implantation rates were significantly higher in the r-hFSH-mass group. The shorter duration of gonadotrophin treatment in the latter group further supports the higher efficacy of the new gonadotrophin preparation. The reduction in stimulation days may be due to the reduced variation in batch content of FSH compared with the bioassay vials where the amount of FSH delivered from the different batches presumably used may have been on average lower. Thus, follicular recruitment could be slightly enhanced in the r-hFSH-mass group. In addition, the improvement in embryo quality may be linked to the presence of a more consistent FSH isoform profile that is biologically more conducive to follicular maturation. In this regard, Nayudu et al. (2002) have reported that follicle development and oocyte quality is strongly influenced by the FSH glycoform range.

There were potential limitations to this study. It was a retrospective investigation, and thus there was the possibility of selection and/or observer bias. However, there was an almost identical distribution between the two treatment groups for age, BMI, duration of infertility, cause of infertility, basal hormone measurements, and type of assisted reproduction used. This supports the comparability of the two study groups with respect to the main demographics and baseline characteristics of patients. In addition, none of the following assisted reproduction programme staff, the ultrasonographer measuring follicular development, the biologist handling oocytes and embryos, and the clinician carrying out the embryo transfer, was aware of the FSH treatment given to each patient once follitropin alfa filled-by-mass was launched in Spain. This is because the operators downstream from the stimulation were not aware of which medication was prescribed, a fact that may explained because in the patient notes it did mention only r-hFSH but not ‘bio’ or ‘mass’. Also, the fact that the treatment cycles were similar with respect to follicular size, number, and oestradiol serum concentrations on the day of HCG administration indicates that the stimulation procedures were carried out in a similar way with both treatments. Finally, the use of the same and well-standardized IVF/ICSI laboratory techniques during the short time frame of this study (4.5 months) precludes any bias in this respect. In fact, no changes were made during the study time on culture media, laboratory practice, or laboratory personal or equipment that could explain the differences observed.

In conclusion, the results suggest that in women undergoing multiple follicular development for IVF/ICSI, the new formulation of follitropin alfa filled-by-mass is more effective than the standard filled-by-bioassay follitropin alfa in terms of embryo quality, implantation rates, and number of days of stimulation. Further prospective, randomized studies are warranted to confirm these results.

Acknowledgements

We are grateful to Ms Paquita Antonell for her technical assistance. This work as supported in part by grant RCMN (C03/08) from the Instituto de Salud Carlos III, and Grant 2001SGR 00372 from Generalitat de Catalunya.
References


Received 22 December 2003; refereed 12 January 2004; accepted 27 January 2004.