Improving the consistency of ovarian stimulation: follitropin alfa filled-by-mass

Dr Matts Wikland, MD, PhD, is Specialist and Associate Professor in obstetrics and gynaecology at the University of Göteborg, Sweden and has directed the ART unit Fertilitetscentrum, at Carlander’s Hospital, Göteborg since 1987. In 1978 he began by developing techniques for ultrasound-guided oocyte pick-up. In 1984, among others, he introduced vaginal ultrasound for monitoring ovarian stimulation and for guiding oocyte aspiration in IVF. The latter technique has become the gold standard for oocyte aspiration. His main research and clinical interest has focused on simplifying the clinical management of ART, especially ovarian stimulation. He has published 120 scientific papers in international journals.

Dr Matts Wikland
Matts Wikland1,4, Jean-Noël Hugues2, Colin Howles3
1Fertility Centre Scandinavia, Carlanders Hospital, Box 5918, S-401 29 Gothenburg, Sweden; 2Centre of Reproductive Medicine, Jean Verdier Hospital, Bondy, University Paris XIII, France; 3Serono Singapore Pte. 9 Temasek Boulevard, #24–01 Suntec Tower 2, Singapore
4Correspondence: Tel: +46 31 7104615; Fax: +46 31 7104610; e-mail: matts.wikland@fcivf.com

Abstract

In their quest for a child, infertile couples embark on a journey that is full of expectations and hopes. Over recent years, treatment procedures for assisted conception have become safer and more efficient. However, couples undergoing treatment can still experience some degree of emotional stress due to disappointment if pregnancy is not achieved, or if treatment cycles may have to be cancelled due to a low- or hyper-response. Strategies aimed at minimizing the variability of ovarian response or overall treatment outcome can be expected to significantly reduce this emotional stress. New developments have led to the production of follitropin alfa filled by mass. This is a highly consistent FSH preparation improving the consistency of ovarian stimulation and reducing the risk of cycle cancellation. The impact of this new FSH preparation for assisted reproduction treatments is discussed in this review.

Keywords: assisted conception, filled by mass, ovarian stimulation, quality control, recombinant FSH, urinary FSH

Introduction

Infertile couples that need the help of assisted reproductive technologies in order to achieve a pregnancy embark on a journey that is full of expectations and hopes. Over the last few decades, assisted reproduction treatment has become much safer, more efficient and more successful. However, even today, situations such as low response and ovarian hyperstimulation cannot be ruled out completely. This is partially due to patient-related factors and partially due to the fact that different types of drugs used for ovarian stimulation could reveal different degrees of consistency in their bio-potency that result in variable ovarian responses.

An estimated 23–60% of patients drop out of treatment, even though their prognosis may have been evaluated as favourable (Gleicher et al., 1996; Penzias, 2004). There is currently an ongoing debate as to the most appropriate and relevant outcome measure that defines ‘success’ within the context of assisted reproductive treatment (Bhattacharya and Templeton, 2004; Heijnen et al., 2004; Messinis and Domali, 2004; Wennerholm and Berg, 2004). Centres that offer assisted reproductive treatment are often assessed according to their published pregnancy rates, but it is well recognized that this simplistic evaluation is inadequate, since it is a figure that is readily influenced by patient selection criteria, and by accepting high order multiple pregnancies or an excessive incidence of complications such as ovarian hyperstimulation as ‘success’, instead of failure (Alper et al., 2002). Recently, Thuring et al. (2004) reported the results of a large multicentre study in Scandinavia demonstrating that in young patients (<36 years) the transfer of a single fresh embryo, followed by a subsequent frozen embryo if needed, dramatically reduced the multiple birth rate without a substantial reduction in the overall live birth rate. For this approach to be successful, careful patient counselling and the collection of a sufficient number of high quality oocytes for two single embryo transfer cycles are mandatory.

Birth emphasizing a successful singleton at term (Min et al., 2004) has been suggested as the ideal outcome, but this
end-point can still be further defined within contexts of treatment cycles started, or per patient within a given time period; ‘live birth per woman’ has also been proposed as an alternative to ‘live birth per cycle’ (Vail and Gardner, 2003), but differing time scales make this difficult to apply in practice.

The purpose of this review is to address parameters that contribute to the ‘quality of assisted reproduction treatment’ and to define their impact on treatment decisions.

Defining ‘quality’ in assisted reproduction

Alper et al. (2002) have examined factors that should be considered in assessing the overall quality of an IVF centre. They showed that although national registries were initially developed in order to measure ‘quality’ between centres, their focus on pregnancy rates has instead resulted in pressure to publish high pregnancy rates, sometimes at the cost of high multiple gestation and OHSS rates. International standards in other fields usually measure ‘quality’ in terms of the end product, and the authors suggest that the ‘product’ for assisted reproduction should be measured in terms of a service – the quality of service that is provided to couples seeking help.

A fundamental goal of quality management is to establish, document and standardize all procedures and protocols, ensuring that they are reproducible and consistent throughout. This quality evaluation should include not only laboratory and clinical aspects (including staff training and development), but also procedures and patient satisfaction (Wikland and Sjoblom, 2000). Patient satisfaction may be difficult to measure; those who take home a healthy baby may be dissatisfied with the treatment they received, and, conversely, those who had unsuccessful treatment may feel that their management was satisfactory. This aspect has been evaluated in a number of studies (Halman et al., 1993; Schmidt, 1998; Souter et al., 1998; Hammarberg et al., 2001), and many clinics ask patients to record their satisfaction at the end of a treatment cycle, as a routine part of quality management. The results of such studies and questionnaires can be used to minimize stress and improve the overall ‘quality of life’ for patients during treatment. Penzias (2004) suggests that the ‘pursuit of excellence should be the goal of every IVF centre’, and has outlined a number of strategies that may be used to address patient satisfaction, by implementing simplified, tailored treatments and reduced monitoring to minimize stress and inconvenience. At the same time, the efficiency and safety of the treatment need to be considered as primary end-points.

Safety of medicines

Ovarian stimulation for multiple follicular development in assisted reproduction is now routinely achieved with the use of gonadotrophins. The history of their therapeutic evolution has recently been reviewed by Lunenfeld (2004); this review traces the constant quest to reduce the potential risks associated with urinary gonadotrophin preparations, and to improve safety and efficacy for the patients, leading to the evolution of new pure products manufactured by recombinant technology.

The gonadotrophins used in assisted reproduction were originally prepared from human menopausal urine, and the early crude methods of preparation yielded a product (human menopausal gonadotrophin, HMG) containing only 5% of FSH and LH in a mixture of unidentified urinary proteins (Donini and Montezemolo, 1949; Donini et al., 1964; Lunenfeld and Donini, 1966). These methods were subsequently refined so that products of at least 95% purity (FSH-HP) became available by the early 1990s. When the process of extracting gonadotrophins from urine was started, the menopausal urine was collected in only four centres, located in the Netherlands, Spain, Israel and Italy (Lunenfeld, 2004). The women participating in these collection centres were known to the centres, and samples could be rejected if there was any indication to do so, such as illness or drug treatment. By the beginning of this millennium, the demand for gonadotrophins had increased 100-fold, and the urine collection process could no longer be reliably traced. Sources had to be extended to many different countries, in Europe, Korea, China, India and South America, so that tracing a donor source became impossible.

Advances in biotechnology during the past 30 years have made it possible to replace urinary-derived products with pure formulations manufactured by recombinant DNA technology. The first recombinant human FSH (r-hFSH; follitropin alfa) preparation for clinical use was produced by Serono Laboratories in 1988, and was licensed for marketing in the European Union as Gonal-f® in 1995. Another r-hFSH (follitropin beta, Puregon®) product was licensed by Organon Laboratories in 1996. Recombinant HCG (Ovitrelle/Ovidrel®) and LH (Luwfer®) are now also available for clinical use.

Concerns about the hazards and potential risks of urinary preparations (Shaked et al., 2001; Hill and Collinge, 2003; Seeger et al., 2005) led a number of countries to apply the ‘precautionary principle’: France, Switzerland and the UK have published warnings regarding the risks associated with urinary products; in 1996, the Australian Drug Evaluation Committee published its resolution on replacement of urinary with recombinant gonadotrophins in view of their higher standard of purity and safety.

Controlling the variables

Assisted reproduction treatment cycles involve a series of different steps, from initial consultation and evaluation, to pituitary down-regulation, ovarian stimulation, monitoring of follicular growth, induction of ovulation, oocyte retrieval, embryo transfer, luteal support, day 15 pregnancy test, and, hopefully, ultrasound assessment of a single viable gestation. Numerous variables are involved at each step, many of which are beyond the control of the patient or the clinical team that manages the treatment. One of the aspects inherent in a total quality management system is to identify variables that can be controlled, and ensure that the different steps during treatment are carried out in a consistent manner. As described below, the development of recombinant DNA technology to produce highly purified hormone preparations, together with refinements in quality control and production processes (Loumaye et al., 1994; Howles, 1996), now allows one key variable in the
treatment cycle, the consistency of the drug administered, to be better controlled.

FSH is a heterodimer glycoprotein hormone with two non-covalently linked subunits, alpha and beta, glycosylated by post-translational modification (Flack et al., 1994). In vivo, the native FSH hormone consists of at least 20 different iso-hormones that differ in their pattern of glycosylation.

During the manufacture of commercial preparations, the source of raw material and the steps involved in the purification process affect the iso-hormone composition of the final product. Commercial preparations consist of a mixture of isoform profiles, and urinary products additionally contain a significant level of contaminating proteins (Guidice et al., 1994, 2001; van de Weijer et al., 2003). Until recently, quantification of the final product relied upon an in-vivo bioassay that measures bioactivity. The in-vivo bioassay for FSH accepted by regulatory agencies is the classic Steelman–Pohley assay (Steelman and Pohley, 1953), which involves the use of immature female rats. The rats are pre-treated with HCG, and then injected with FSH subcutaneously once daily for 3 days. The ovaries are weighed at autopsy 72 h later, and FSH bioactivity, measured in terms of international units (IU), is estimated based upon ovarian weight gain. This assay has a number of important limitations, the main one being the lack of analytical precision, with a coefficient of variation (CV) of 10–20% for each determination. These limitations have been recognized internationally and the US and EU Pharmacopoeias accept a specification range of 80–125% of the potential activity. Thus, ampoules of FSH that have been filled on the basis of the bioassay can contain a highly variable amount of the hormone: an ampoule labelled as containing 75 IU FSH could actually have a measured FSH bioactivity between 60 and 94 IU.

The technology used to manufacture recombinant proteins leads to the isolation of a pure rhFSH that can be reliably characterized and quantified by precise physicochemical methods. This molecule has been characterized by a combination of techniques, including sophisticated chromatography and mass spectrometry. The different isoforms of FSH can be separated according to the distribution of their carbohydrate moieties, by glycan mapping (Gervais et al., 2003), and the number and distribution of sialic acid residues by isoelectric focusing (IEF). These techniques have been extensively validated and are complemented with further physicochemical methods, and are now applied routinely to monitor and control the consistency of the commercial manufacturing process for recombinant gonadotrophins: follitropin alfa, (rhFSH, Gonal-f®), Lutropin alfa (rhLH, Luveris®), choriogonadotrophin alfa (r-HCG = Ovidrel®) and follitropin beta (rhFSH, Puregon®). For instance, this is illustrated by an assessment of the follitropin alfa manufacturing history, where an analysis of the results for 309 batches of rhFSH over a 6-year period showed a high degree of consistency between batches (Bassett and Driebergen, 2005).

The ability to produce a hormone preparation that can be characterized and confirmed as pure and consistent in specific activity means that this product can now be quantified in terms of its mass, in μg protein. The quantity of follitropin alfa in any given sample can be measured by using an optimized biophysical method, size-exclusion high performance liquid chromatography (SE-HPLC). Specific activity (ratio of bioactivity to protein content) is expressed in IU/mg protein, and detailed specific activity data have been analysed for 100 follitropin alfa rhFSH batches manufactured over a 3-year period, from nine different bioreactor runs. This analysis showed that the specific activity of follitropin alfa batches was normally distributed, stable, and that there was no effect of different bioreactor runs. The average specific activity of the product was 13,745 IU/mg, and therefore a mass of 5.5 μg is equivalent to 75 IU. These values were subsequently confirmed on 120 batches (Driebergen and Baer, 2003) and 309 batches (Bassett and Driebergen, 2005).

There is a general regulatory trend for European regulatory agencies (European Directive 86/609) as well as a commitment of the European Pharmacopoeia Commission to recommend replacing the International Unit as a standard of measurement with mass in micrograms, by using reliable and precise physicochemical analytical methods instead of in-vivo bioassays. Recombinant human growth hormone and recombinant human insulin are now tested by physicochemical methods. As a result of the tightly controlled manufacturing process and sophisticated characterization methodology that ensures the integrity and consistency of each batch of the final product, follitropin alfa can now be filled according to mass in μg protein, instead of relying on bioactivity as measured by an imprecise in-vivo bioassay. A recent detailed analysis assessed the manufacturing consistency of the rhFSH follitropin alfa bulk product, determined the consistency and quality of the follitropin alfa filled-by-mass (FBoM) drug product, and compared the analytical data with follitropin beta. Batches of follitropin alfa FBM, 75 IU, presented as a lyophilized powder in a glass vial for reconstitution with 1 ml of water injection prior to administration was compared with follitropin beta (Puregon®/Follistim®) presented as a solution of rhFSH in a cartridge to be installed in a pen device prior to administration (Basset and Driebergen, 2005). The data showed high purity and batch-to-batch consistency for follitropin alfa FBM in terms of specific activity, protein content, glycan mapping, isoform patterns and purity. The batch-to-batch variability in protein content was lower for follitropin alfa FBM (1.6%) than for follitropin beta (12%), and follitropin beta showed more batch-to-batch variability in isoform patterns than follitropin alfa FBM. Concentrations of oxidized alpha subunit were slightly lower for follitropin alfa FBM (1–2.5%) than in follitropin beta (3.5–5.3%). The thorough characterization of Serono’s three recombinant gonadotrophins now allows them to be reliably quantified by mass, so that one of the major goals in controlling the variables, the quest for consistency, has been fulfilled (Keck et al., 2005).

Clinical benefits of follitropin alfa FBM

Treatment efficiency in IVF/ICSI

Since follitropin alfa FBM became available for clinical use in 2002, a number of studies have investigated its efficacy in IVF/ICSI and ovulation induction treatments, compared with preparations filled by bioassay. Hugues et al. (2003) reported a prospective double-blind randomized study where four lots of rhFSH were each divided into one batch of follitropin alfa filled
by bioassay (FbB) and one batch of follitropin alfa FbM and were compared. Their results showed that patients had a more consistent response to stimulation with the FbM formulation, both within and between centres, in terms of number of oocytes retrieved, as well as a more consistent clinical outcome in terms of pregnancy rate. Balasch et al. (2004) used a study group of women undergoing their first IVF/ICSI cycle, and compared 125 consecutive cycles using follitropin alfa FbB with 125 cycles using the new follitropin alfa FbM formulation. The results of this study showed that treatment with FbM was more effective, in particular with respect to a significantly shorter duration of ovarian stimulation (10.1 versus 11.1 days, \( P = 0.02 \)) and a trend for higher implantation rate and clinical pregnancy rates (44.0 versus 35.2%, \( P = 0.1 \)). A multicentre prospective randomized study in 32 assisted reproduction centres in the USA and Latin America comparing the two formulations also reported that the FbM product is safe and effective, delivering a better clinical response, i.e. more oocytes retrieved and higher embryo quality, with a reduction in total FSH administered and number of treatment days (Abuzeid et al., 2001). Data from these studies are summarized in Table 1. Similar results have been reported from a large observational study from UK (Lass et al., 2004).

### Table 1. Comparison of FbM with FbB in IVF/intracytoplasmic sperm injection (ICSI) assisted reproduction; clinical studies.

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. patients</th>
<th>No. days stimulation</th>
<th>Mean no. ampoules</th>
<th>No. oocytes</th>
<th>Clinical pregnancy rate (%)</th>
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<tr>
<td>Balasch et al., 2004</td>
<td>125/125</td>
<td>10.1 ± 0.6 versus 11.1 ± 2.7 ( (P = 0.02) )</td>
<td>32.6 ± 11.5 versus 34.4 ± 12 (NS)</td>
<td>10.1 ± 4.7 versus 9.7 ± 4.6 (NS)</td>
<td>44.0 versus 35.2 (NS)</td>
</tr>
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<td>Abuzeid et al., 2001</td>
<td>220/223</td>
<td>9.7 ± 1.8 versus 10.2 ± 1.8 ( (P = 0.004) )</td>
<td>26.1 ± 10.3 versus 29.3 ± 11.9 ( (P &lt; 0.01) )</td>
<td>11.9 ± 6.3 versus 10.8 ± 6.8 ( (P = 0.052) ) in patients ≤35 years ( (12.5 \pm 10.6) ) ( (P &lt; 0.05) )</td>
<td>n/a</td>
</tr>
<tr>
<td>Hugues et al., 2003</td>
<td>66/65</td>
<td>10.4 ± 1.3 versus 10.5 ± 1.4 ( (P = 0.7) )</td>
<td>Not shown</td>
<td>10.8 ± 4.5 versus 11.3 ± 5.5 ( (P = 0.5) )</td>
<td>30 versus 26.2</td>
</tr>
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NS = not significant; n/a = not applicable.

### Table 2. Follitropin alfa FbM versus FbB for ovulation induction; clinical studies. Results are presented as FbM/FbB.

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. patients</th>
<th>No. cycles</th>
<th>No. days stimulation</th>
<th>Mean no. ampoules</th>
<th>Cumulative ovulation rate three cycles (%)</th>
<th>Cycles requiring dose adjustment &gt;75 IU (%)</th>
<th>Cancellations due to poor response</th>
<th>Cumulative pregnancy rate over three cycles</th>
</tr>
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<tr>
<td>Yeko et al., 2004</td>
<td>83/94</td>
<td>176/207</td>
<td>12.5 ± 5.9 versus 15.7 ± 7.1</td>
<td>14.4 ± 7.1 versus 18.8 ± 9.3 (NS)</td>
<td>91.6 versus 7.2 ( (P = 0.0004) )</td>
<td>37.5 versus 60.4 ( (P &lt; 0.0001) )</td>
<td>2.3 versus 7.7 ( (P &lt; 0.02) )</td>
<td>44.6 versus 41.4 (NS)</td>
</tr>
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NS = not significant.

## Treatment efficiency in ovulation induction

Follitropin alfa FbM has also been demonstrated to have an advantage in the treatment of WHO II anovulatory patients for ovulation induction. Data presented by Tredway et al. (2002) and Yeko et al. (2004) (Table 2) showed that the new follitropin alfa FbM formulation allows more precise dosing of FSH, which is critical in anovulatory patients. Improved control of follicular development is reflected by better precision in choosing the starting dose, and fewer cancelled cycles due to poor response. The number of treatment days and mean dosage required was less for groups treated with FbM than for those treated with FbB, and the results overall confirm that the use of FbM achieves improved control, fewer treatment days and a lower total dose of administered gonadotrophin compared with FbB.

## Multiple pregnancies in controlled ovarian stimulation

Assisted reproduction procedures are associated with an increased multiple pregnancy rate compared with spontaneous
Reduced incidence of cancelled cycles and OHSS

Cancelled cycles due to inadequate or excessive ovarian response represent a major source of disappointment for the couple treated, even more in countries where treatment costs are not reimbursed. Reducing the incidence of cancelled cycles is a major concern in assisted reproduction, and significant benefit can be gained in this respect by using a drug that can be relied upon as consistently effective. In a similar manner, patients who have shown an excessive response to stimulation in a previous cycle can be more carefully managed with the accurate dosing that FbM preparations allow.

Conclusions

Over the last few decades, major improvements in assisted reproduction procedures have been achieved. This does not only refer to the technical aspects of the treatment but even more to the drugs used for ovarian stimulation: in the beginning of assisted reproduction treatments urine-derived gonadotrophins were used for ovarian stimulation. These preparations were associated with low purity, low specific activity and a lack of traceability. Recombinant gonadotrophins have come into the market in the early 1990s and their higher purity and consistency have resulted in higher treatment efficiency compared with urine-derived gonadotrophins in terms of pregnancy rates and total dose of gonadotrophin per live birth (Ludwig et al., 2004). The latest achievement in ovarian stimulation treatment was the development of gonadotrophins FbM.

The use of these refined and tested products allows a safer and more streamlined approach to assisted reproduction cycle management, with tailored patient-friendly protocols and reduced monitoring based upon confidence in a consistent response to stimulation. This superior control can minimize the risks of cancelled cycles, OHSS and multiple births after assisted reproduction as well as after ovulation induction therapy.

“Quality is never an accident; it is always the result of high intention, sincere effort, intelligent direction and skilful execution; it represents the wise choice of many alternatives.”

William A Foster.

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