Polycystic ovary syndrome. A therapeutic challenge

Bayram, N.

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CHAPTER 2

Recombinant FSH in alternative doses
or versus urinary gonadotrophins for
ovulation induction in subfertility associated
with polycystic ovary syndrome

Neriman Bayram, Madelon van Wely, Fulco van der Veen

Update Cochrane review
Abstract

Background
Over the last four decades, various urinary FSH (uFSH) products of different purity have been developed. In 1988 recombinant FSH (rFSH) was prepared by transfecting Chinese hamster ovary cell lines with both FSH subunit genes. It is unknown which of these FSH preparations to prefer, regarding effectiveness and safety. Essential in ovulation induction with gonadotrophins is the time during which the serum FSH is above the threshold at which recruitment of follicles occurs. Extending this time carries the risk of multiple follicle development and multiple pregnancies. Because of this, various dose regimens have been developed to minimize these complications.

Objectives
To compare in women with clomiphene-resistant polycystic ovary syndrome (PCOS) the effectiveness and safety of 1) rFSH with uFSH and 2) various dose regimens of rFSH.

Search strategy
We searched the Cochrane Menstrual Disorders and Subfertility Group trials register (searched 1st May 2004), PubMed, MEDLINE, Web of Science (all searched 1985 to May 1 2004), and reference lists of articles. We also contacted manufacturers and researchers in the field.

Selection criteria
All relevant published RCT’s were selected. Randomised controlled trials were eligible for inclusion if treatment consisted of recombinant FSH versus urinary FSH or recombinant FSH in different dose regimens, to induce ovulation in women with clomiphene citrate resistant PCOS.

Data collection & analysis
The main outcome measure was ongoing pregnancy/live birth per woman. Secondary outcomes were ovulation, pregnancy, miscarriage, multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). Relevant data were extracted independently by two reviewers (NB, MW). Odds ratios were generated using the Peto modified Mantel-Haenszel technique. The main outcome measure was ongoing pregnancy/live birth per woman. Secondary outcomes were ovulation, pregnancy, miscarriage, multiple pregnancy and ovarian hyperstimulation syndrome (OHSS). Relevant data were extracted independently by two reviewers (NB, MW). Odds ratios were generated using the Peto modified Mantel-Haenszel technique.

Main results
Three randomised trials comparing rFSH versus uFSH were identified. Only one trial provided data on the primary outcome, ongoing pregnancy, while all three studies provided data on the secondary outcomes. No difference was found in ongoing pregnancy rate (OR 1.10, 95% CI 0.51 to 2.35). There was no difference in clinical pregnancy rate (OR 0.95, 95% CI 0.64 to 1.41), nor between any of the other secondary outcomes. Four small trials with different dose regimens were identified. As each of the four included trials compared different regimens, no trial results could be combined. None of the trials
presented data on ongoing pregnancy/live birth. In the first trial that compared chronic low dose step up regimen versus conventional regimen no statistically significant differences were found for clinical pregnancy rate (OR 1.62, 95% CI 0.64 to 4.07), nor in the other secondary pregnancy outcomes. However, significantly fewer follicles >10 mm diameter (WMD -3.30, 95% CI -5.34 to -1.26) were found in the group treated with the chronic low dose step up regimen. The second trial compared two starting doses in a chronic low dose step up regimen. The clinical pregnancy rate was comparable in both stimulation regimens (OR 0.79, 95% CI 0.12 to 4.96). The third trial compared a chronic low dose step up regimen with modified step down regimen. No difference in clinical pregnancy rate could be proven (OR 1.90, 95% CI 0.18 to 19.97). The fourth trial compared a chronic low dose step up regimen with a step down regimen revealed also no statistically significant differences in clinical pregnancy rate (OR 1.41, 95% CI 0.57 to 3.46). Similarly no difference could be proven in any of the other pregnancy outcomes. However, significantly more monofollicular development (OR 4.24, 95% CI 2.27 to 7.92) and less multifollicular development (OR 0.13, 95% CI 0.06 to 0.29) was observed in the chronic low dose step up group.

**Reviewers' conclusions**

At this moment there is not sufficient evidence to determine whether rFSH or uFSH is preferable for ovulation induction in women with clomiphene citrate resistant PCOS. No difference could be proven between the four different dose regimens. However, the conventional regimen is known to be plagued by OHSS and multiple pregnancies and usage should therefore be discouraged.
Background

Polycystic ovary syndrome (PCOS) is the most common cause of female anovulatory infertility. To induce ovulation, clomiphene citrate (CC) is the first line of treatment. However, about 20% of patients are CC resistant i.e. they do not ovulate after a maximum dose of clomiphene citrate (100-200 mg) (Imani et al., 1998). Since 1958, these patients are treated with follicle stimulating hormone (FSH), originally extracted from pituitary glands (Gemzell et al., 1958) and later extracted from post-menopausal urine (Lunenfeld et al., 1960).

Over the last four decades, various urinary FSH products have been developed. Menotropin (human menopausal gonadotrophins (hMG), available since the early 1960s, contains FSH, LH and large quantities of potentially allergic urinary proteins. Urofollitropin (FSH), available since the mid 1980s, is devoid of LH, but is still contaminated with urinary proteins. Highly purified urofollitropin (FSH-HP), available since the mid 1990s contains very small amounts of urinary proteins. Lack of urinary proteins diminishes adverse reactions such as local allergy or hypersensitivity (Biffoni et al., 1994; Albano et al., 1996) whereas the absence of LH has no negative influence on folliculogenesis of PCOS patients (van Weissenbruch et al., 1993; Hayden et al., 1999).

To obtain higher purity, complete absence of LH, high specific bioactivity, absolute source control, independence of urine collection and batch to batch consistency, recombinant FSH (rFSH) was synthesized in 1988. This was realised by transfecting Chinese hamster ovary cell lines with both FSH subunit genes (Keene et al., 1989; Howles et al., 1996).

At present, two preparations of rFSH are available. The first one, marketed in 1995, is follitropin alpha (Gonal F®), soon followed by follitropin beta (Puregon®). Both preparations are similar to pituitary and urinary FSH, although they show minor differences in the structure of the carbohydrate side chains and contain more basic and less acidic isohormones than the natural hormones (Hard et al., 1990, de Leeuw et al., 1996, Andersen, 2004 in press).

Ovulation induction with FSH bears the risk of multiple follicle development and multiple pregnancies (Nugent et al., 2004). To reduce these complications, various dose regimens have been developed (Brown 1978; Hamilton-Fairley et al., 1991, van Santbrink et al., 1995).

At present, the most frequently used dose regimens are the chronic low dose step up and step down regimens. In a previous review the effectiveness of urinary FSH was compared with hMG in patients with PCOS (Nugent et al., 2004). In this review we compare the effectiveness and safety of rFSH with urinary gonadotrophins and the effectiveness and safety of various regimens using rFSH.

Objectives

1 To determine the safety and effectiveness of recombinant FSH compared with urinary gonadotrophins used for ovulation induction in women with clomiphene citrate resistant PCOS.
2 To determine the safety and effectiveness of various dose regimens of recombinant FSH used for ovulation induction in women with clomiphene citrate resistant PCOS.
Criteria for considering studies for this review

Types of studies
A trial was eligible for inclusion if it dealt with the use of a recombinant FSH versus urinary gonadotrophins or recombinant FSH in various dose regimens, and if primary outcomes of ovulation rate and pregnancy rate were specified. The participants were allocated by a randomisation procedure.

Types of participants
Women with clomiphene citrate resistant PCOS undergoing ovulation induction. Clinical and endocrinological characteristics, infertility work-up, age, duration of infertility and previous treatment(s) if available were specified in the trial characteristics table.

Types of interventions
1) Recombinant FSH versus Urinary FSH
2) Recombinant FSH versus Human menopausal gonadotrophin (HMG)
3) Recombinant FSH versus Recombinant FSH (various dose regimens)

Types of outcome measures
Primary Outcome:
Ongoing pregnancy or live birth rate per woman

Secondary Outcomes:
Clinical pregnancy rate (per woman)
Ovulation rate (per woman, if available, otherwise per cycle)
Incidence of ovarian hyperstimulation syndrome (OHSS) (per woman)
Incidence of multiple pregnancy (per woman)
Miscarriage rate (per woman)
Incidence of multifollicular growth (per woman)
Total gonadotrophin dose
Total duration of stimulation
Oestradiol levels on day hCG administration
Number of follicles on the day of hCG administration

Search strategy for identification of studies
This review has drawn on the search strategy developed for the Cochrane menstrual Disorders and Subfertility Group as a whole.

1. The following electronic databases were searched:
   - MEDLINE (1966-April 2004)
   - Cochrane Controlled Clinical Trials Register (CCTR)
The following keywords were used;
polycystic ovary syndrome, oligomenorrhea, oligo-menorrhea, amenorrhea, anovulation,
ovulation induction, gonadotrophins, recombinant FSH and FSH.

2. Handsearching of the references mentioned in the obtained studies was performed

3. Serono Benelux BV and NV Organon, the manufacturers of follitropin alpha (Gonal F®) and follitropin beta (Puregon®) respectively, were asked for ongoing studies and unpublished data.

**Methods of the review**

**Selection of studies & quality assessment**

Data from included trials was processed as described in the Cochrane Handbook (Clarke and Oxman, 2003). All selected studies were assessed and evaluated for methodological quality and appropriateness for inclusion without consideration of their results. Trials were screened and analysed for the following quality criteria and methodological details: method and timing of randomisation; number of patients randomised and analysed; whether they were single or multicenter studies; parallel or cross-over design; blinding of treatment; the use of sequential analysis or factorial design; the presence of a power calculation; duration of follow-up; whether pregnancy was a measured outcome and, if so, how this was diagnosed; how pregnancy results were presented (particularly whether cumulative conception curves with the use of life table analysis were employed); and the source of any funding.

Relevant trials were screened independently by two authors (NB and MW). Differences of opinion were registered and resolved by consensus with the senior author (FV). If crossover studies were identified, data were included in the review from the pre-crossover period where possible. If pre-crossover data could not be extracted, the results of pre- and post-crossover study periods were given descriptively. The data were extracted into two-by-two tables and checked for accuracy by N. Bayram and M. van Wely. If necessary, additional data was requested from the authors. For dichotomous data, odds ratios with 95% confidence intervals were calculated for each individual trial using the Peto modification of the Mantel-Haenszel method (Peto, 1987). For continuous data the weighted mean was calculated. When median and range were given instead of mean and SD, the mean was estimated by logarithmic transformation of the minimum and maximum values and the SD was imputed from the overall SD of other studies. In the absence of heterogeneity of treatment effect among trials, which was tested using the Breslow-Day chi-square test, the data were pooled. For pooled dichotomous data an overall combined OR with 95% CIs was calculated using the Peto method and for continuous data a weighted mean difference (WMD) with 95% CI was calculated using the inverse variance method. Negative values in WMD indicate a benefit of "treatment group" over controls.
Description of studies

Please see Appendix I for details of the study methods and participants of the seven trials included in the analysis.

Types of studies

**Recombinant FSH versus urinary FSH**

Three trials were identified meeting the inclusion criteria (Loumaye et al., 1996; Coelingh Bennink et al., 1998; Yarali et al., 1999). All trials compared ovulation induction with rFSH versus uFSH in clomiphene citrate resistant patients. The study of Loumaye 1996 was described in a review on human gonadotrophins produced by recombinant DNA technology. By personal communication additional data on this trial was obtained and all these data was incorporated in this review. In total 451 women were included in the trials comparing rFSH with uFSH.

**Recombinant FSH versus HMG**

No trials could be identified comparing rFSH with hMG.

**Recombinant FSH versus recombinant FSH - various dose regimens**

Four trials were identified meeting the inclusion criteria (Hedon et al., 1998; Balasch et al., 2000; Balasch et al., 2001; Christin-Maitre et al., 2003). Trials comparing various dose regimens using urinary FSH were excluded as these are evaluated in another systematic review (Nugent et al., 2004). In total 237 women were included in the trials.

Types of participants

Polycystic ovary syndrome was defined as a combination of at least two of three criteria; clinical features (oligo/amenorrhoea, chronic anovulation), endocrinological abnormalities (hyperandrogenism and/or high LH/FSH ratio) and polycystic ovaries diagnosed with ultrasonography. Chronic anovulation was mainly defined by cycle length of >35 days or amenorrhea. The participants were all clomiphene citrate-resistant, anovulatory women. Clomiphene citrate resistance was defined as failure to ovulate or failure to conceive if ovulation occurred after various numbers of cycles. The maximum dose of clomiphene citrate differed in the trials. In one trial no definitions on PCOS or clomiphene citrate resistance were given (Loumaye et al., 1996).

Types of intervention

Three trials compared recombinant FSH with urinary FSH using the chronic low dose step up regimen. Loumaye et al., 1996 and Yarali et al., 1999 compared Gonal-F with Metrodin and Coelingh Bennink 98 compared Puregon with Metrodin. Loumaye 1996 did not describe cancellation criteria. The cycles were cancelled by Coelingh Bennink et al., 1998 if more than three follicles of ≥ 15 mm were present or if there was no follicular response after 42 days of treatment. Yarali et al., 1999 cancelled cycles if more than 4 follicles ≥ 15 mm were present or if there was no follicular response after 35 days of treatment.

Four trials compared various dose regimens of recombinant FSH. Hedon et al., 1998 compared a chronic low dose step up versus conventional step up protocol (Gonal-F) after
Injection of hCG was withheld if four or more follicles >14 mm were present. Two trials used a crossover design. The first trial compared two starting doses in a chronic low dose step up protocol (Gonal-F and Puregon) (Balash et al., 2000) and the second trial compared a chronic low dose step up regimen with modified step down regimen (Gonal-F) (Balash et al., 2001). No cancellation criteria were described in the first trial. In the second trial ovulation was triggered by one subcutaneous injection of 5000 IU hCG when no more than three follicles with a mean diameter of 16 mm were present. The last trial compared a chronic low dose step up regimen with the step down regimen (Puregon) (Christin-Maitre et al., 2003). Patients were treated for three consecutive cycles. HCG administration was withheld if four or more follicles > 16 mm in diameter were present and/or if the serum oestradiol level was >1000 pg/ml. In addition ovarian stimulation was cancelled in the absence of follicular development after 21 days of stimulation or in case of cyst development.

**Types of outcome measures**

Pregnancy rate, ovulation rate, miscarriage rate, multiple pregnancy rate, ovarian hyperstimulation syndrome rate, total FSH dose, duration of stimulation, cancellation rate, single follicle development, number of follicles >10 mm on day of HCG administration, oestradiol concentration on day of HCG were reported. In the trials comparing different regimens no definitions of any of the outcome parameters was given. In the trials comparing rFSH with uFSH definitions of ovulation and clinical pregnancy were given. In one trial only a definition of ongoing pregnancy was given (Coelingh Bennink et al., 1998).

**Methodological quality of included studies**

All seven trials included were randomised clinical trials. Three studies were single center studies (Yarali et al., 1999; Balash et al., 2000; Balash et al., 2001) and the other four studies were multicenter studies (Loumaye et al., 1996; Coelingh Bennink et al., 1998; Hedon et al., 1998; Christin-Maitre et al., 2003). Two studies used a randomisation list that corresponded with patient drug codes (Coelingh Bennink et al., 1998; Yarali et al., 1999), two studies used sealed envelopes (Loumaye et al., 1996; Christin-Maitre et al., 2003), one study used computerized allocation stratified by center (Hedon et al., 1998) and for two studies the method of randomisation is unknown (Balash et al., 2000; Balash et al., 2001). Only one study performed a power calculation (Loumaye et al., 1996). An intention-to-treat analysis was performed in one trial only (Coelingh Bennink et al., 1998). This trial also was assessor-blind, whereas the other six were open-label. Double blinding should have been possible for the three trials comparing rFSH with uFSH, but this was not done as rFSH was supplied in vials and uFSH in ampoules. Withdrawals after randomisation were mentioned in all trials. In the three trials comparing rFSH with uFSH women were treated for a maximum of three cycles. For these trials no information was present on losses to follow-up after the first cycle. Only three trials gave information on duration and timing of the trial (Coelingh Bennink et al., 1998; Hedon et al., 1998; Yarali et al., 1999). Of the trials comparing different dose regimens, two were crossover trials (Balash et al., 2000; Balash et al., 2001). The sample size of the studies ranged between 22 to 222 women and the follow up was short (1 to 3 cycles).
Results

Recombinant FSH versus urinary FSH
(Loumaye et al., 1996; Coelingh Bennink et al., 1998; Yarali et al., 1999)

Data per woman could be extracted for all outcome measures except for ovulation rate which is reported per cycle. There was no evidence of statistical heterogeneity. The primary outcome i.e. live birth rate per woman was not reported in any of the trials (Loumaye et al., 1996; Coelingh Bennink et al., 1998; Yarali et al., 1999). Only one trial (Coelingh Bennink et al., 1998) provided data on ongoing pregnancy, while all three studies provided data on the secondary outcomes. No difference was found in ongoing pregnancy rate (OR 1.10, 95% CI 0.51 to 2.35). After pooling the data, the clinical pregnancy rate was not statistically significant different (OR 0.95, 95% CI, 0.64 to 1.41). Ovulation rate per cycle was available in all studies but because of absence of the exact number of cycles in the study by Loumaye et al., 1996 only the data from Coelingh Bennink et al., 1998 and Yarali et al., 1999 could be pooled with an overall OR 1.19 (95% CI, 0.78 to 1.80).

The miscarriage rate per woman was comparable in all studies with an overall OR 1.22, (95% CI 0.60 to 2.46). Ovarian hyperstimulation syndrome occurred in 0.9% in both groups (Loumaye et al., 1996) and in 7.6% in the rFSH group versus 4.5% in the uFSH group (Coelingh Bennink et al., 1998). No cases of OHSS occurred in the study of Yarali et al., 1999. Overall, the pooled OR was not statistically significant different (OR 1.55, 95% CI 0.50 to 4.84).

Loumaye et al., 1996 found 3% versus 7% multiple pregnancies for rFSH and uFSH respectively. From this study data on the order of multiple pregnancies could not be retraced. In the other two studies one twin and one triplet pregnancy (2%) versus one twin pregnancy (1.5%) (Coelingh Bennink et al., 1998) and no multiple pregnancies (0%) versus three twin pregnancies (8.5%) (Yarali et al., 1999) were found in the rFSH group and the uFSH group respectively. There was no difference in multiple pregnancy in the rFSH group compared with uFSH in these trials (OR 0.44, 95% CI 0.16 to 1.19). Continuous outcome measures like total FSH dose, duration of stimulation, oestradiol (E2) level and number of follicles of various diameters on the day of hCG administration were only reported by Coelingh Bennink et al., 1998 and Yarali et al., 1999. Total FSH dose and duration of stimulation and E2 level did not differ significantly between rFSH and uFSH with a WMD of -216, 95% CI -733 to 312, -2.6, 95% CI -5.6 to 0.3 and 67, 95% CI -67 to 200 for rFSH and uFSH respectively.

As various diameters for number of follicles were used, these data could not be pooled. Coelingh Bennink et al., 1998 reported three different groups of follicle diameter after one, two and three consecutive cycles. In the first group mean number (SD) of follicles ≥12 mm and in the second group number of follicles ≥15 mm and in the third group number of follicles ≥18 mm were described. Only in the group of follicles ≥12 mm after the first cycle a statistically significant difference was found: (WMD 1.00, 95% CI 0.17 to 1.83) between the rFSH and uFSH group.

Yarali et al., 1999 reported two different groups of follicle diameter after one cycle. In the first group mean number of follicles of 10-14mm and in the second group number of follicles > 14 mm were described. Only in the second group a statistically significant difference was found: (WMD 1.10, 95% CI 0.39 to 1.81) between rFSH and uFSH group.
Recombinant FSH versus recombinant FSH - various dose regimens

As each of the four included trials compared various dose regimens, no trial results could be combined. Analyses are therefore presented separately for each comparison.

1. Chronic low dose step up regimen versus conventional step up regimen (Hedon et al., 1998)
The primary outcome i.e. live birth rate or ongoing pregnancy was not reported. No statistically significant difference in clinical pregnancy rate was found: 14 of 53 women (26%) versus 9 of 50 women (18%) (OR 1.62, 95% CI, 0.64 to 4.07). Ovulation rate per woman was similar comparing the regimens during one cycle. Thirty of 53 women (57%) and 29 of 50 women (58%) ovulated respectively (OR 0.95, 95% CI, 0.43 to 2.06). Miscarriage rates per woman were 1.9% and 2% respectively (OR 0.62, 95% CI 0.03 to 11.34). OHSS occurred in one patient in each rFSH treatment group. There was no difference in multiple pregnancy rate per woman. Two twins (4%) in the chronic low dose step up regimen and two twins (4%) in the conventional regimen were observed (OR 0.94, 95% CI 0.13 to 6.89).

Continuous outcome measures like total FSH dose, duration of stimulation, oestradiol (E2) level and number of follicles of different measures on the day of hCG gift were also reported. The results of mean (SD) total FSH dose used and the mean (SD) duration of stimulation did not differ significantly with a WMD of -45 (95% CI -346 to 255) and 1.5 (95% CI -0.87 to 3.87) respectively. Mean E2 (SD) concentration after one cycle was significantly lower in the chronic low dose regimen, 504 (477) pg/ml compared with the conventional regimen 989 (740) pg/ml (WMD -485, 95% CI -743 to -227).

Two different groups of follicle diameter after one cycle were reported. In the first group mean number (SD) of follicles >10 mm and in the second group mean number (SD) of follicles ≥16 were described. Only in the group of follicles >10 mm was a statistically significant difference found (WMD -3.30, 95% CI -5.34 to -1.26) in favour of the chronic low dose.

2. Two starting doses (37.5 IU versus 50 IU) in a chronic low dose step up regimen (Balasch et al., 2000)

The primary outcome i.e. live birth rate or ongoing pregnancy was not reported. No statistically significant difference in clinical pregnancy rate per woman were found: three of 12 women (25%) versus three of 10 women (30%) respectively (OR 0.79, 95% CI 0.12 to 4.96). All 22 participating women ovulated in the first cycle in both groups. No data was reported for the outcomes miscarriage, OHSS or multiple pregnancy. As no data from the pre-crossover period could be obtained on the continuous outcome measures like mean total FSH dose, mean duration of stimulation, mean oestradiol (E2) level and mean number of follicles of different measures on the day of hCG administration, these parameters were not included in the MetaView analysis.

3. Chronic low dose step up regimen versus modified step down regimen (Balasch et al., 2001)

The primary outcome i.e. live birth rate or ongoing pregnancy was not reported. No statistically significant difference in clinical pregnancy rate per woman was found: two of 15 women (13%) and one of 14 women (7%) (OR 1.90, 95% CI 0.18 to 19.97). All 29
participating women ovulated after the first cycle. No data was reported for the outcomes miscarriage and multiple pregnancy. No cases of OHSS occurred. As no data from the pre-crossover period could be obtained on the continuous outcome measures like mean total FSH dose, mean duration of stimulation, mean oestradiol (E2) level and mean number of follicles of different measures on the day of hCG administration, these parameters were not included in the MetaView analysis.

4. Chronic low dose step up regimen versus step down regimen (Christin-Maitre et al., 2003)

The primary outcome i.e. live birth rate or ongoing pregnancy was not reported. No statistically significant difference in clinical pregnancy rate per woman were found: 17 of 44 women (38.6%) and 12 of 39 (31%) (OR 1.41, 95% CI 0.57 to 3.46). Ovulation rate per cycle was significantly different (OR 2.24, 95% CI, 1.18 to 4.27). It is unclear how the miscarriages were calculated, but no differences were found: 12.5% versus 16.7%. Three cases of moderate OHSS were reported but it is not clear in which treatment group. Two twin pregnancies (4.5% per woman) in the step up protocol and two twin and one triplet pregnancy (8% per woman) in the step down protocol were observed (OR 0.58, 95% CI 0.10 to 3.50).

Continuous outcome measures like total FSH dose, duration of stimulation, oestradiol (E2) level and number of follicles of different measures on the day of hCG gift were also reported. The mean (SD) total FSH dose used was not statistically different (WMD -16, 95% CI -241 to 209). A significant difference was found for mean duration of stimulation (OR 5.50, 95% CI 3.21-7.79) and E2 levels on day of hCG gift (WMD -395, 95% CI -770 to -19).

In this study the rate of monofollicular (1 follicle >16mm), bifollicular (2 follicles >16mm) and multifollicular (at least 3 follicles >16mm) development was described. No difference was observed in bifollicular development (OR 0.59, 95% CI 0.26 to 1.30), whereas a significant difference was found in monofollicular (OR 4.24, 95% CI 2.27 to 7.92) and multifollicular development (OR 0.13, 95% CI 0.06 to 0.29).

**Discussion**

Ovulation induction involves the use of medication or surgery to stimulate development of a mature follicle in the ovary of women who have chronic anovulation and infertility. At this moment recombinant FSH and urinary FSH are commonly used for this purpose. The main difference between these two types of gonadotrophins is the presence or urinary protein contaminants in uFSH.

Yet, impurity affects safety only if it should be demonstrated that any of the potential contaminants adversely affect either the patients' health or treatment outcome. The urinary preparations of human gonadotrophins have been widely used for 40 years and no infections have been associated with their injection, even in the past when the urinary extracts were rather impure (Balen, 2002, Gleicher et al., 2003). In spite of this, urinary FSH is more immunogenetic leading to local and allergic reactions (Biffoni et al., 1998, Battaglia et al., 2000).

The bio-potency of rFSH and uFSH depends on their glycoform distribution.
Recombinant FSH is more basic and resembles Metrodin more closely than Metrodin HP, which is more acidic (Lambert et al., 1995). Therefore the bio-potency of FSH increases in the order highly purified urofollitropin (Metrodin HP), urofollitropin (Metrodin) and rFSH (Lambert et al., 1995). Loumaye et al., 1996 and Yarali et al., 1999 compared Gonal-F with Metrodin and Coelingh Bennink et al., 1998 compared Puregon with Metrodin in a chronic low dose step up regimen. Several parameters are commonly used as indicators for the biopotency of FSH: number of follicles >12 mm, E2 level on the day of HCG administration, total dose of FSH required to induce follicular development and the duration of stimulation.

Indeed, (Coelingh Bennink et al., 1998) found significantly more follicles in the range of 12-14mm in the rFSH group, although this was not found for follicles larger than 15 mm. Furthermore, rFSH required a significantly shorter treatment period to induce ovulation in the first cycle. There was no proof of a difference in mean E2 level and total dose required between rFSH and uFSH. Yarali et al., 1999 also studied the indicators for bioactivity mentioned above. This trial however found no difference in number of follicles in the range of 10-14 mm in the rFSH group. No differences were observed in E2 level, total FSH dose and treatment period required to induce ovulation. More importantly, results from the pooled data showed no difference in ovulation rate per cycle (OR 1.19, 95% CI 0.78 to 1.80), clinical pregnancy rate per woman (OR 0.95, 95% CI 0.64 to 1.41), multiple pregnancy rate per woman (OR 0.44, 95% CI 0.16 to 1.21), miscarriage rate per woman (OR 1.26, 95% CI 0.59 to 2.70) and OHSS per woman (OR 1.55, 95% CI 0.50 to 4.84).

Apart from the gonadotrophin used, the stimulation regimen is of importance in ovulation induction. The conventional regimen used to be the standard stimulation protocol but has now been abandoned. It is well known that the use of the conventional regimen results in high rates of multiple pregnancies (33%) and ovarian hyperstimulation syndrome (up to 14%) (Homburg, 2003; Yarali, 2004). These complications are due to the supraphysiological doses of FSH that are administered in the conventional regimen. The chronic low dose step up has been introduced to prevent the development of multiple follicles, multiple pregnancies and OHSS as seen in conventional step up regimens. Basic thinking behind the chronic low dose step up regimen is the "threshold theory", that states that FSH recruits and maintains one follicle only if it does not exceed a certain level (Brown 1978). The principle of the chronic low dose step up regimen is to employ a low starting dose for 14 days and then to use small incremental dose rises (usually half an ampoule) when necessary, at intervals of not less than 7 days, until follicular development is initiated (Seibel et al., 1984; Hamilton-Fairley et al., 1991). In the one trial that compared the chronic low dose step up with the conventional step up regimen no differences were found in effectiveness which may be due to the small size of this study (Hedon et al., 1998). Although a difference in OHSS and multiple pregnancies was not found there were significantly fewer follicles >10 mm diameter (WMD -3.30, 95% CI -5.34 to -1.26) in the group treated with the chronic low dose step up regimen.

A possible criticism of the chronic low dose step up regimen is that, unlike the events of the normal ovulatory cycle in which decreasing FSH concentrations are seen throughout the follicular phase, FSH levels may be elevated during the follicular phase (Dale et al., 1993). In order to mimic more closely the events of the normal ovulatory cycle the step down regimen has been introduced with a starting dose of 150 IU and decreasing the dose by 0.5 ampoules when a follicle of 10 mm develops and by the same amount every 3 days.
if follicular growth continues (Fauser et al., 1993). A key issue of the step down regimen is to estimate the FSH threshold of follicular development in order to determine the FSH starting dose. Therefore a "dose-finding" low-dose step up regimen is first offered for these patients followed by the step down regimen. A comparison of the step down regimen with the chronic low dose step-up regimen in a small randomised controlled trial with uFSH demonstrated a higher monofollicular growth rate of cycles in the step down regimen (88%) compared with step up regimen (56%) (van Santbrink and Fauser 1997). In the step down-group, duration of treatment and gonadotrophin requirement were significantly reduced. However, the study by Christin-Maitre et al., 2003 with a larger sample size, comparing the chronic low dose step up regimen versus the step down regimen with rFSH, demonstrated superiority of the step up regimen with regard to the rates of monofollicular development (OR 4.24, 95% CI 2.27 to 7.92), ovulation (OR 2.24, 95% CI 1.18 to 4.27) and multifollicular development (OR 0.13, 95% CI 0.06 to 0.29). Although the mean duration of stimulation was significantly longer (OR 5.50, 95% CI 3.21-7.79) in the step up regimen, no significant difference was found in the total dose of FSH used (WMD -16, 95% CI -24 to 209).

In the chronic low dose step up regimen a starting dose of no higher than 75 IU is used. In theory, risk of multifollicular development and multiple pregnancies can be prevented when an even lower starting dose is used. From the largest cohort study of the chronic low dose step up regimen (White et al., 1996) it was possible to compare the results of a starting dose of 75 IU with that of 52.5 IU, with an incremental dose rise of 37.5IU or 22.5IU respectively. No significant differences between the two groups were found but pregnancy rate per patient, monofollicular growth rate and miscarriage rate were all in favour of the lower starting dose. Although having a very small sample size with a crossover design similar clinical pregnancy rates and ovulation rates were observed in the randomized controlled trial comparing 37.5 IU with 50 IU (Balasch et al., 2000).

In summary, there is as yet insufficient evidence to conclude that rFSH is more effective than uFSH. Therefore, it is questionable whether its high costs should be traded off its purity, independence of urine collection, absolute source control and batch to batch consistency. No difference could be proven between the four different dose regimens. However, the conventional regimen is known to be plagued by OHSS and multiple pregnancies and its usage should therefore be discouraged.

**Reviewers' conclusions**

**Implications for practice**

The newly developed recombinant gonadotrophins have clear advantages, particularly availability, batch to batch consistency and self-administration. Based on the differences in terminal sialic acid residues, which confer different charge patterns to the gonadotropin preparations, differences in biological activity are expected. The role of biological activity in ovulation induction still has to be resolved. However, at this moment this does not have clear clinical consequence as both rFSH and uFSH are equally effective for ovulation induction in women with PCOS. Furthermore, attention should be given to the costs involved in these treatments. The higher costs associated with the rFSH can present a problem, particularly in a society with decreasing health resources.

For ovulation induction the chronic low dose step up regimen as well as step down
regimen should be preferred to the now outdated conventional regimen.

**Implications for research**

More randomised controlled trials with sufficient power are necessary to estimate the difference of rFSH and uFSH, if one exists. The theoretical advantage of a "dose finding" step up cycle preceding the step down cycle has to be determined in a randomized controlled trial. Evaluations of the outcomes should relate to effectiveness, as well as to adverse effects and cost-effectiveness. There is also a need to report all relevant outcomes, including life birth, multiple follicular development, OHSS, multiple pregnancy and miscarriage rates. Parallel rather than crossover design and an intention to treat analysis should be used. The need for large numbers means that such trials would be best organised on a multicentre basis.

**References**

**References to included studies**


Coelingh Bennink HJT, Fauser BCJM, Out HJ. (1998) Recombinant follicle-stimulating hormone (FSH; Puregon) is more efficient than urinary FSH (Metrodin) in women with clomiphene citrate-resistant, normogonadotropic, chronic anovulation: a prospective, multicenter, assessor-blind, randomized, clinical trial. Fertil. Steril. 69, 19-25.


References to excluded studies


Additional references


Characteristics of excluded studies

Attria et al., 2003: Not truly randomised and the starting dose differed between groups, i.e. 50 IE in the rFSH group and 75IE in the uFSH group.

Fulghesu et al., 2001: Non-randomized controlled trial.

Szilagy et al., 2004: Participating patients were treated with clomiphene citrate and gonadotrophins before randomization.