

VU Research Portal

Clomiphene Citrate in PCOS

Weiss, N.S.

2018

document version

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

citation for published version (APA)

Weiss, N. S. (2018). *Clomiphene Citrate in PCOS*. [, Vrije Universiteit Amsterdam].

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy

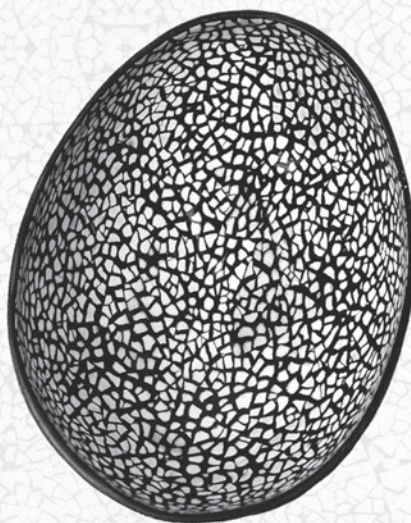
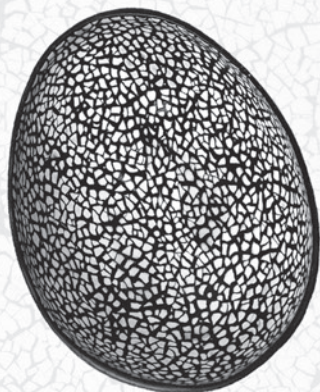
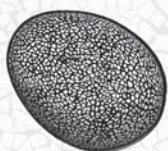
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

vuresearchportal.ub@vu.nl

Gonadotrophins for ovulation induction in women with polycystic ovarian syndrome

Weiss NS, Nahuis MJ, Bayram N, Mol BWJ,
van der Veen F, van Wely M



ABSTRACT

Background: Ovulation induction with follicle stimulating hormone (FSH) is the second-line treatment in women with polycystic ovary syndrome (PCOS) who do not ovulate or conceive on clomiphene citrate (CC).

Objectives: To compare the effectiveness and safety of gonadotrophins as a second-line treatment for ovulation induction in women with CC-resistant PCOS.

Search methods: We searched the Menstrual Disorders & Subfertility Group's Specialist Register of controlled trials, the Cochrane Central Register of Controlled Trials, MEDLINE (1966 to October 2014), EMBASE (1980 to October 2014), CINAHL (1982 to October 2014), National Research Register and web-based trials databases such as Current Controlled Trials. There was no language restriction.

Selection criteria: All randomised controlled trials reporting data on comparing clinical outcomes in women with PCOS who did not ovulate or conceive on CC, and undergoing ovulation induction with urinary FSH (uFSH: FSH-P or FSH-HP), HMG/HP-HMG or recombinant FSH. We included trials reporting on ovulation induction followed by intercourse or intrauterine insemination. We excluded studies that used co-treatment with CC, metformin, LH or letrozole.

Data collection and analysis: Three review authors (NW, MN and MvW) independently selected studies for inclusion, assessed study quality and extracted study data. Primary outcomes were live birth rate per woman (effectiveness outcome) and incidence of ovarian hyperstimulation syndrome (OHSS) per woman (safety outcome). Secondary outcomes were clinical pregnancy, miscarriage, multiple pregnancy, total gonadotrophin dose and total duration of stimulation per woman. We combined data using a fixed-effect model to calculate the odds ratio (OR). We summarised the overall quality of evidence for the main outcomes using GRADE criteria.

Main results: The review includes 14 trials with 1726 women. Ten trials compared rFSH versus urinary-derived gonadotrophins (three rFSH versus HMG and seven rFSH versus FSH-HP), four trials compared FSH-P with HMG. We found no trials that compared FSH-HP with FSH-P.

We found no evidence of a difference in live birth for rFSH versus urinary-derived gonadotrophins (OR 1.26, 95% CI 0.80 to 1.99, 5 trials, 505 women, $I^2 = 0\%$, low-quality evidence) or clinical pregnancy rate (OR 1.08, 95% CI 0.83 to 1.39, 8 trials, 1330 women,

$I^2 = 0$, low-quality evidence). This suggests that for the observed average live birth per woman with urinary-derived FSH of 16%, the chance of live birth following rFSH is between 13% and 26%.

For the comparison HMG or HP-HMG versus FSH-P there was also no difference in the evidence on live birth rate (OR 1.36, 95% CI 0.58 to 3.18, 3 trials, 138 women, $I^2 = 0\%$, low-quality evidence). This suggests that for a woman with a live birth rate of 18% with HMG or HP-HMG, the chance of live birth following uFSH is between 9% and 37%.

Trial authors used various definitions for OHSS. Pooling the data, we found no evidence of a difference for rFSH versus urinary-derived gonadotrophins (OR 1.52, 95% CI 0.81 to 2.84, 10 trials, 1565 women, $I^2 = 0\%$, very low-quality evidence) and for HMG or HP-HMG versus FSH-P (OR 9.95, 95% CI 0.47 to 210.19, 2 trials, 53 women, $I^2 = 0\%$, very low-quality evidence).

Authors' conclusions: In women with PCOS and CC resistance or CC failure, we found no evidence of a difference in live birth and OHSS rates between urinary-derived gonadotrophins and rFSH or HMG/HP-HMG. Evidence for all outcomes was of low or very low quality. We suggest weighing costs and convenience in the decision to use one or the other.

BACKGROUND

Description of the condition

Subfertility occurs in one in 10 couples world-wide. In about one-third of couples this is based on polycystic ovarian syndrome (PCOS). PCOS is characterised by oligo-anovulation, clinical or biochemical hyperandrogenism and polycystic ovaries^{1,2}. The syndrome affects approximately 6% to 10% of women of childbearing age.

Infertility due to chronic anovulation is the most common reason for women with PCOS to seek counselling or treatment. The first line of treatment in these women is ovulation induction with clomiphene citrate (CC), with or without metformin.

About 15% to 20% of women do not ovulate on CC and require alternative or second-line ovulation induction strategies. This failure to ovulate with CC is termed 'clomiphene resistance'. The most common treatment in women with CC-resistant PCOS is ovulation induction with gonadotrophins³. A recent review showed that laparoscopic electrocautery of the ovaries is an effective alternative treatment.⁴ If women fail to conceive with CC despite regular ovulation, the term 'clomiphene failure' is used. Also in this case, CC treatment is also often changed to second-line ovulation induction with gonadotrophins.

Description of the intervention

The strategy of stimulating follicle development and growth with exogenous gonadotrophins for ovulation induction in women with CC-resistant PCOS is well established.

FSH is found in the pituitary gland and in the circulation in different molecular forms. This molecular heterogeneity is due to the variation in the structures of the carbohydrate moieties, in particular of sialic acid. It is the configuration of these carbohydrate moieties that determines the FSH isoform. The configuration depends on which glycosylation enzymes are available in the cell during synthesis.⁵ Each molecular glycoform has a different molecular weight, net charge, circulating half-life and metabolic clearance.⁶⁻⁹ Gonadotrophins were originally extracted from pituitary gland and later extracted from the urine of post-menopausal women.^{10,11}

Over the last five decades, various urinary-derived follicle stimulating hormone (FSH) products, or urofollitropins, have been developed. Menotropin (human menopausal gonadotrophin (HMG)) has been available since the early 1960s and contains FSH, luteinising hormone (LH) and large quantities of potentially allergenic urinary proteins. Purified urofollitropin (FSH-P) has been available since the mid-1980s. FSH-P is devoid of LH but still contains urinary proteins. Highly purified urofollitropin (FSH-HP) has been available since the mid-1990s and contains very small amounts of urinary proteins. The absence of urinary proteins diminishes rare adverse reactions such as local allergy or hypersensitivity.^{12,13} The

most recent development in urinary gonadotrophins is highly purified menotropin (HP-HMG), containing equal amounts of FSH and LH activity.

To obtain even higher purity, gonadotrophins were developed using recombinant DNA technology (recombinant FSH (rFSH)) in 1988.^{14,15} The production of rFSH is independent of urine collection, thus guaranteeing a high availability of a biochemical pure FSH preparation that is free from LH and urinary protein contaminants. The production process also yields FSH with high specific bioactivity (roughly 100 times higher than for urine-derived FSH products), minimal batch-to-batch discrepancies and low immunogenicity.¹⁶ There is evidence that rFSH has a higher bioactivity than urinary products.¹⁷

At present two preparations of rFSH are available: follitropin alpha and follitropin beta. 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 fewer acidic isohormones than the urinary-derived gonadotrophin preparations.¹⁸⁻²⁰

How the intervention might work

In the follicular phase of a normal menstrual cycle, between 10 and 20 antral follicles develop. Of this cohort, one follicle will obtain dominance over the others and will continue to grow until ovulation takes place. In women with PCOS this dominance does not occur. The aim in ovulation induction is to induce growth of up to three follicles. This is accomplished by ovarian stimulation with FSH containing gonadotrophins. Too forceful a regimen will result in overstimulation and hence in an increased risk of multiple pregnancy and ovarian hyperstimulation syndrome (OHSS); a stimulation regimen with too low a dosage of FSH will not result in a dominant follicle and thereby will not lead to ovulation.

Why it is important to do this review

Gonadotrophins are the standard drugs in medical ovulation induction in women with CC-resistant PCOS. The present review is an update and extension of two previous Cochrane reviews.^{21,22} Bayram 2001 had compared rFSH with FSH-P and FSH-HP; Nugent 2000 had compared HMG with purified FSH. No Cochrane review has yet compared HMG with rFSH in CC-resistant women. Summarising the evidence on the effectiveness and safety of the various gonadotrophins will help gynaecologists and women to make informed decisions on their use for ovulation induction.

OBJECTIVES

To compare the effectiveness and safety of gonadotrophins as a second-line treatment for ovulation induction in women with CC-resistant PCOS.

METHODS

Criteria for considering studies for this review

Types of studies

We have included randomised controlled trials. We excluded quasi-randomised controlled trials in which allocation was, for example, by alternation, reference to case record numbers or to dates of birth. We also excluded cross-over trials, which are not appropriate in this context.²³

Types of participants

1. Subfertile women with CC-resistant PCOS undergoing ovulation induction. We define CC resistance as a failure to ovulate with CC doses of at least 100 mg/day for at least five days.
2. Subfertile women with PCOS and CC failure undergoing ovulation induction. We define CC failure as a failure to conceive after three cycles of ovulation induction with CC.
3. Women treated in the past by metformin with or without CC.
4. Women with prior treatment with electrocautery of the ovaries.

Types of interventions

1. Ovulation induction with rFSH versus any other urinary gonadotrophin (HMG, FSH-P, FSH-HP)
2. Ovulation induction with FSH-HP versus FSH-P
3. Ovulation induction with HMG or HP-HMG versus FSH-P or FSH-HP

For all interventions, ovulation induction could include intrauterine insemination. We excluded trials involving co-treatment with CC, metformin, LH or letrozole.

Types of outcome measures

Primary outcomes

1. Live birth rate per woman
2. Incidence of ovarian hyperstimulation syndrome (OHSS) per woman (safety outcome)

Secondary outcomes

3. Clinical pregnancy rate (per woman)
4. Miscarriage rate (per woman)
5. Incidence of multiple pregnancy (per woman and per clinical pregnancy)
6. Total gonadotrophin dose per woman (IU)
7. Total duration of stimulation per woman

Search methods for identification of studies

This review has drawn on the search strategy developed for the Cochrane Menstrual Disorders and Subfertility Group (MDSG) as a whole.

Electronic searches

Marian Showell (Trials Search Co-ordinator of the MDSG) developed the search strategies. See Appendix 1, Appendix 2, Appendix 3, Appendix 4, Appendix 5, Appendix 6 (all available online).

We searched the following electronic sources to 22 October 2014:

- Cochrane Central Register of Controlled Trials (CENTRAL) (current issue)
- MEDLINE (from 1966 onwards)
- EMBASE (from 1988 onwards)
- Trial registers for ongoing and registered trials - clinicaltrials.gov, a service of the US National Institutes of Health, or the WHO ICTRP (World Health Organization International Trials Registry Platform search portal)
- Reference lists from reviews and trials
- the Cochrane Library for Database of Abstracts of Reviews of Effects (DARE) (reference lists from non-Cochrane reviews on similar topics)
- Handsearching of appropriate journals
- Conference abstracts on the Web of Knowledge
- OpenGrey for unpublished literature from Europe
- LILACS database, a source of trials from the Portuguese- and Spanish-speaking world
- PubMed and Google for any recent trials that have not yet been indexed in MEDLINE

Searching other resources

We searched the following conference abstracts:

- American Society for Reproductive Medicine and Canadian Fertility and Andrology Society (ASRM/CFAS) Conjoint Annual Meeting (2001 to 2014), Abstracts of the Scientific Oral and Poster Sessions, Program Supplement;
- European Society of Human Reproduction and Embryology (ESHRE) Annual Meeting (2001 to 2014), Abstracts of the Scientific Oral and Poster Sessions, Program Supplement.

We hand searched the references cited in all obtained studies.

We asked Serono Benelux BV and Merck, Ferring and IBSA, the manufacturers of gonadotrophins, for ongoing studies and unpublished data.

Data collection and analysis

Selection of studies

Three review authors (NW, MN and MvW) independently examined the electronic search results for reports of possibly relevant trials, and retrieved these reports in full. All review authors independently applied the selection criteria to the trial reports, rechecking trial eligibility and resolving disagreements by discussion with the other authors.

Data extraction and management

Three review authors (NW, MN and MvW) independently extracted the outcome data and information on funding, location, clinical and design details, and participants. We resolved any differences by discussion. We entered details of the studies into the table Characteristics of included studies (available online). We presented studies that appeared to meet the inclusion criteria but were excluded from the review in the table Characteristics of excluded studies (available online), briefly stating the reason for exclusion but giving no further information.

Assessment of risk of bias in included studies

Three review authors (NW, MN and MvW) extracted information regarding the risk of bias (threats to internal validity) under six domains (also see the Cochrane 'Risk of bias' assessment tool in Appendix 7).²⁴ We resolved any differences by discussion.

1. Sequence generation. Evidence that an unpredictable random process was used.
2. Allocation concealment. Evidence that the allocation list was not available to anyone involved in the recruitment process.
3. Blinding of participants, clinicians and outcome assessors. Evidence that knowledge of allocation was not available to those involved in subsequent treatment decisions or follow-up efforts.
4. Completeness of outcome data. Evidence that any losses to follow-up were low and comparable between groups.
5. Selective outcome reporting. Evidence that major outcomes had been reported in sufficient detail to allow analysis, independently of their apparent statistical significance.
6. Other potential sources. Evidence of miscellaneous errors or circumstances that might influence the internal validity of trial results.

We sought missing details from the authors of the original publications. We present all details in the 'Risk of bias' table following each included study.

Measures of treatment effect

We summarised all binary outcomes using the odds ratio (OR) with a 95% confidence interval (CI).

We treated ordinal scales, such as amount of gonadotrophin used and duration of ovarian stimulation, as continuous outcomes. We abstracted, calculated or requested means and standard deviations.

Unit of analysis issues

We expressed all outcomes per woman randomised.

We also expressed the secondary outcome of multiple pregnancy per clinical pregnancy.

Dealing with missing data

Where there was insufficient information in the published report, we attempted to contact the authors for clarification. If missing data became available, we included them in the analysis. We anticipated that trials conducted over 10 years ago might not have data on live birth rates. We analysed data extracted from the trials on an intention-to-treat basis. Where randomised participants were missing from outcome assessment, we first contacted the authors for additional data. If further data were not available, we assumed that missing participants had failed to achieve pregnancy and had not suffered any of the reported adverse events.

Assessment of heterogeneity

The presence of statistical heterogeneity of treatment effect among trials was determined using the I^2 statistic.²⁵ We considered whether clinical and methodological characteristics of the included studies were sufficiently similar for meta-analysis to provide a clinically meaningful summary. We assessed statistical heterogeneity by the measure of the I^2 statistic. We took an I^2 measurement greater than 50% to indicate substantial heterogeneity, in which case we tested the effect of using a random-effects model.²⁴

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases, we aimed to minimise their potential impact by ensuring a comprehensive search for eligible studies and by being alert for duplication of data. If we included 10 or more studies in an analysis, we planned to use a funnel plot to explore the possibility of small-study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies).

Data synthesis

When multiple studies were available on a similar comparison, we used Review Manager 5 software to perform the meta-analyses, using the Mantel-Haenszel method with a fixed-effect model. For reporting purposes, we translated primary outcomes to absolute risks. We combined results for continuous outcomes using the mean difference.

Subgroup analysis and investigation of heterogeneity

If excessive heterogeneity existed within strata, we planned to explore this informally using the clinical and design details recorded in the table Characteristics of included studies which is found online. Prospectively, we planned to undertake three different stratifications of the primary outcomes: type of urinary gonadotrophin (HMG, FSH-P and FSH-HP); single or multiple cycles; sponsorship (commercial, non-commercial).²⁶

Sensitivity analysis

We conducted sensitivity analyses for the primary outcomes to determine whether the conclusions were robust to arbitrary decisions made regarding study eligibility and analysis. These analyses included consideration of whether the review conclusions would have differed if:

- we had used a random-effects model
- we had reported risk ratios rather than odds ratios

Overall quality of the body of evidence: 'Summary of findings' table

We generated 'Summary of findings' tables using GRADEPRO software. These tables evaluate the overall quality of the body of evidence for main review outcomes using GRADE criteria: study limitations (i.e. risk of bias), consistency of effect, imprecision, indirectness and publication bias. We justified judgements about evidence quality (high, moderate or low), documented them, and incorporated them into the reporting of results for each outcome.

RESULTS

Description of studies

For details of the studies please see: Characteristics of included studies; Characteristics of excluded studies, both available online.

Results of the search

We identified 18 RCTs, four of which we excluded from analysis. See Figure 1.

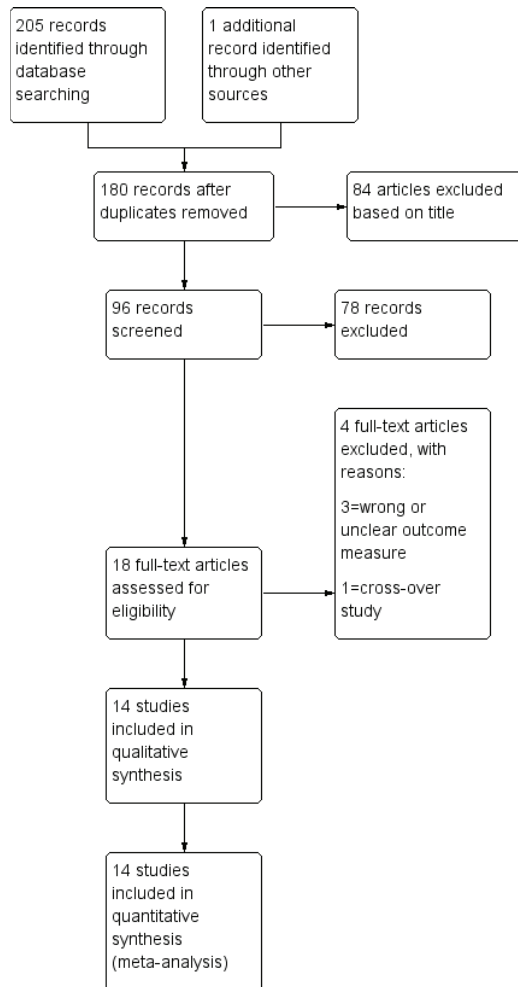


Figure 1. Study flow diagram.

Included studies

We included 14 trials.

1. Ten studies compared the effects of rFSH versus urinary derived gonadotrophins (HMG: Balen 2007; Platteau 2006; Revelli 2006¹⁻³; uFSH: Coelingh Bennink 1998; Feigenbaum 2001; Gerli 2004; Loumaye 1996; Szilágyi 2004⁴⁻⁸, Taketani 2010; Yarali 1999).^{9,10} Loumaye 1996 was described in a review on human gonadotrophins produced by recombinant DNA technology.⁷ The authors of the 2001 Cochrane review (Bayram 2001)²¹ collected the data for this trial by personal communication, and we now used them again.

2. There were no studies that compared FSH-HP with FSH-P.

3. Four studies compared FSH-P with HMG (Gadir 1990; McFaul 1990; Sagle 1991; Seibel 1985). Gadir 1990 made an extra comparison with laparoscopic electrocautery of the ovaries. One trial also included normo-ovulatory women with unexplained subfertility (Revelli 2006). For this review, we used only the data of women with PCOS. For Seibel 1985, we included pre-cross-over data.

Seven trials reported data on live birth, and 10 trials (Balén 2007; Coelingh Bennink 1998; Feigenbaum 2001; Gerli 2004; Loumaye 1996; Platteau 2006; Revelli 2006; Szilágyi 2004; Taketani 2010; Yarali 1999) reported the incidence of OHSS. The definition of OHSS varied between trials, as is detailed in the Characteristics of included studies tables (available online). Some studies did not give a definition.

All studies included women who were CC-resistant; six of them also included women with CC failure (Balén 2007; Coelingh Bennink 1998; Gerli 2004; Platteau 2006; Seibel 1985; Yarali 1999). None of the women included in this review had been treated with electrocautery in the past. Nine trials analysed more than one cycle per woman, whereas five trials only analysed one cycle per woman (Balén 2007; Feigenbaum 2001; Platteau 2006; Revelli 2006; Taketani 2010). In three trials intra-uterine insemination (IUI) was performed in some cases (Balén 2007; Gerli 2004; Platteau 2006). All trials used a low-dose step-up protocol, but the protocol used in Loumaye 1996 was unknown. Ten trials reported a commercial sponsor (Balén 2007, Loumaye 1996, Coelingh Bennink 1998; Feigenbaum 2001; Platteau 2006; Sagle 1991; Seibel 1985; Szilágyi 2004; Taketani 2010; Yarali 1999).

Only five trials reported a power calculation (Balén 2007; Coelingh Bennink 1998; Loumaye 1996; Platteau 2006; Revelli 2006).

Excluded studies

We excluded four trials: one trial because the outcome measure was the effect of FSH on haemostasis (Ricci 2004)¹; two studies because the outcome 'pregnancy' was not defined and this outcome was only presented per cycle (Homburg 1990; Jacobs 1987), and one study because it was a cross-over design and it was not possible to extract the pre-cross-over data per woman (Larsen 1990).

Risk of bias in included studies

We summarise the risks of bias in the included studies in Figure 2 and Figure 3.

Allocation

Allocation to the intervention or control group was adequately concealed in three trials (Balén 2007; Loumaye 1996; Platteau 2006). The allocation concealment was inadequate in two trials (Gadir 1990; Gerli 2004) and unclear in the remaining trials.

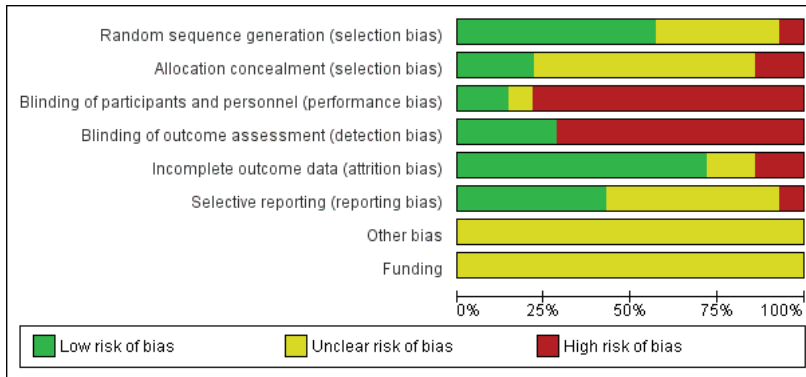


Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias	Funding
Balen 2007	+	+	+	+	+	+	?	?
Coelingh Bennink 1998	+	?	?	+	+	+	?	?
Feigenbaum 2001	+	?	-	-	+	+	?	?
Gadir 1990	-	-	-	-	+	?	?	?
Gerli 2004	+	?	-	-	+	?	?	?
Loumaye 1996	+	+	-	-	?	?	?	?
McFaul 1990	?	?	-	-	+	?	?	?
Platteau 2006	+	+	+	+	+	+	?	?
Revelli 2006	+	?	-	-	+	+	?	?
Sagle 1991	?	?	-	-	+	?	?	?
Seibel 1985	?	?	-	-	-	?	?	?
Szilágyi 2004	?	?	-	-	-	-	?	?
Taketani 2010	?	?	-	+	?	?	?	?
Yarali 1999	+	-	-	-	+	+	?	?

Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Blinding

Four trials were assessor-blinded (Balén 2007; Coelingh Bennink 1998; Platteau 2006; Taketani 2010). Blinding was not performed in the remaining studies.

Incomplete outcome data

Two trials had a high risk of attrition bias (Seibel 1985; Szilágyi 2004). For another two trials this was unclear (Loumaye 1996; Taketani 2010). All other trials had a low risk for this domain.

Selective reporting

We rated six studies as having a low risk of selective reporting bias; seven as having an unclear risk of bias in this domain, and one study as having high risk (Szilágyi 2004).

Other potential sources of bias

We rated this as unclear for all studies. Some studies provided too few details to make a judgement. Within all the trials, the baseline characteristics appeared balanced over the two treatment groups. Only five of the 14 trials mentioned the duration of the trial (Balén 2007; Coelingh Bennink 1998; Loumaye 1996; Platteau 2006; Taketani 2010).

Effects of interventions

rFSH versus urinary-derived gonadotrophins

Live birth rate per woman (Figure 4)

Five trials including 505 women reported on live birth (Balén 2007; Feigenbaum 2001; Platteau 2006; Revelli 2006; Szilágyi 2004). After pooling the results, the overall OR per woman was 1.26 (95% CI 0.80 to 1.99, 5 RCTs, $n = 505$, $I^2 = 17\%$, low-quality evidence), indicating no evidence of a difference. Translated into absolute risks, this means that for a woman with a 16% chance of achieving live birth with the use of urinary-derived FSH, the chance of a live birth with the use of rFSH would be between 13% and 26%. Statistical heterogeneity for this outcome was low. The live birth rate varied from 16% to 40% in the rFSH group and from 0% to 25% in the urinary gonadotrophin group.

When dividing the urinary-derived gonadotrophins into subgroups (three trials compared rFSH versus HP-HMG, two trials compared rFSH versus FSH-HP), we found no evidence of a difference between the subgroups ($P = 0.09$). The OR for rFSH versus HP-HMG/HMG was 1.04 (95% CI 0.63 to 1.73, 3 RCTs, $n = 409$, $I^2 = 0\%$, low-quality evidence) and for rFSH versus FSH-HP was 3.11 (95% CI 0.98 to 9.91, 2 RCTs, $n = 96$, $I^2 = 26\%$, low-quality evidence).

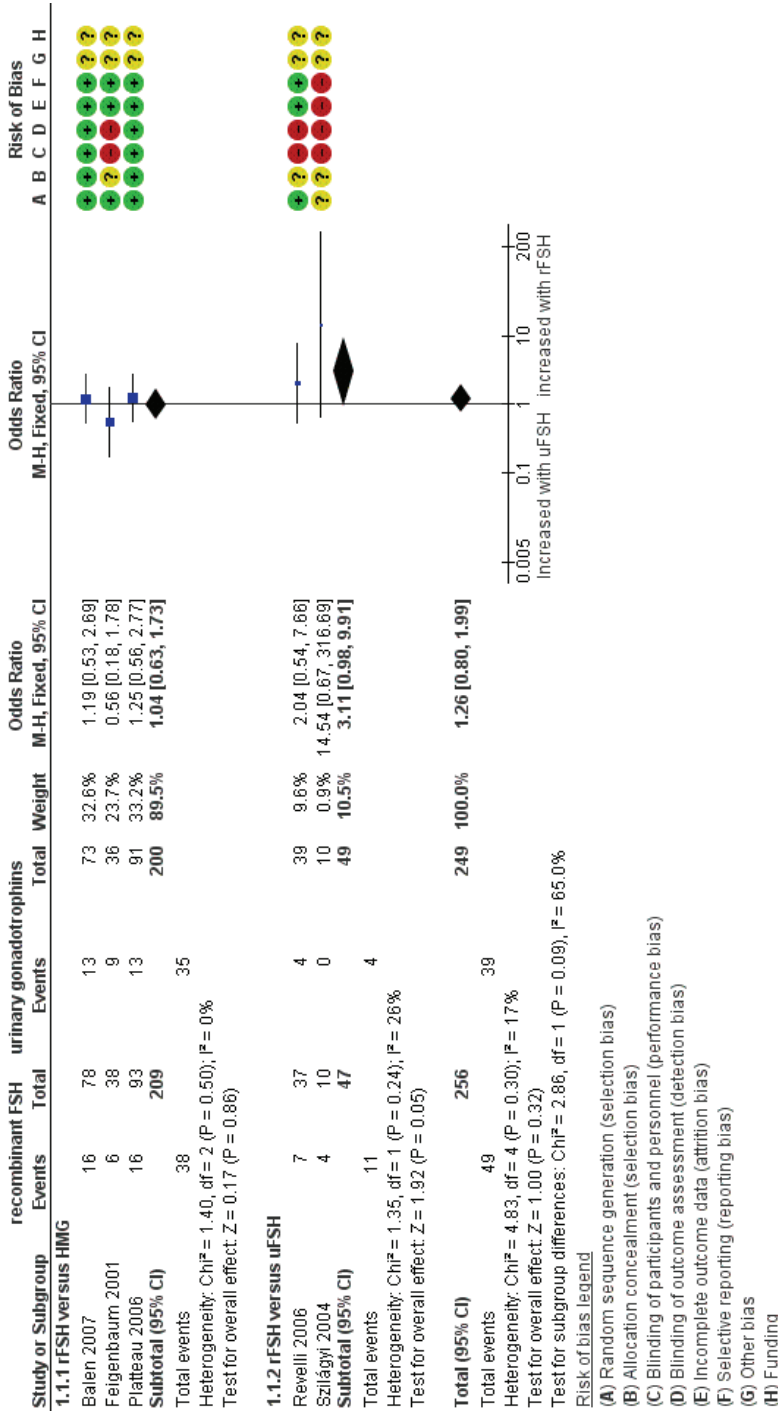


Figure 4. Forest plot of comparison 1: recombinant FSH versus urinary gonadotrophins, outcome: Live birth rate per woman.

All trials comparing rFSH and HP-HMG were sponsored by Ferring, while for the other two trials comparing rFSH and FSH-P the sponsor was not reported. Subgrouped results per sponsor were therefore similar to the gonadotrophin comparison, i.e. subgrouping did not result in differences between subgroups ($P = 0.09$)

Incidence of ovarian hyperstimulation syndrome (OHSS) per woman

Ten studies reported OHSS, including 1565 women (Balén 2007; Coelingh Bennink 1998; Feigenbaum 2001; Gerli 2004; Loumaye 1996; Platteau 2006; Revelli 2006; Szilágyi 2004; Taketani 2010; Yarali 1999). After pooling the results, the overall OR for OHSS per woman was 1.52 (95% CI 0.81 to 2.84, 10 RCTs, $n = 1565$, $I^2 = 0\%$, very low-quality evidence), indicating no evidence of a difference). This means that for a woman with a 2.2% chance of OHSS urinary-derived gonadotrophins, the chance of OHSS with the use of rFSH would be between 1.2% and 5.2%. The OHSS rate varied from 0% to 20% in both groups.

When dividing the urinary-derived gonadotrophins into subgroups (three trials compared rFSH versus HP-HMG, seven trials compared rFSH versus FSH-HP), we found no evidence of a difference between the subgroups ($P = 0.53$). The OR for rFSH versus HP-HMG was 1.12 (95% CI 0.37 to 3.44, 3 RCTs, $n = 409$, $I^2 = 0\%$, very low-quality evidence) and for rFSH versus FSH-HP was 1.74 (95% CI 0.81 to 3.72, 7 RCTs, $n = 1156$, $I^2 = 0\%$, very low-quality evidence).

Clinical pregnancy rate per woman

Eight studies including 1330 women reported on clinical pregnancy (Balén 2007; Coelingh Bennink 1998; Feigenbaum 2001; Gerli 2004; Loumaye 1996; Platteau 2006; Taketani 2010; Yarali 1999). There was no evidence of a difference in clinical pregnancy (OR 1.08, 95% CI 0.83 to 1.39; 8 RCTs, $n = 1330$, $I^2 = 0\%$, low-quality evidence).

When dividing the urinary-derived gonadotrophins into subgroups (three trials compared rFSH versus HP-HMG, five trials compared rFSH versus FSH-HP), there was no evidence of a difference between the subgroups ($P = 0.49$). The OR for rFSH versus HP-HMG was 1.25 (95% CI 0.76 to 2.04, 3 RCTs, $n = 409$, $I^2 = 0\%$, low-quality evidence) and for rFSH versus FSH-HP 1.02 (95% CI 0.75 to 1.38, 5 RCTs, $n = 921$, $I^2 = 0\%$, low-quality evidence).

Miscarriage rate per woman

Seven studies including 970 women reported on miscarriage (Balén 2007; Coelingh Bennink 1998; Gerli 2004; Loumaye 1996; Platteau 2006; Szilágyi 2004; Yarali 1999). There was no evidence of a difference in miscarriage (OR 1.22, 95% CI 0.69 to 2.15; 7 RCTs, $n = 970$, $I^2 = 0\%$, low-quality evidence).

When dividing the urinary-derived gonadotrophins into subgroups (two trials compared rFSH versus HP-HMG, five trials compared rFSH versus FSH-HP), we found no evidence of a difference between the subgroups ($P = 0.70$).

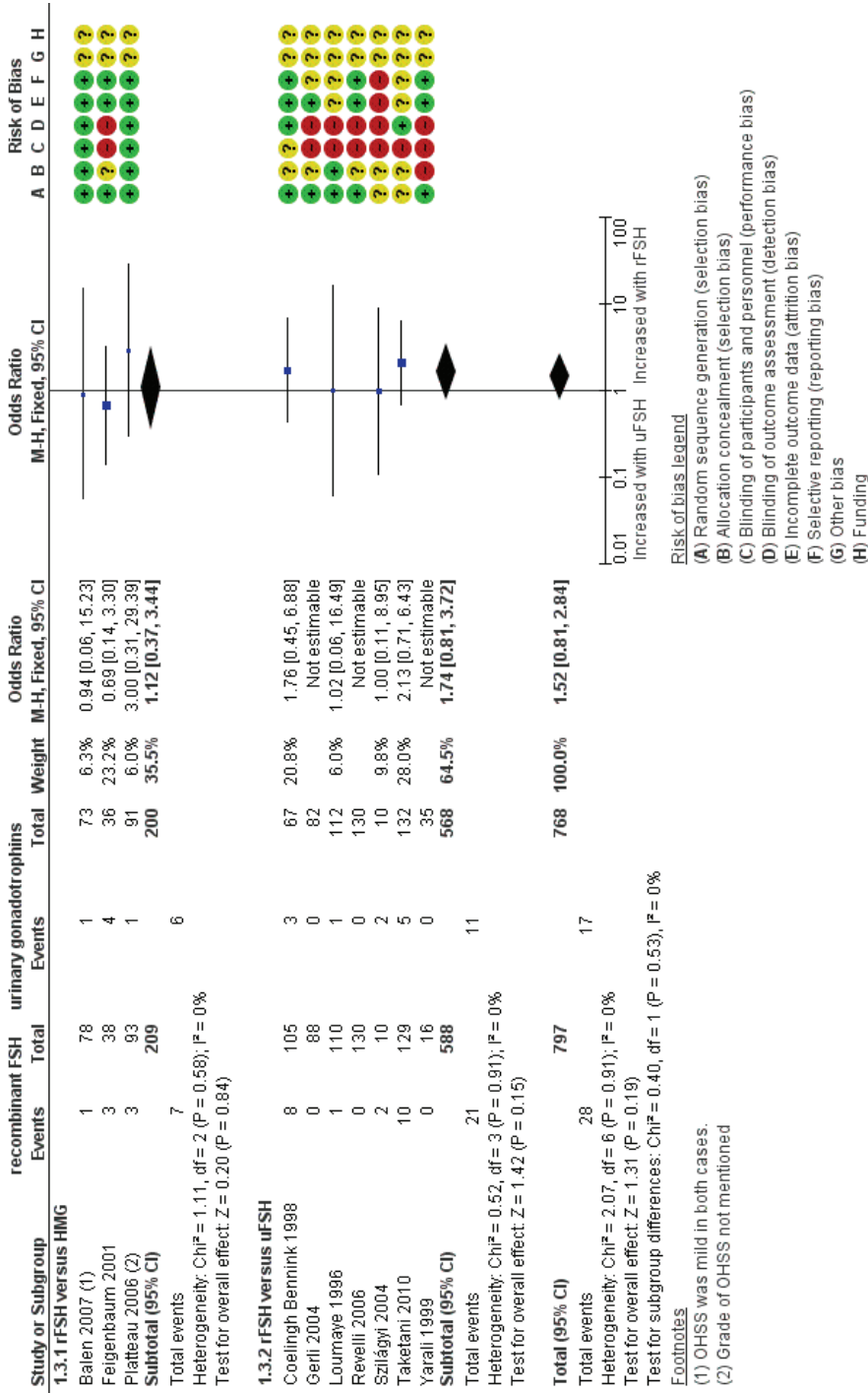


Figure 5. Forest plot of comparison 1: recombinant FSH versus urinary-derived gonadotrophins, outcome: Incidence of OHSS per woman. Subgrouping by sponsor did not result in differences between subgroups (P = 0.88).

Incidence of multiple pregnancy per woman

Eight studies including 1368 women reported on multiple pregnancy (Balen 2007; Coelingh Bennink 1998; Feigenbaum 2001; Gerli 2004; Platteau 2006; Revelli 2006; Taketani 2010; Yarali 1999). There was no evidence of a difference in multiple pregnancy (OR 0.86, 95% CI 0.44 to 1.65; 8 RCTs, n = 1368, I² = 0%, low-quality evidence).

When dividing the urinary-derived gonadotrophins into subgroups (three trials compared rFSH versus HP-HMG, five trials compared rFSH versus FSH-HP), there was no evidence of a difference between the subgroups (P = 0.34).

Table I. Summary of findings for the main comparison: recombinant FSH versus urinary-derived gonadotrophins for ovulation induction in women with polycystic ovarian syndrome.

Patient or population: women with polycystic ovarian syndrome					
Settings: women visiting the outpatient clinic					
Intervention: recombinant FSH versus urinary-derived gonadotrophins as second-line treatment					
Outcomes	Illustrative comparative risks* (95% CI)		Relative risk (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)
	Assumed risk	Corresponding risk			
	Urinary-derived gonadotrophins	Recombinant FSH			
Live birth rate per woman	157 per 1000	191 per 1000 (127 to 257)	OR 1.26 (0.80 to 1.99)	505 (5 studies)	⊕⊕⊕⊖ low ^{1,2}
Incidence of OHSS per woman	22 per 1000	33 per 1000 (18 to 60)	OR 1.52 (0.81 to 2.84)	1565 (10 studies)	⊕⊖⊖⊖ very low ^{1,2,3}
Clinical pregnancy rate per woman	239 per 1000	253 per 1000 (207 to 304)	OR 1.08 (0.83 to 1.39)	1330 (8 studies)	⊕⊕⊕⊖ low ^{1,2}
Miscarriage rate per woman	47 per 1000	57 per 1000 (33 to 95)	OR 1.22 (0.69 to 2.15)	970 (7 studies)	⊕⊕⊕⊖ low ^{1,2}
Incidence of multiple pregnancy (per woman)	30 per 1000	26 per 1000 (13 to 48)	OR 0.86 (0.44 to 1.65)	1368 (8 studies)	⊕⊕⊕⊖ low ^{1,2}

*The basis for the **assumed risk** is the median risk in the control groups. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; OR: Odds ratio

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

¹ Imprecision around the absolute effect

² Inconsistency in results across studies

³ In each study a different definition or no definition of OHSS, downgraded one further level

Incidence of multiple pregnancy per clinical pregnancy

We found no evidence of a difference in multiple pregnancy per clinical pregnancy (OR 0.69, 95% CI 0.33 to 1.43; 8 RCTs, 315 pregnancies, $I^2 = 0\%$).

Mean total gonadotrophin dose per woman

We found evidence of a statistically significant mean difference in total gonadotrophin use in favour of rFSH (MD -105 IU, 95% CI -154 to -57, 6 RCTs, $n = 1046$, $I^2 = 81\%$). Use of a random-effects model in view of the high statistical heterogeneity resulted in no evidence of a difference (MD -127 IU, 95% CI -258 to 3.26).

Total duration of stimulation per woman (days)

We found evidence of a statistically significant mean difference in total duration of stimulation favour of rFSH (MD -0.66 days, 95% CI -1.04 to -0.28, 6 RCTs, $n = 1122$, $I^2 = 72\%$). Use of a random-effects model in view of the high statistical heterogeneity resulted in no evidence of a difference (MD -0.80 days, 95% CI -1.66 to 0.05).

HMG or HP-HMG versus uFSH***Live birth per woman***

Three trials including 138 women reported on live birth (Gadir 1990; McFaul 1990; Sagle 1991). We found no evidence of a difference in live birth rate (OR 1.36, 95% CI 0.58 to 3.18, 3 RCTs, $n = 138$, $I^2 = 0\%$, low-quality evidence) (Figure 6).

Incidence of OHSS per woman

Two studies reported OHSS including 53 women (Sagle 1991; Seibel 1985). We found no evidence of a difference in OHSS (OR 9.95, 95% CI 0.47 to 210, 2 RCTs, $n = 53$, very low-quality evidence).

Clinical pregnancy rate per woman

One study reported clinical pregnancy rate per woman (Sagle 1991). McFaul 1990 presented pregnancy rates without defining this outcome. For this study, we calculated the clinical pregnancy rates by adding the number of live births to the number of miscarriages in each group. Seibel 1985 reported conception rates, which we used as clinical pregnancy rate. This analysis covers 102 women. After pooling the data, we found no evidence of a difference (OR 1.44, 95% CI 0.55 to 3.77, 3 RCTs, $n = 102$, $I^2 = 0\%$, low-quality evidence).

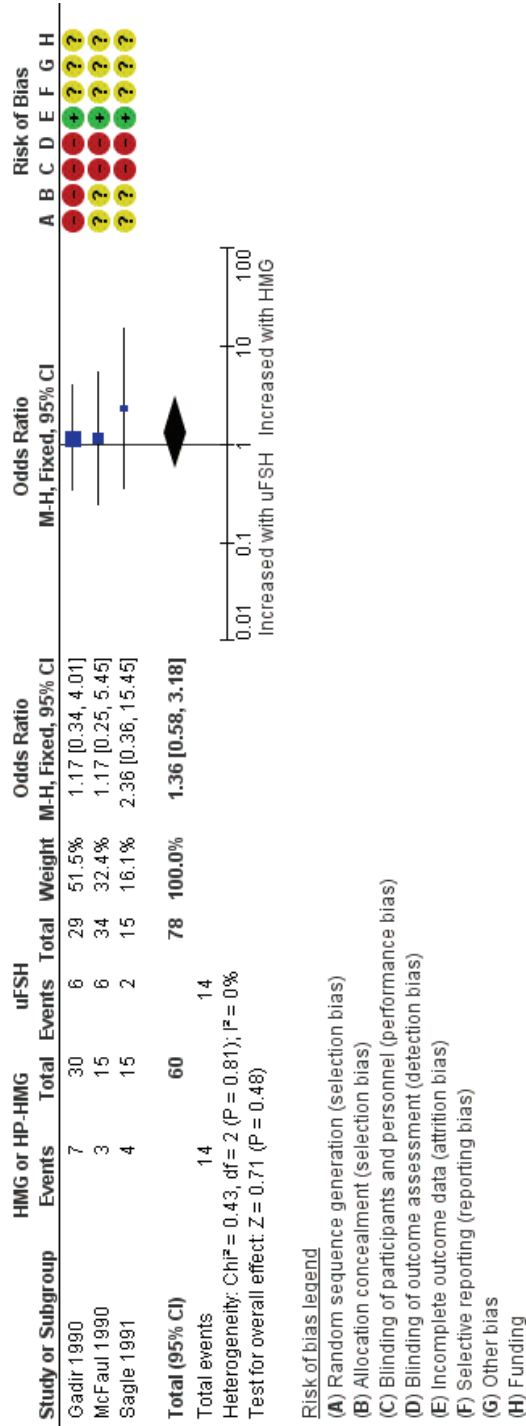


Figure 6. Forest plot of comparison 2: HMG or HP-HMG versus uFSH, outcome: Live birth rate per woman.

Miscarriage rate per woman

We found no evidence of a difference in miscarriage rate (OR 0.30, 95% CI 0.04 to 2.05, 2 RCTs, n = 98, $I^2 = 0\%$, low-quality evidence).

Incidence of multiple pregnancy per woman

We found no evidence of a difference in multiple pregnancy rate per woman (OR 2.26, 95% CI 0.47 to 10.95, 4 RCTs, n = 161, $I^2 = 0\%$).

Table II. Additional summary of findings for the comparison HMG or HP-HMG versus uFSH for ovulation induction in women with polycystic ovarian syndrome.

Outcomes	Illustrative comparative risks* (95% CI)		Relative risk (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)
	Assumed risk	Corresponding risk			
	uFSH	HMG or HP-HMG			
Live birth rate per woman	179 per 1000	230 per 1000 (90 to 370)	OR 1.36 (0.58 to 3.18)	138 (3 studies)	⊕⊖⊖⊖ low ^{1,2}
Incidence of OHSS per woman	No events	4/28 ³	OR 9.95 (0.47 to 210)	53 (2 studies)	⊕⊖⊖⊖ very low ^{1,2,4}
Clinical pregnancy rate per woman	203 per 1000	269 per 1000 (123 to 490)	OR 1.44 (0.55 to 3.77)	102 (3 studies)	⊕⊖⊖⊖ low ^{1,2}
Miscarriage rate per woman	82 per 1000	26 per 1000 (4 to 154)	OR 0.30 (0.04 to 2.05)	98 (2 studies)	⊕⊖⊖⊖ low ^{1,2}
Incidence of multiple pregnancy (per woman)	23 per 1000	50 per 1000 (11 to 203)	OR 2.26 (0.47 to 10.95)	161 (4 studies)	⊕⊖⊖⊖ low ^{1,2}

*The basis for the **assumed risk** is the median risk in the control groups. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; OR: Odds ratio

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

¹ Imprecision around the absolute effect

² Inconsistency in results across studies

³ Event rate derived from the raw data. A 'per thousand' rate is non-informative in view of the scarcity of evidence and zero events in the control group

⁴ In each study a different definition of OHSS, downgraded one further level. Two of our studies did not report this outcome

Incidence of multiple pregnancy per clinical pregnancy

The total number of multiple pregnancies within the studies that reported clinical pregnancies were too small to compare.

Mean total gonadotrophin dose per woman

The studies of Gadir 1990 and McFaul 1990 reported mean values for total doses but they did not state standard deviations (HMG/HP-HMG versus uFSH): 1568 versus 1478 (Gadir 1990) and 1770 versus 1995 (McFaul 1990). The authors declared that they found no significant difference.

Sagle 1991 also observed no significant difference. They reported values in mean total dose per cycle: 1080 (min - max: 525 - 1950) versus 1447.5 (min - max: 675 - 2887.5).

Total duration of stimulation per woman (days)

McFaul 1990 reported no significant mean difference between HMG and uFSH (11.8 versus 11.9 days respectively). They did not provide standard deviations.

DISCUSSION

Summary of main results

This review compared the effectiveness of recombinant gonadotrophin (rFSH) with the three main types of urinary gonadotrophins (i.e. HMG, FSH-P and FSH-HP) as a second-line treatment for ovulation induction in women with CC-resistant PCOS. We found 10 studies that compared rFSH versus urinary-derived gonadotrophins, and four trials that compared uFSH with HMG. There was no evidence of a difference in pregnancy outcomes when rFSH was compared to urinary gonadotrophins as a whole, nor when comparing rFSH with HMG/HP-HMG or rFSH with FSH-HP. There was no evidence of a difference observed in OHSS for any of the comparisons. We found no trials which compared rFSH and FSH-P or FSH-HP with FSH-P.

Overall completeness and applicability of evidence

For the trials that compared rFSH and urinary-derived gonadotrophins, outcome data needed to make the planned comparisons were largely available; these trials were all published after 1996. The data of trials that compared rFSH and uFSH-P and uFSH-HP were incomplete, probably because these trials had been published between 1985 and 1991 when there were no CONSORT or PRISMA guidelines and clinical pregnancy or ovulation rate were still accepted endpoints.

Seven trials did not define the outcome OHSS. The remaining studies used very different definitions (see Characteristics of included studies, available online). Nowadays, it is common to categorise cases of OHSS by three degrees; mild, moderate or severe.²⁷ Since this ranking was almost never used in the included studies of this review, it may be inappropriate to pool the data on OHSS. Also, different starting dosages were used varying from 50 to 150 IE per day, with various criteria to withhold from injecting human chorion gonadotrophin (hCG). This may influence the incidence of OHSS, regardless of the type of gonadotrophin used.

The data on gonadotrophin dose used and duration of stimulation were never presented per woman randomised, and showed high statistical heterogeneity. These outcomes are therefore likely to be biased, and conclusions on the basis of these data should not be drawn.

Three of the included studies used IUI in addition to ovulation induction with gonadotrophins. IUI may or may not have increased the pregnancy rate, but as in these studies IUI was always provided in both study arms, its effect on differential pregnancy rates is likely to be small. No women were included that had been treated with electrocautery in the past. We can therefore draw no conclusions on this specific population.

The included population represents women with PCOS who are either CC-resistant or failed to conceive with CC. The evidence is broadly applicable as a second-line treatment for ovulation induction in these women.

Quality of the evidence

GRADE assessment found that evidence for most outcomes was of low to very low quality, due to the limited amount of studies, small study size, statistical heterogeneity, and the quality of the individual studies.

Potential biases in the review process

Strengths of this review include comprehensive systematic searching for eligible studies, rigid inclusion criteria for RCTs and data extraction, and analysis by three independent review authors. The possibility of publication bias was minimised by inclusion of both published and unpublished studies (such as abstracts from meetings). However, as with any review, we cannot guarantee that we found all eligible studies.

Agreements and disagreements with other studies or reviews

Our results are in line with the outcomes of the previous Cochrane review of Bayram 2001,²¹ in concluding that rFSH and urinary-derived gonadotrophins are equally effective for ovulation induction in women with PCOS in terms of ovulation rate, pregnancy rate, miscarriage rate and multiple pregnancy rate. Our results are also in line with the outcomes

of the previous Cochrane review of Nugent 2000,²² who concluded that comparing FSH and HMG showed no evidence of a difference in pregnancy rate. Nugent 2000 did find a significant reduction in OHSS rate per cycle in women treated with FSH-P compared to HMG. We focused on OHSS rate per woman and did not find any difference, although only two trials were available for this analysis.

Bayram 2001 and Nugent 2000 did not evaluate the outcome of live birth. We found no evidence of a difference in live birth rate for the comparisons rFSH versus urinary gonadotrophins and HMG or HP-HMG versus uFSH, but the quality of evidence was low.

Another review compared rFSH with urinary-derived FSH products. The authors found that follitropin alpha, beta and urinary FSH products appeared to be as effective in terms of clinical, ongoing and multiple pregnancy rates as in live birth rates. This review did not pool data on OHSS.²⁸

AUTHORS' CONCLUSIONS

Implications for practice

It appears that differences in effectiveness and safety between the available gonadotrophins are small. The choice of one or the other product will depend upon the availability of the product, the convenience of its use, and the associated costs.

Implications for research

We chose ovarian hyperstimulation syndrome (OHSS) as a safety outcome, since this review is an update of two older Cochrane reviews which have also used this outcome. Both reviews and the current review included mainly older studies, performed at a time when OHSS was a complication after ovulation induction. At present, OHSS is mainly a complication that occurs after treatment with IVF.²⁷ Nowadays it seems more relevant to investigate the occurrence of multiple pregnancies after ovulation induction. In this respect, we found no evidence of a difference in multiple pregnancy rates per woman when comparing the different types of gonadotrophins, but the included studies were of low quality. We therefore feel that new research should be specifically directed at preventing multiple pregnancies while retaining the highest live birth chances. Another reason for the need for new research is the low quality of most of the included studies in this review.

REFERENCES

References to studies included in this review

- Balen A, Platteau P, Andersen AN, et al. Highly purified FSH is as efficacious as recombinant FSH for ovulation induction in women with WHO Group II anovulatory infertility: a randomized controlled non-inferiority trial. *Hum Reprod* 2007; 22(7): 1816-23.
- Coelingh Bennink HJ, Fauser BC, Out HJ. 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. European Puregon Collaborative Anovulation Study Group. *Fertil Steril* 1998; 69(1): 19-25.
- Feigenbaum SL MP, Kaufmann R, Elkind-Hirsch K, Fein SH, Marshall DC. A new highly purified humanderived FSH, Bravelle, is as effective and well tolerated as recombinant follitropin beta in ovulation induction in infertile women with ovulatory dysfunction. *Today's Therapeutic Trends* 2001; 19(4): 297-313.
- Gadir A, Mowafi RS, Alnaser HM, Alrashid AH, Alonezi OM, Shaw RW. Ovarian electrocautery versus human menopausal gonadotrophins and pure follicle stimulating hormone therapy in the treatment of patients with polycystic ovarian disease. *Clin Endocrinol (Oxf)* 1990; 33(5): 585-92.
- Gerli S, Casini ML, Unfer V, Costabile L, Mignosa M, Di Renzo GC. Ovulation induction with urinary FSH or recombinant FSH in polycystic ovary syndrome patients: a prospective randomized analysis of cost-effectiveness. *Reprod Biomed Online* 2004; 9(5): 494-9.
- Loumaye E, Martineau I, Piazza A, et al. Clinical assessment of human gonadotrophins produced by recombinant DNA technology. *Hum Reprod* 1996; 11 Suppl 1: 95-107; discussion 17-9.
- McFaul PB, Traub AI, Thompson W. Treatment of clomiphene citrate-resistant polycystic ovarian syndrome with pure follicle-stimulating hormone or human menopausal gonadotropin. *Fertil Steril* 1990; 53(5): 792-7.
- Platteau P, Andersen AN, Balen A, et al. Similar ovulation rates, but different follicular development with highly purified menotrophin compared with recombinant FSH in WHO Group II anovulatory infertility: a randomized controlled study. *Hum Reprod* 2006; 21(7): 1798-804.
- Revelli A, Poso F, Gennarelli G, Moffa F, Grassi G, Massobrio M. Recombinant versus highly-purified, urinary follicle-stimulating hormone (r-FSH vs. HP-uFSH) in ovulation induction: a prospective, randomized study with cost-minimization analysis. *Reprod Biol Endocrinol* 2006; 4: 38.
- Sagle MA, Hamilton-Fairley D, Kiddy DS, Franks S. A comparative, randomized study of low-dose human menopausal gonadotropin and follicle-stimulating hormone in women with polycystic ovarian syndrome. *Fertil Steril* 1991; 55(1): 56-60.
- Seibel MM, McArdle C, Smith D, Taymor ML. Ovulation induction in polycystic ovary syndrome with urinary follicle-stimulating hormone or human menopausal gonadotropin. *Fertil Steril* 1985; 43(5): 703-8.
- Szilagyi A, Bartfai G, Manfai A, Koloszar S, Pal A, Szabo I. Low-dose ovulation induction with urinary gonadotropins or recombinant follicle stimulating hormone in patients with polycystic ovary syndrome. *Gynecol Endocrinol* 2004; 18(1): 17-22.
- Taketani Y KE, Yoshimura Y, Hoshiai H, Irahara M, Mizunuma H. Recombinant follicle stimulating hormone (follitropin alfa) versus purified urinary follicle stimulating hormone in a low dose step up regimen to induce ovulation in japanese women with anti-estrogenineffective oligo- or anovulatory infertility. *Reproductive Medicine and Biology* 2010; 9: 99-106.

- Yarali H, Bukulmez O, Gurgan T. Urinary follicle-stimulating hormone (FSH) versus recombinant FSH in clomiphene citrate-resistant, normogonadotropic, chronic anovulation: a prospective randomized study. *Fertil Steril* 1999; 72(2): 276-81.

References to studies excluded from this review

- Homburg R, Eshel A, Kilborn J, Adams J, Jacobs HS. Combined luteinizing hormone releasing hormone analogue and exogenous gonadotrophins for the treatment of infertility associated with polycystic ovaries. *Hum Reprod* 1990; 5(1): 32-5.
- Jacobs H PR, Eshel A, Craft I. Profertility uses of LHRH agonist analogues. *LHRH and its Analogues; Contraceptive and Therapeutic Application Part 2* 1987: 303–22.
- Larsen T, Larsen JF, Schioler V, Bostofte E, Felding C. Comparison of urinary human follicle-stimulating hormone and human menopausal gonadotropin for ovarian stimulation in polycystic ovarian syndrome. *Fertil Steril* 1990; 53(3): 426-31.
- Ricci G, Cerneca F, Simeone R, et al. Impact of highly purified urinary FSH and recombinant FSH on haemostasis: an open-label, randomized, controlled trial. *Hum Reprod* 2004; 19(4): 838-48.

Additional references

1. Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004; 81(1): 19-25.
2. Rotterdam EA-SPCWG. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 2004; 19(1): 41-7.
3. Balen AH. Ovulation induction in the management of anovulatory polycystic ovary syndrome. *Mol Cell Endocrinol* 2013; 373(1-2): 77-82.
4. Farquhar C, Brown J, Marjoribanks J. Laparoscopic drilling by diathermy or laser for ovulation induction in anovulatory polycystic ovary syndrome. *Cochrane Database Syst Rev* 2012; (6): CD001122.
5. Wide L. In: Isoforms of human gonadotrophins under different physiological conditions. *Gonadotrophin Isoforms: Facts and Future* 1997: 43–8.
6. Baenziger JU, Green ED. Pituitary glycoprotein hormone oligosaccharides: structure, synthesis and function of the asparagine-linked oligosaccharides on lutropin, follitropin and thyrotropin. *Biochim Biophys Acta* 1988; 947(2): 287-306.
7. Gray C. Glycoprotein gonadotrophins. Structure and synthesis. *Acta Endocrinologica Supplementum* 1988; 288: 20–7.
8. Stockell Hartree A, Renwick AG. Molecular structures of glycoprotein hormones and functions of their carbohydrate components. *Biochem J* 1992; 287 (Pt 3): 665-79.
9. Wilson CA, Leigh AJ, Chapman AJ. Gonadotrophin glycosylation and function. *J Endocrinol* 1990; 125(1): 3-14.
10. Gemzell CA, Diczfalusy E, Tillinger G. Clinical effect of human pituitary follicle-stimulating hormone (FSH). *J Clin Endocrinol Metab* 1958; 18(12): 1333-48.
11. Lunenfeld B, Menzi A, Volet B. [Clinical effects of a human postmenopausal gonadotropin]. *Rass Clin Ter* 1960; 59: 213-6.

12. Albano C, Smitz J, Camus M, Bennink HC, Van Steirteghem AC, Devroey P. Pregnancy and birth in an in-vitro fertilization cycle after controlled ovarian stimulation in a woman with a history of allergic reaction to human menopausal gonadotrophin. *Hum Reprod* 1996; 11(8): 1632-4.
13. Biffoni M, Battaglia A, Borrelli F, Cantelmo A, Galli G, Eshkol A. Allergenic potential of gonadotrophic preparations in experimental animals: relevance of purity. *Hum Reprod* 1994; 9(10): 1845-8.
14. Howles CM. Genetic engineering of human FSH (Gonal-F). *Hum Reprod Update* 1996; 2(2): 172-91.
15. Keene JL, Matzuk MM, Otani T, et al. Expression of biologically active human follitropin in Chinese hamster ovary cells. *J Biol Chem* 1989; 264(9): 4769-75.
16. Bergh C. What are the clinical benefits of recombinant gonadotrophins? Recombinant follicle stimulating hormone. *Hum Reprod* 1999; 14(6): 1418-20.
17. Andersen CY, Westergaard LG, van Wely M. FSH isoform composition of commercial gonadotrophin preparations: a neglected aspect? *Reprod Biomed Online* 2004; 9(2): 231-6.
18. de Leeuw R, Mulders J, Voortman G, Rombout F, Damm J, Kloosterboer L. Structure-function relationship of recombinant follicle stimulating hormone (Puregon). *Mol Hum Reprod* 1996; 2(5): 361-9.
19. Hard K, Mekking A, Damm JB, et al. Isolation and structure determination of the intact sialylated N-linked carbohydrate chains of recombinant human follitropin expressed in Chinese hamster ovary cells. *Eur J Biochem* 1990; 193(1): 263-71.
20. Lambert A, Rodgers M, Mitchell R, et al. In-vitro biopotency and glycoform distribution of recombinant human follicle stimulating hormone (Org 32489), Metrodin and Metrodin-HP. *Hum Reprod* 1995; 10(7): 1928-35.
21. Bayram N, van Wely M, van der Veen F. Recombinant FSH versus urinary gonadotrophins or recombinant FSH for ovulation induction in subfertility associated with polycystic ovary syndrome. *Cochrane Database Syst Rev* 2001; (2): CD002121.
22. Nugent D, Vandekerckhove P, Hughes E, Arnot M, Lilford R. Gonadotrophin therapy for ovulation induction in subfertility associated with polycystic ovary syndrome. *Cochrane Database Syst Rev* 2000; (4): CD000410.
23. Vail A, Gardener E. Common statistical errors in the design and analysis of subfertility trials. *Hum Reprod* 2003; 18(5): 1000-4.
24. Higgins JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. *The Cochrane Collaboration* 2011.
25. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; 327(7414): 557-60.
26. Lexchin J, Bero LA, Djulbegovic B, Clark O. Pharmaceutical industry sponsorship and research outcome and quality: systematic review. *BMJ* 2003; 326(7400): 1167-70.
27. Youssef MA, van der Veen F, Al-Hnany HG, et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. *Cochrane Database Syst Rev* 2014; (10): CD008046.
28. Nahuis M, van der Veen F, Oosterhuis J, Mol BW, Hompes P, van Wely M. Review of the safety, efficacy, costs and patient acceptability of recombinant follicle-stimulating hormone for injection in assisting ovulation induction in infertile women. *Int J Womens Health* 2010; 1: 205-11.