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FSH treatment of male idiopathic infertility improves pregnancy rate: a meta-analysis

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Abstract

Introduction: The aim of this study is to comprehensively evaluate whether FSH administration to the male partner of infertile couples improves pregnancy rate, spontaneously and/or after assisted reproductive techniques (ART).
Methods: Meta-analysis of controlled clinical trials in which FSH was administered for male idiopathic infertility, compared with placebo or no treatment. Randomization was not considered as an inclusion criterion.
Results: We found 15 controlled clinical studies (614 men treated with FSH and 661 treated with placebo or untreated). Concerning the type of FSH, eight studies used recombinant FSH, whereas seven studies used purified FSH. Nine studies evaluated spontaneous pregnancy rate, resulting in an overall odds ratio (OR) of about 4.5 (CI: 2.17–9.33). Eight studies evaluated pregnancy rate after ART, showing a significant OR of 1.60 (CI: 1.08–2.37). Sub-dividing studies according to the FSH preparations (purified/recombinant), pregnancy rate improvement remained significant for each preparation. Eleven studies considered sperm quality after FSH treatment, finding a significant improvement of sperm concentration (2.66×10^6/ml, CI: 0.47–4.84), but not of concentration of sperm with progressive motility (1.22×10^6/ml, CI: −0.07 to 2.52). Three trials evaluated testicular volume, showing a non-significant increase in men treated (1.35 ml, CI: −0.44 to 3.14).
Conclusion: The results of controlled clinical trials available in the literature indicate an improvement of pregnancy rate after FSH administration to the male partner of infertile couples, both spontaneously and after ART. However, the heterogeneity of studies, the high risk of bias and the lack of precise criteria to guide FSH administration limit the strength of these results. Future studies should be designed to identify the markers of FSH response which are helpful in the decision-making process. Meanwhile, the use of FSH in the treatment of male infertility should be cautious.

Key Words
- FSH
- male infertility
- infertile couples

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Introduction

Rationale

Infertility is conventionally defined as a clinical condition affecting a couple failing to conceive after a period of 12 months of regular intercourse without contraception (1). Male infertility plays a significant role in ~50% of couples experiencing a delay in conceiving (2). A careful and complete diagnostic workup allows for recognition of the most important male infertility causes (3). However, the underlying pathophysiological mechanism remains idiopathic in about 30% of cases, which fall in the category of 'male idiopathic infertility'(2). The medical treatment of infertility may be specific or non-specific. Specific treatments are used for certain etiologies, such as hypogonadotropic hypogonadism, male accessory gland infection, retrograde ejaculation, and positive antisperm antibody (4). Non-specific treatments are hormonal and non-hormonal therapies, proposed for the treatment of idiopathic infertile men (5, 6).

Assisted reproductive techniques (ART) based on IVF were introduced to clinical practice in 1978 (7), and progressively extended its indication from female, tubal infertility to unexplained couple infertility. Currently, intracytoplasmatic-sperm injection (ICSI) is the most frequently used ART (8). The outcome of ICSI seems to be influenced by sperm structure and quality (9). Thus, it seems reasonable that an improvement in sperm quality could effect ICSI outcomes.

The empirical administration of follicle stimulating hormone (FSH) to infertile men has been reported in the literature since 1991 to variably improve fertilization and pregnancy rate (10, 11). This therapy is popular in some countries, although doubts remain on its efficacy in the treatment of infertile men, particularly in the ART setting. A significant increase in pregnancy rate after IVF and male treatment with FSH was shown in some studies (12, 13), while other trials did not find the same improvement (14). Recently, the Cochrane Collaboration estimated the overall effect of FSH treatment of the man in couples attending ART enrolled in randomised, controlled, clinical trials (15). This meta-analysis demonstrates that FSH treatment significantly improves spontaneous pregnancy rate, whereas no improvement of pregnancy rate is observed after ART, probably because only one randomized clinical trial (RCT) was included (15). These results were obtained by evaluating only randomized, controlled, clinical trials, using fixed and strict inclusion criteria (15). The authors excluded quasi-RCTs, cross-over trials (if data before the cross-over was not available), and excluded all trials in which randomization was not carried out (15). Therefore, only six RCTs, the most recent thereof published in 2006 (16), were included in the meta-analysis. The absence of further RCTs in this field of medicine in the last decade reflects the difficulty of conducting a randomized approach in infertile couples, in which the woman is nearing the end of her reproductive age, as a swift successful pregnancy is required. Finally, Attia *et al.* (15) evaluated pregnancy rate as an outcome of FSH treatment without assessing separately purified and recombinant FSH formulations.

Objectives

The aim of this study was to comprehensively evaluate whether FSH administration to the male partner of infertile couples improves pregnancy rate, spontaneously and/or after ART, by performing a meta-analysis of all controlled clinical trials.

Materials and methods

We performed a meta-analysis according to the Cochrane Collaboration and PRISMA statement (17, 18).

Data sources and searches

We conducted a comprehensive literature search for English-language articles in MEDLINE (PubMed), EMBASE, the Cochrane Library, SCOPUS and UpToDate. Search key words were: FSH, FSH treatment, FSH therapy, follicle-stimulating hormone, gonadotropin, infertility, male infertility, IVF, intracytoplasmatic sperm injection, IVF, ICSI and the Boolean functions AND and OR.

Study selection and inclusion criteria

Types of studies ▸ Controlled clinical trials in which FSH was administered for male idiopathic infertility, compared with placebo or no treatment. Randomization was not considered as inclusion criterion, thus both randomized and non-RCTs were reviewed.

Type of participants ▸ Men with idiopathic infertility or subfertility. All semen abnormalities (from mild oligozoospermia to severe oligo-astheno-teratospermia)

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were considered eligible, independently of the changes over time of the normality ranges for semen analysis by the World Health Organization (WHO). Azoospermic men were not considered eligible. Controls were not treated or placebo-treated idiopathic infertile or sub-fertile men. No inclusion criteria were applied for the female partner of the infertile couple.

Type of interventions ▸ Chronic treatment with any type of FSH, compared with placebo or no treatment.

Data collection process and quality

One author (D S) extracted the abstracts from all studies found through the literature search. All abstracts were evaluated for inclusion criteria and data were extracted from each study considered eligible, with regard to study design, year of publication, number of included/excluded subjects, number of dropped-out patients and the use of intention to treat/per protocol analysis. Furthermore, D S extracted study subjects' demographics and underlying diseases, with particular attention to the time elapsed from the first attempt to conceive.

All controlled study designs were considered eligible. We included studies assessing efficacy of FSH administration to the male partner of infertile couples in order to improve fertilization. The quality of trials was assessed using the parameters proposed by Jadad *et al.* (19) and Table 1 summarizes the features of the selected studies.

Studies considered in the meta-analysis used different endpoints. Some studies evaluated spontaneous pregnancy rate, occurring after unprotected intercourse, whereas other studies evaluated fertilization and pregnancy rate occurring after ART. We performed an overall meta-analysis which considered all studies that evaluated pregnancy rate. Subsequently, we separately evaluated pregnancy rate, either spontaneously or after ART.

Semen analysis, a surrogate fertility marker, was routinely performed using light microscopy. In all studies, semen samples were collected by masturbation, generally after 3–4 days of sexual abstinence, and liquefied at 37 °C.

The other investigators (M S, A R G) performed quality control checks on the extracted data (Fig. 1). The investigators, using the Cochrane risk-of-bias algorithm, independently assessed the risk of bias for all trials (20). The following quality criteria and methodological details were evaluated for each trial included in the meta-analysis: i) method of randomization, even if the randomization was not an inclusion criterion, ii) concealment of allocation, iii) presence or absence of blinding to treatment allocation, iv) duration and type of treatment and follow-up phases, v) number of participants recruited, analyzed or lost to follow-up, vi) timing of trial, vii) whether an intention to treat analysis was done, viii) whether a power calculation was done, ix) source of funding, and x) criteria for including participants and assessing outcomes.

Summary measures

The primary outcome was pregnancy rate, evaluated as an odds ratio (OR) between treated and control men. Secondary outcomes were semen analysis parameters. Sperm quality (i.e. sperm concentration and progressive motility) and testicular volume were considered as mean differences. Sperm motility was assessed as the number of progressive motile sperm, calculated considering only the percentage of sperm with progressive motility out of the total sperm number.

Data synthesis and analysis

The meta-analysis was conducted using the Review Manager (RevMan) 5.3 Software (Version 5.3.1 Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Data were combined using the fixed effect model for the primary endpoint, considering the reliability in the evaluation of pregnancy. However, the random effect model was used for secondary endpoints, providing a more conservative estimate of the overall effect, considering the intrinsic inaccuracy of parameters such as sperm quality. Weighted mean differences and 95% CIs were estimated for each endpoint. Heterogeneity among the results of different studies was examined by inspecting both the scatter in the data points and the overlap in their CIs, and by performing χ^2 tests and I^2 statistics. The I^2 statistics answer the question: what proportion of the observed variance reflects real differences in effect size? It is a measure of inconsistency across the findings of the studies, not a measure of the real variation across the underlying true effects. Values of $P<0.05$ were considered statistically significant.

Risk of bias across studies

The authors (D S, A R G, M S) independently evaluated risk of bias. Publication bias is a bias towards reporting significant results, despite the fact that studies with significant results do not appear to be superior to studies with a null result with respect to result quality (21, 22). A simple analysis of funnel plots provides a useful test for

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Table 1 Characteristics of studies included in the meta-analysis.

References	Number of treated patients	Number of control patients	Mean age (years)	Baseline sperm concentration of treated patients (million/ml)	Baseline sperm morphology of treated patients (%)	Baseline FSH of treated patient (IU/l)	Baseline Sperm concentration of controls (million/ml)	Baseline sperm morphology of controls (%)	Baseline FSH of controls (IU/l)	Drugs	Cumulative FSH dose (IU)	Duration (weeks)	Parameters evaluated
(12)	39	39	–	–	–	–	–	–	–	Purified FSH (Metrodin)	6300	12	PR
(14)	24	20	–	8.81	–	–	12.42	–	–	Purified FSH (Fertinorm)	12 600	12	PR, FR, semen analysis
(35)	31	101	31.5	–	–	–	–	–	–	Purified FSH (Metrodin)	2100	4	PR, semen analysis
(36)	20	20	31.7	–	–	6.4±2.4	–	–	6.4±2.6	Purified FSH (Metrodin)	–	2 IVF cycles	PR, FR
(37)	23	10	35.3	1.3±2.2	23.9±8.2	9.7±6.05	2.5±2.2	30.0±12.9	10.5±6.8	Recombinant FSH (Gonal F)	5400	12	PR, FR, TV, semen analysis
(38)	65	63	32.6	7.8±2.7	20.6±5.0	5.9±1.3	8.1±2.2	21.8±4.7	6.1±1.6	Recombinant FSH (Gonal F)	6750	12	Semen analysis, sDF
(41)[a]	77	20	32	4.7±5.1	–	9.0±8.9	61.2±38.8	–	2.6±1.6	Purified FSH (Metrodin)	3375	12	Semen analysis
(40)[a]	30	55	32.6	4.8±2.6	–	4.1±2.2	63.2±39.2		2.8±1.5	Recombinant FSH (Gonal F)	2250	12	Semen analysis
(39)	65	90	34.2	6.4±3.5	25.3±6.2	4.6±1.2	6.8±3.2	26.2±6.8	4.8±1.4	Recombinant FSH (Gonal F)	4500	12	PR, semen analysis
(42)	57	62	34.2	1.8±0.7	20.7±8.4	15.1±5.8	1.7±0.9	18.1±9.9	14.7±6.6	Recombinant FSH (Gonal F)	6750	12	PR, semen analysis
(13)	34	33	32.8	8.7±1.5	33.4±3.0	5.0±0.4	8.7±1.6	29.6±3.0	5.6±0.6	Recombinant FSH (Gonal F)	12 600	12	PR, semen analysis, TV
(43)	19	20	–	–	–	–	–	–	–	hMG	–	13	PR
(44)[a]	58	78	–	69.5±40.7	27.5±11.8	–	72.3±43.2	30.3±12.3	–	Purified FSH (Fertinorm)	–	unclear	PR, semen analysis
(16)	15	15	–	7.6±3.6	9.4±6.1	4.1±1.6	7.4±4.1	8.7±5.5	4.3±1.8	Recombinant FSH (Gonal F)	18 000	16	PR, semen analysis
(45)	70	35	–	4.0±4.2	20.8±15.4	5.0±1.8	3.8±4.0	20.1±16.3	4.9±2.0	Recombinant FSH (Gonal F)	5400	12	PR, semen analysis, TV

hMG, human menopausal gonadotropin, FR, fertilization rate, FSH, follicle-stimulating hormone, PR, pregnancy rate, sDF, sperm-DNA fragmentation, TV, testicular volume.
[a]Studies in which untreated normozoospermic infertile men of infertile couples were enrolled in the control group.

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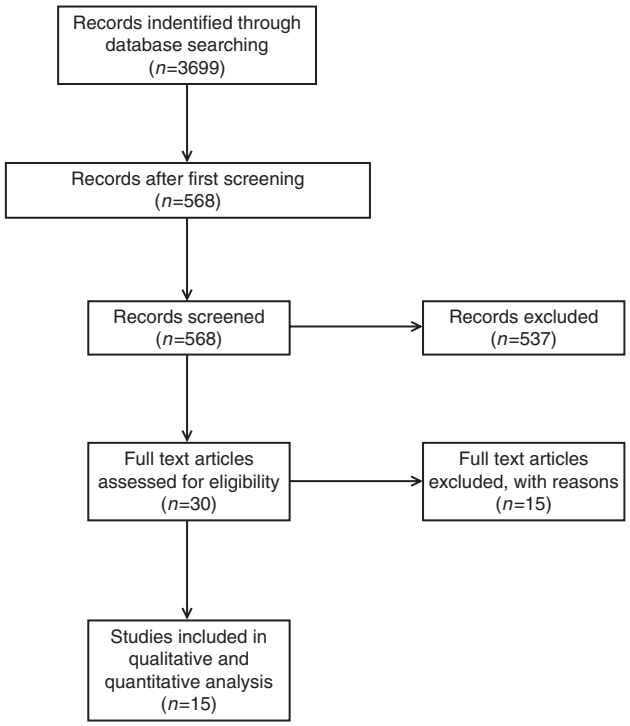

Figure 1
Study flow chart showing the search results for the studies included in the meta-analysis.

the likely presence of bias in meta-analyses, but as the capacity to detect bias will be limited when meta-analyses are based on a limited number of small trials, the results from such analyses should be treated with considerable caution (22). Funnel and Egger's plots were performed using RevMan Software.

Additional analysis

Comparison between variables was performed with the Mann–Whitney *U* test. Meta-regression analysis was conducted by comparing sperm parameters to pregnancy rate. Number needed to treat (NNT) was calculated as the statistical inverse ratio of the absolute OR reduction. All additional analyses were performed using 'Statistical Package for the Social Sciences' Software for Macintosh (version 20.0; SPSS, Inc.).

Results

Study selection

Figure 1 shows the literature searching process, conducted from September 2014 to January 2015. From 568 studies initially found according to the research strategy, we identified 30 potentially relevant studies, based on the information given in the abstract. All trials were thoroughly appraised for eligibility in the meta-analysis and methodological quality. Fifteen studies were excluded from the final analysis since they did not fulfill the inclusion criteria (9, 10, 11, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34). Four studies were excluded because they were neither controlled nor cross-over studies (10, 11, 33, 34). Three studies were excluded because, although they aimed at evaluating the effect of gonadotropins in infertile couples, the study design did not provide the enrollment and the treatment criteria for the male partner (23, 25, 27). Four studies were excluded because they evaluated pregnancy rate in hypogonadotropic hypogonadic men (24, 30, 31, 32). Another trial was excluded because it evaluated the effect on sperm retrieval after testicular sperm extraction in azoospermic men after treatment with clomiphene citrate (26). Furthermore, another work was excluded because it was only a letter and not an original article (28). Finally, two studies were excluded because they were not clinical trials but reviews of sperm characteristics related to fertility (9, 29). Fifteen studies met the inclusion criteria (12, 13, 14, 16, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45) (Figure 1).

Study characteristics

All trials included were controlled against placebo or no treatment and enrolled the male partners of infertile couples attending ART Centers. By definition, the term 'infertility' was used after at least 1 year of unsuccessful intercourse (1). However, the duration of infertility considered in each study was different. Some trials considered infertile couples failing to conceive after 1 year, others after 2 years of unprotected intercourse. Furthermore, the definition of infertility in each trial was different. Some studies enrolled men with severe oligo-asthenoteratozoospermia, whereas others enrolled men with mild to moderate semen abnormalities, i.e. astheno-zoospermia or teratozoospermia. The definition of these semen abnormalities was different, reflecting the WHO criteria, which changed during the time frame of the studies included.

Concerning the type of FSH, eight studies included in the meta-analysis used recombinant FSH (13, 16, 37, 38, 39, 40, 42, 45), whereas seven studies used purified FSH (12, 14, 36, 41, 43, 44). Since the efficacy of recombinant and purified FSH was demonstrated to be similar (46) the type of FSH was not considered a source of bias.

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Pregnancy rate, when evaluated, was considered spontaneous or after ART.

The different inclusion and exclusion criteria used in the studies included represent the most important source of heterogeneity in the estimation of the overall effect. This is a typical selection bias in studies involving infertile patients, considering several factors could influence couple fertility, from either the male or from the female side. The selected trials gave details about 1275 infertile men, 614 treated with FSH and 661 untreated (545) or placebo-treated (116).

Risk of bias in included studies

Figure 2 summarizes the risk of bias.

Allocation (selection bias) ▸ Nine studies were RCTs and precisely specified the methodology of randomization (12, 13, 14, 38, 39, 40, 42, 43, 45). The other six studies included were non-randomized, controlled, clinical trials, with potential selection bias due to the lack of randomization.

Blinding (performance bias and detection bias) ▸ The nine RCTs were double-blinded (12, 13, 14, 38, 39, 40, 42, 43, 45).

Incomplete outcome data (attrition bias) ▸ The drop-out rate of patients enrolled in the trials included was similar. Only five trials correctly reported the drop-out rate, and the evaluation of data after drop-out (13, 38, 39, 42, 44). The remaining studies included in the meta-analysis neither reported the drop-out rate nor gave information about the evaluation of patient drop-out.

Selective reporting (reporting bias) ▸ The primary endpoints described in the aims were reported in the results section of each study. Several studies included secondary endpoints, which, however, were not always completely reported in the results and discussion sections.

Other potential sources of bias ▸ None of the trials reported a calculation of the sample size in the study design.

Synthesis of results

Considering the lack of a unique, validated pharmacodynamics marker of FSH administration in men, the major endpoint of FSH treatment efficacy in male infertility is pregnancy rate. Among the 15 studies included, 12 trials considered pregnancy rate after FSH administration to the male partner, for a total of 482 infertile men treated, compared to 393 control men (12, 13, 14, 16, 35, 36, 37, 39, 42, 43, 44, 45) (Fig. 3A). First of all, baseline FSH levels were generally within or slightly above the normal range. There were no significant differences in basal FSH serum levels between treated and not-treated men (6.89+3.48 vs 6.27+3.69 mIU/ml, $P=0.052$). On the contrary, sperm concentration at baseline was significantly higher in the group of men not treated with FSH, compared to those treated (22.56+27.91 vs 11.40+19.44×106/ml, $P<0.001$), confirming a lack of randomization.

A significant improvement in pregnancy rate was found in treated men. The overall OR was 2.09 with CI 1.46–3.01 ($P<0.001$) (Fig. 3A). χ^2 was 10.09 and I^2 statistics 0% (Fig. 3A). The I^2 statistics showed a low degree of inconsistency of this result. Significant improvement in pregnancy rate remained dividing the analysis in randomized (OR 1.55, CI 1.0–2.4, $P=0.05$), and not-RCTs (OR 3.96, CI 1.87–8.37, $P<0.001$).

Assessing pregnancy rate, we further performed two subgroup meta-analyses, keeping spontaneous and ART pregnancies seperate. The first subgroup analysis included

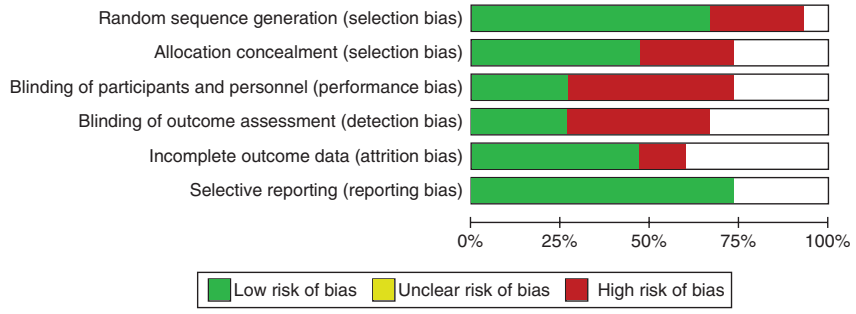

Figure 2
Risk of bias graph: the authors' judgement about each risk of bias item is presented as percentages across all included studies.

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nine studies in which spontaneous pregnancy rate was reported or evaluated as the main outcome (12, 13, 16, 37, 39, 42, 43, 44, 45). A total of 384 men were treated, compared to 308 controls during the study protocol. The pregnancy rate improved significantly after FSH treatment, with an OR of about 4.5, with a CI 2.17–9.33 ($P<0.001$) (Fig. 3B). χ^2 was 2.29 and I^2 statistics 0% (Fig. 3B). This increased pregnancy rate was confirmed both in five RCTs (OR 5.15, CI 2.01–13.15, $P<0.001$), and in four not-RCTs (OR 3.70, CI 1.17–11.73, $P=0.003$), suggesting that the lack of randomization did not impair the result. The second subgroup meta-analysis considered eight studies evaluating pregnancy rate after ART, independently of the ART methods applied (12, 13, 14, 36, 37, 39, 42, 44). A total of 322 men were treated, compared to 275 controls. Pregnancy rate significantly improved after FSH treatment, with an OR of about 1.60 and CI 1.08–2.37 ($P=0.002$) (Fig. 3C). χ^2 was 12.22 and I^2 statistics 43% (Fig. 3C). Pregnancy rate improved in not-RCTs (OR 1.57, CI 1.04–2.37, $P=0.03$), whereas it did not change in RCTs. This lack of a significant increase in randomized trials is probably due to the very low number (only one) of RCTs included.

NNT calculation indicated that ten men should be treated to achieve one spontaneous pregnancy and 18 men to achieve pregnancy after ART.

Considering FSH dosages, regimens and formulations, the studies included in the meta-analytic process were heterogeneous. Table 1 shows the FSH formulation used in each study and the cumulative FSH dose. The mean duration of FSH administration was 11.77 ± 2.59 weeks and the mean cumulative FSH dose used was 7168.75 ± 4815.47 IU. No linear correlation between mean duration of FSH administration and pregnancy rate was found ($P=0.581$). Similarly, cumulative FSH dose did not significantly correlate with pregnancy rate ($P=0.076$). We subdivided the analysis into two sub-analyses, considering studies using recombinant (Fig. 3D) and purified FSH (Fig. 3E), respectively. The significant increase of pregnancy rate was confirmed, independently of the FSH preparation chosen ($P=0.007$, OR 3.49 and $P=0.002$, OR 7.11 for recombinant and purified FSH, respectively).

Another meta-analysis was performed in order to assess the overall effect of FSH administration on semen parameters, evaluated by light microscopy. We considered 11 studies reporting sperm concentration after FSH treatment (13, 14, 16, 37, 38, 39, 40, 41, 42, 44, 45). A total of 520 men were treated, compared to 427 controls. The meta-analysis showed a significant improvement of sperm concentration after FSH administration, with a mean improvement of 2.66×10^6/ml (CI 0.47–4.84) ($P=0.02$) (Fig. 4A). χ^2 was 206.08 and I^2 statistics 95% (Fig. 4A). Furthermore, we analyzed six studies which considered progressive sperm motility (13, 37, 38, 41, 44, 45). A total of 332 men were treated, compared to 297 controls. The meta-analysis showed a non-significant improvement of progressive sperm motility after FSH and the mean improvement was 1.22×10^6/ml (CI -0.07 to 2.52) ($P=0.06$) (Fig. 4B). χ^2 was 1851.9 and I^2 statistics 100% (Fig. 4B).

Three trials reported testicular volume (13, 37, 45). A total of 127 FSH-treated men and 76 controls were compared. The results showed an increase of testicular volume in FSH-treated men, but this increase was not statistically significant ($P=0.14$). The mean increase was 1.35 ml, with CI -0.44 to 3.14. χ^2 was 2.68 and I^2 statistics 25%. Considering the two studies in which testicular volume was evaluated by ultrasonography, this parameter did not change after FSH treatment ($P=0.44$) (13, 45). In particular, Selice *et al.* (45) gave neither any information about the mathematical formula used for testicular volume calculation by ultrasonography, nor found any significant variation in this parameter after treatment. In contrast, Kamischke *et al.* (13) reported the method applied for testicular volume calculation and found a significant increase after FSH treatment. These discrepancies do not demonstrate a clear FSH effect on testicular size.

Additional analysis

Meta-regression analysis showed no significant correlation between pregnancy rate and sperm concentration, progressive motility and testicular volume ($P=0.502$, $P=0.175$ and $P=0.854$, respectively). Sperm concentration correlations were not found with progressive motility, nor with testicular volume ($P=0.925$ and $P=0.203$, respectively).

The visual evaluation of funnel plots did not reveal publication bias in the studies considered.

Discussion

Here we confirm the beneficial effect of FSH administration to the male partner of couples with idiopathic infertility in terms of pregnancy rate improvement, either spontaneously or after ART. This finding extends that recently obtained by the Cochrane Collaboration which was limited to spontaneous pregnancy (15). Some features

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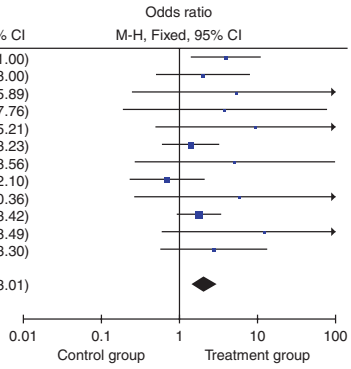
A

Reference	FSH treatment Events	Total	Control group Events	Total	Weight (%)	Odds ratio M-H, Fixed, 95% CI
(12)	18	39	7	39	9.0	3.92 (1.40, 11.00)
(14)	8	24	4	20	6.9	2.00 (0.50, 8.00)
(35)	2	31	0	31	1.1	5.34 (0.25, 115.89)
(36)	3	40	0	20	1.4	3.83 (0.19, 77.76)
(37)	7	23	0	10	1.1	9.55 (0.49, 185.21)
(39)	16	65	12	63	21.9	1.39 (0.60, 3.23)
(42)	4	57	0	30	1.4	5.13 (0.27, 98.56)
(13)	8	31	10	30	17.9	0.70 (0.23, 2.10)
(43)	2	19	0	20	1.0	5.86 (0.26, 130.36)
(44)	36	68	31	80	31.9	1.78 (0.92, 3.42)
(16)	4	15	0	15	0.9	12.13 (0.59, 248.49)
(45)	10	70	2	35	5.4	2.75 (0.57, 13.30)
Total (95% CI)		482		393	100.0	2.09 (1.46, 3.01)
Total events	118		66			

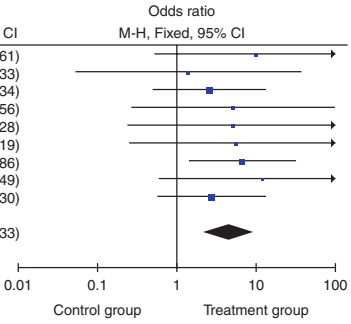
Heterogeneity: χ^2=10.09, df=11 (P=0.52); I^2=0%
Test for overall effect: Z=3.99 (P<0.0001)



B

Reference	FSH treatment Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, Fixed, 95% CI
(12)	4	39	0	39	5.0	10.01 (0.52, 192.61)
(37)	1	23	0	10	7.3	1.40 (0.05, 37.33)
(39)	6	62	2	50	22.7	2.57 (0.50, 13.34)
(42)	4	57	0	30	6.8	5.13 (0.27, 98.56)
(13)	2	31	0	30	5.3	5.17 (0.24, 112.28)
(43)	2	19	0	19	5.0	5.57 0.25, 124.19)
(44)	10	68	2	80	17.8	6.72 (1.42, 31.86)
(16)	4	15	0	15	4.1	12.13 (0.59, 248.49)
(45)	10	70	2	35	26.0	2.75 (0.57, 13.30)
Total (95% CI)		384		308	100.0	4.50 (2.17, 9.33)
Total events	43		6			

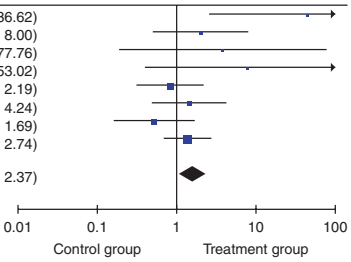
Heterogeneity: χ^2=2.29, df=8 (P=0.97); I^2=0%
Test for overall effect: Z=4.04 (P<0.0001)



C

Reference	FSH treatment Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, Fixed, 95% CI
(12)	14	39	0	39	0.8	44.92 (2.57, 786.62)
(14)	8	24	4	20	7.2	2.00 (0.50, 8.00)
(36)	3	40	0	20	1.5	3.83 (0.19, 77.76)
(37)	6	23	0	10	1.2	7.80 (0.40, 153.02)
(39)	10	56	10	48	22.0	0.83 (0.31, 2.19)
(42)	14	53	6	30	14.0	1.44 (0.49, 4.24)
(13)	6	29	10	30	19.4	0.52 (0.16, 1.69)
(44)	26	58	29	78	33.9	1.37 (0.69, 2.74)
Total (95% CI)		322		275	100.0	1.60 (1.08, 2.37)
Total events	87		59			

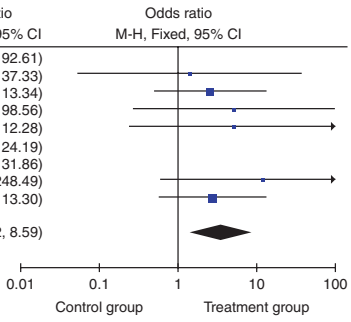
Heterogeneity: χ^2=12.22, df=7 (P=0.09); I^2=43%
Test for overall effect: Z=2.36 (P=0.02)



D

Reference	FSH treatment Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, Fixed, 95% CI
(12)	4	39	0	39	0.0	10.01 (0.52, 192.61)
(37)	1	23	0	10	10.1	1.40 (0.05, 37.33)
(39)	6	62	2	50	31.5	2.57 (0.50, 13.34)
(42)	4	57	0	30	9.5	5.13 (0.27, 98.56)
(13)	2	31	0	30	7.4	5.17 (0.24, 112.28)
(43)	2	19	0	19	0.0	5.57 (0.25, 124.19)
(44)	10	68	2	80	0.0	6.72 (1.42, 31.86)
(16)	4	15	0	15	5.7	12.13 (0.59, 248.49)
(45)	10	70	2	35	36.0	2.75 (0.57, 13.30)
Total (95% CI)		258		170	100.0	3.49 (1.42, 8.59)
Total events	27		4			

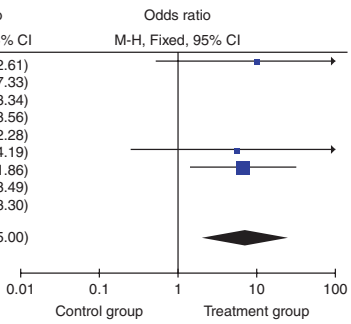
Heterogeneity: χ^2=1.30, df=5 (P=0.94); I^2=0%
Test for overall effect: Z=2.72 (P=0.007)



E

Reference	FSH treatment Events	Total	Control Events	Total	Weight (%)	Odds ratio M-H, Fixed, 95% CI
(12)	4	39	0	39	18.1	10.01 (0.52, 192.61)
(37)	1	23	0	10	0.0	1.40 (0.05, 37.33)
(39)	6	62	2	50	0.0	2.57 (0.50, 13.34)
(42)	4	57	0	30	0.0	5.13 (0.27, 98.56)
(13)	2	31	0	30	0.0	5.17 (0.24, 112.28)
(43)	2	19	0	19	17.9	5.57 (0.25, 124.19)
(44)	10	68	2	80	64.0	6.72 (1.42, 31.86)
(16)	4	15	0	15	0.0	12.13 (0.59, 248.49)
(45)	10	70	2	35	0.0	2.75 (0.57, 13.30)
Total (95% CI)		126		138	100.0	7.11 (2.02, 25.00)
Total events	16		2			

Heterogeneity: χ^2=0.08, df=2 (P=0.96); I^2=0%
Test for overall effect: Z=3.06 (P=0.002)



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of our meta-analysis are, however, different. In particular, we used broader inclusion criteria, due to the difficulty in performing RCTs in the field of infertility, demonstrated by the lack of randomized studies on FSH for male infertility published in the last decade. Furthermore, we analysed the treatment effect on pregnancy rate while distinguishing between the FSH formulations used. Finally, we estimated the overall effect of FSH treatment on other outcomes, i.e sperm concentration, progressive sperm motility and testicular volume.

Considering pregnancy rate, this meta-analysis confirms the beneficial effect of FSH administration to the male partner of infertile couples, demonstrating an improvement in spontaneous pregnancies (15). Furthermore, we demonstrate, for the first time, that FSH administration to the male partner is able to increase pregnancy rate after ART, independent of the ART methodology. This improvement in pregnancy rate remains even when recombinant or purified FSH are considered separately, suggesting that the two FSH preparations have a similar efficacy (Fig. 3D and E). Similarly, an improved pregnancy rate was confirmed when randomized and not-RCTs were considered separately, suggesting that lack of randomization does not impair the quality of the results. However, these results should be viewed in light of the broad inclusion criteria and high risk of bias. With this in mind, pregnancy rate increases in men treated with FSH, even if the OR is low, and additional analysis suggests that 10–18 men should be treated to achieve one additional pregnancy. This might sound marginal, but considering the costs of ART and the woman's burden for the treatment of male infertility versus the cost of FSH treatment (irrespective of recombinant or extractive), this option should be given a trial.

The best tool for the evaluation of male fertility in clinical practice remains semen analysis. However, its interpretation is difficult, because of the inherent variability of the parameters and lack of clear-cut threshold values (47, 48). Several studies demonstrated a beneficial effect of FSH on the quality of spermatozoa (9, 14, 49), and an algorithm was proposed to improve the interpretation of semen analysis (49). Our results demonstrate an improvement of sperm concentration after treatment with FSH (Fig. 4A and B). However, a high degree of heterogeneity was evident (I^2 of 95%) and progressive sperm motility did not significantly improve after treatment. We interpret this difference as a sign of methodological inconsistency in semen analysis itself (48), as well as a heterogeneity of the inclusion criteria of the patients.

Even if conventional semen analysis does not provide accurate information about the spermatozoa's ability to fertilize the egg, this meta-analysis demonstrates that FSH administration to infertile men improves sperm concentration and increases pregnancy rate. However, no correlation between sperm parameters and pregnancy rate was found. Evaluating the baseline characteristics of patients enrolled, we found that basal FSH serum levels were not different between study and control groups. Men not treated with FSH have higher baseline sperm concentration compared to treated men, reflecting the fact that some of the included studies were not randomized. Considering these limits, FSH administration increases sperm concentration irrespective of the basal FSH serum levels and absolute sperm number. This result suggests that basal FSH serum levels and sperm concentration do not represent a useful marker in the decision making process of whether to treat or not. Sperm morphology is a weak parameter, considering the spontaneous variability over the years, as evident from the different thresholds proposed by the various editions of the WHO manual to define normal morphology. Considering that studies included in the meta-analysis were conducted using different WHO manuals, it is not possible to compare this parameter with a statistical approach. Finally, other parameters, such as sperm DNA fragmentation, could be useful in the future to direct the decision-making process of whether or not to treat infertile men (38).

In animal models, FSH administration stimulates spermatogenesis and increases Sertoli cell secretions (50). In monkeys, the proliferation of spermatogenetic cells, together with Sertoli cells secretions, results in a testicular volume increase after 6 weeks of FSH administration (50). Some studies in men reported a testicular enlargement caused by a FSH-secreting pituitary adenoma (51) followed by a significant volume reduction after normalization of FSH levels (51). Since Sertoli cell number is constant after

Figure 3

Pregnancy rate. (A) Forest plot of 15 studies evaluating pregnancy rate after FSH administration. (B) Forest plot of nine studies evaluating spontaneous pregnancy rate after FSH administration. (C) Forest plot of eight studies evaluating pregnancy rate during ART, after FSH administration. The results are divided according to the use of recombinant (D) or purified (E) FSH. The diamond indicates the overall summary estimate for the analysis (width of the diamond represents the 95% CI); boxes indicate the weight of individual studies in the pooled analysis.

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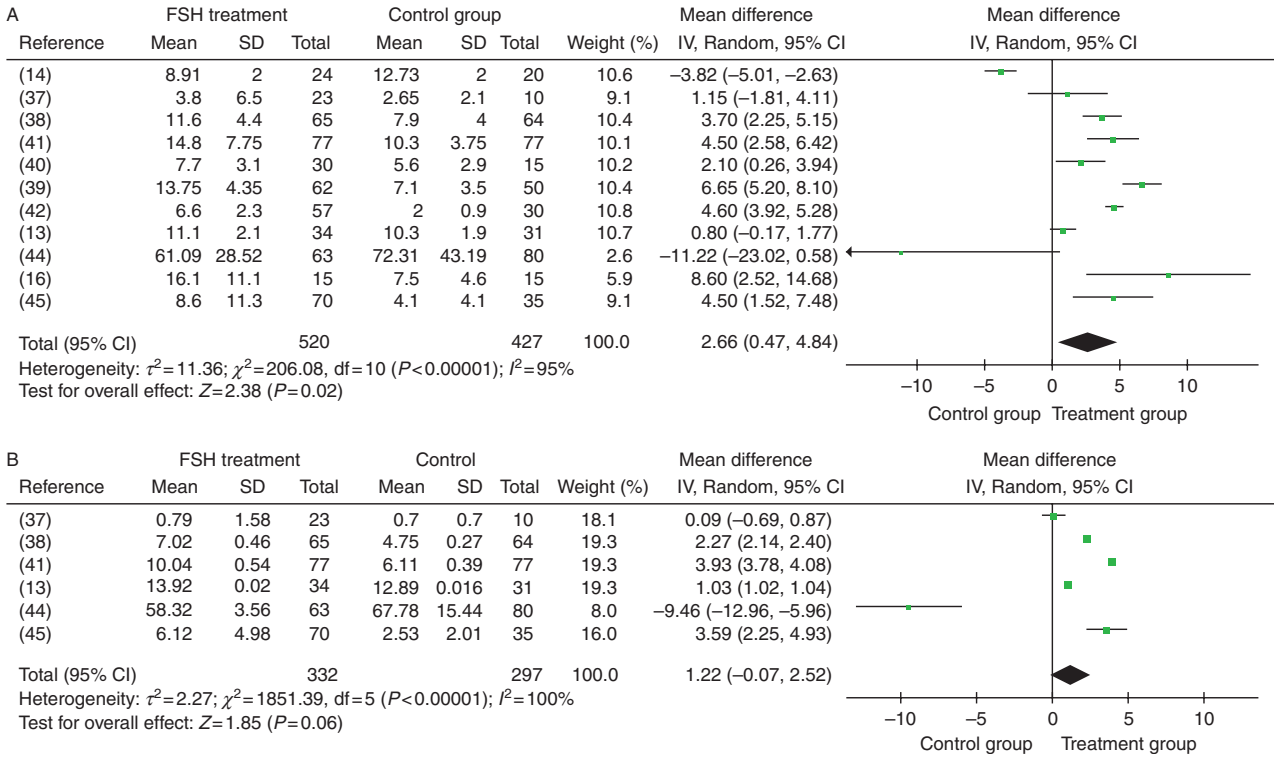

Figure 4

(A) Forest plot of 11 studies evaluating sperm concentration after FSH administration. (B) Forest plot of six studies evaluating progressive sperm motility after FSH administration. The diamond indicates the overall summary estimate for the analysis (width of the diamond represents the 95% CI); boxes indicate the weight of individual studies in the pooled analysis.

puberty, any increase in testicular volume should be primarily due to increased spermatogenesis (13). Therefore, FSH treatment of male infertility could increase testicular volume. This hypothesis was suggested by some clinical trials (13, 32, 37). However, our meta-analysis did not find any significant improvement after FSH treatment. This result is limited by the high heterogeneity of the studies included in this subgroup of the meta-analysis ($I^2=93\%$), possibly related to the inconsistency of methods used for testicular volume measurement (52). Testicular volume calculation by ultrasonography is more accurate and precise than comparative evaluation performed by orchidometry (53). However, several mathematical formula for testicular volume are proposed in the literature, but only one study included in our meta-analysis specified the methodology used (13). In our meta-analysis the lack of testicular volume increase after FSH treatment remains when considering the two studies in which ultrasonography was performed. Thus, the FSH-induced improvement of sperm concentration without a significant increase in testicular size remains intriguing.

In conclusion, FSH administration to infertile men improves pregnancy rate, even if the OR is low and 10–18 men need to be treated to achieve one additional pregnancy. However, treated men were very heterogeneous and the usefulness of FSH treatment probably depends on the precise definition of the baseline condition and selection criteria of the infertile man. The studies included in the meta-analysis are limited by: i) empirical unstandardized FSH treatment use; ii) heterogeneity of the infertile men enrolled; iii) heterogeneity of the studies included, possibly related to an unknown female factor, as suggested by the relative high risk of bias (Fig. 2); iv) different lengths of treatment and follow-up phases used. Our results show the impossibility of defining a basal FSH serum level or basal sperm concentration which could distinguish responders from non-responders. Other predictors of response to FSH treatment should be identified, considering the relevance in clinical practice of distinguishing those men who will respond to FSH treatment from those who will not. A pharmacogenetic approach was suggested (54) and

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could help the decision-making process while new fertility-related markers need to be identified to quantify the efficacy of FSH treatment. New markers, such as sperm DNA fragmentation (38), could help clinicians in the decision-making process, both as a prediction marker and pharmacodynamics parameter of FSH treatment. However, this parameter requires further evaluation.

Conclusions

FSH administration to the male is sometimes used for the empirical treatment of infertile couples. However, its efficacy remains unclear. The results of the clinical trials available in the literature, considered together in this meta-analysis, indicate an improvement of pregnancy rate after FSH administration to the male partner of infertile couples, both spontaneously or after ART. Furthermore, this meta-analysis suggests an improvement of sperm concentration after FSH administration, without a testicular size increase. However, a standardized FSH treatment protocol of male idiopathic infertility does not exist. Since a specific predictor of response to FSH administration is not available, the use of FSH in men with infertility should be judicious. Future clinical trials should be designed to define who will and who will not respond to FSH treatment.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement
All three authors participated in the literature search, analysis, and discussion of the results of the meta-analysis, as well as writing the manuscript.

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