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ORIGINAL ARTICLE

Male Infertility

FSH dosage effect on conventional sperm parameters: a meta-analysis of randomized controlled studies

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Follicle-stimulating hormone (FSH) represents a therapeutic option in normogonadotropic patients with idiopathic oligozoospermia. The aim of this review was to evaluate the possible dose- and drug-dependent efficacy of FSH treatment on conventional sperm parameters. We performed a comprehensive systematic review via a meta-analysis of all available randomized controlled trials, in which FSH administration was compared with placebo or no treatment when administered to normogonadotropic patients with idiopathic oligozoospermia. Of the 971 articles that were retrieved, 5 were finally included, including a total of 372 patients and 294 controls. Overall, FSH treatment was effective in ameliorating the sperm concentration, total count, progressive motility, but not normal forms. On the basis of the weekly dosage, the studies were classified into those using low (175–262.5 IU per week), intermediate (350–525 IU per week), and high (700–1050 IU per week) doses. At low doses, FSH improved only sperm motility. At intermediate doses, FSH ameliorated sperm concentration and morphology. Total sperm count and progressive motility showed a trend toward the increase. At high doses, FSH increased sperm concentration, total sperm count, and progressive motility. Sperm morphology showed a trend toward the increase. Finally, both highly purified FSH (hpFSH) and recombinant human FSH (rhFSH) improved sperm concentration, total sperm count, progressive motility, but not morphology. No different efficacy was observed between these two preparations. This meta-analysis provides evidence in favor of high FSH doses. The FSH efficacy was not related to the preparation type (recombinant *vs* highly purified). Further studies are needed to evaluate the effectiveness of long-standing treatment regimes.

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INTRODUCTION

Infertility, defined as the inability to achieve conception after 1–2 years of unprotected sexual intercourse,[1] affects approximately 15% of couples of childbearing age in industrialized countries.[2] A male factor may be solely responsible for approximately 30% of cases; overall, male factors contribute to approximately 50% of all cases.[3]

Male infertility is characterized by abnormal conventional sperm parameters (concentration, motility, and morphology), resulting in oligozoospermia (sperm count $<15 \times 10^6$ ml^{-1} and total sperm count $<39 \times 10^6$ per ejaculate), asthenozoospermia (progressive sperm motility <32% and total motility <40%), and/or teratozoospermia (normal forms <4%), although biofunctional sperm parameters (mitochondrial function, chromatin compactness, sperm apoptosis, and DNA fragmentation) may also be affected.[3]

Medical treatment covers hormonal and nonhormonal options, with the latter including many different compounds with antioxidant and prokinetic properties, supported by variable degrees of evidence of clinical efficacy.[4] Among the hormonal therapeutic strategies, follicle-stimulating hormone (FSH), given its relevant role in spermatogenesis, is prescribed in oligozoospermic patients with gonadotropin serum levels within the normal range.[3] FSH serum concentrations are considered predictive of responsiveness to FSH treatment: the lower they are the higher is the probability of a positive response.[3] A definitive cutoff value has not been specified.[5] Previous studies considered 12 mUI ml^{-1}.[6–8] However, recent Italian studies included patients with FSH serum concentration <8 mUI ml^{-1},[9–11] mainly due to the Italian legislation, which restricts FSH administration to patients having FSH serum levels lower than 8 IU ml^{-1}.

Various FSH preparations are available. Indeed, FSH can be extracted and purified from the urine of postmenopausal women (so-called highly-purified FSH [hpFSH])[12] or synthesized using recombinant *in vitro* technology (rhFSH).[13]

Although several studies reported FSH effectiveness in increasing sperm count, a portion of oligozoospermic patients are unresponsive to this treatment.[3] Indeed, it is thought that the treatment should be given to the selected patients.[3] Therefore, effort has been made to identify predictors of the efficacy of FSH therapy, such as inhibin B serum levels[3] as well as FSHβ or FSH receptor polymorphisms.[14,15] However, the possible dose-dependent and type of FSH preparation effectiveness of FSH treatment is not known.

Several different therapeutic schemes and dosages have been used in oligozoospermic patients (**Supplementary Table 1**). Notably, in

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some countries, legislation restricts the therapeutic choice in terms of dosage and duration, and it cannot be excluded that unresponsiveness, at least in some patients, is due to insufficient hormonal stimulation. To the best of our knowledge, no conclusive study investigating the possible efficacy of the dosage and FSH preparation type on conventional sperm parameters has been conducted. Therefore, this study aimed to meta-analyze all available data as a means of evaluating the effects of different therapeutic schemes of FSH treatment on sperm concentration, total sperm count, sperm motility, and morphology in normogonadotropic patients with idiopathic oligozoospermia.

MATERIALS AND METHODS

Sources

This study was performed using the Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines.[16] The data were independently extracted by AEC and RC. A systematic search was performed through the PubMed, MEDLINE, Cochrane, Academic One Files, Google Scholar, and Scopus databases from the inception of each database to November 30, 2018, using Medical Subjects Headings (MeSH) indexes and keyword searches.

The search strategy used combined MeSH terms and keywords and was based on the following keywords: FSH therapy, FSH treatment, follicle-stimulating hormone, oligozoospermia, oligoasthenozoospermia, oligoasthenoteratozoospermia, male infertility, semen, sperm, and spermatozoa. Additional manual searches were conducted using the reference lists of relevant studies. No language restriction was applied in any literature search. The authors of individual studies were contacted for missing data.

Study selection

Information on the year of publication, country, continent, study design, and mean age of patients was collected. Studies that met the following inclusion criteria were included in the meta-analysis:

1. Design: randomized controlled trials
2. Studies reporting conventional sperm parameters evaluated after at least 3 months of FSH administration to normogonadotropic (FSH <12 mIU ml^{-1}) patients with idiopathic infertility
3. Studies in which semen samples were analyzed according to the World Health Organization (WHO) criteria
4. No major comorbidities present in patients and controls, including all known causes of male infertility such as male accessory gland infection; varicocele; hypogonadism; Y chromosome microdeletions; testicular torsion or trauma; history of cryptorchidism; thyroid, pituitary, or adrenal disorders; or liver or kidney failure.

Studies that did not meet these criteria were excluded from the analysis. Placebo administration in the control group was not an inclusion criterion. The selection criteria (Population, Intervention, Comparison, Outcome – PICO) are shown in **Supplementary Table 2**. The quality assessment of the studies included in the present meta-analysis was performed using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) system (**Table 1**).[17]

The mean difference (MD) or the standardized mean difference (SMD) for continuous variables was used for data pooling. Meta-analysis was conducted to evaluate whether MD and standard deviation (s.d.) of sperm concentration and total sperm count and SMD and s.d. of sperm motility and morphology differed in patients treated with low, intermediate, or high FSH doses compared with controls. In addition, we evaluated whether these outcomes differed in patients treated with hpFSH or rhFSH and the respective control groups. For each outcome, the 95% confidence interval (CI) was calculated. The Cochran-*Q* and I^2 statistics were used in the assessment of statistical heterogeneity. Specifically, statistical heterogeneity was tested using the Chi-square test. If $I^2 \leq 50\%$, the variation of the studies was considered to be homogenous, and the fixed effects model was adopted. If $I^2 > 50\%$, there was a significant heterogeneity between studies, and the random effects model was used. The analysis was performed using RevMan software version 5.3 (Cochrane Collaboration, Oxford, UK). All $P < 0.05$ were considered statistically significant.

RESULTS

Using the above-mentioned search strategy, 971 articles were retrieved. After the exclusion of duplicate records, 422 articles were screened. Of these, 285 were judged not pertinent upon reading their abstracts. The remaining 137 full texts were carefully read, and 27 were assessed for eligibility. Of the latter, 22 studies were excluded. The reasons for the exclusion of individual studies are presented in **Supplementary Table 3**. Finally, 5 articles[18–22] met our inclusion criteria and were included in the analysis, resulting in a total of 372 patients and 294 controls (**Figure 1**). Baseline FSH serum levels are reported in **Table 1**. The analysis was performed on conventional sperm parameters assessed after 3 months of treatment.

On the basis of the weekly dosage administered, the studies were classified into three groups: those using a low dose (175–262.5 IU per week; therapeutic schemes: 50 IU on alternate days[19,22] and 75 IU on alternate days[18]); those using an intermediate dose (350–525 IU per week; therapeutic schemes: 100 IU on alternate days[19,20,22] and 150 IU on alternate days[21]); and those using a high dose (700–1050 IU per week; therapeutic schemes: 200 IU on alternate days[22] and 300 IU on alternate days[22]).

Overall, FSH treatment was effective in ameliorating the sperm concentration (MD: 4.53×10^6 ml^{-1}, 95% CI: [2.14–6.92] $\times 10^6$ ml^{-1}; $P < 0.01$; **Figure 2a**), total sperm count (MD: 10.74×10^6 per ejaculate, 95% CI: [4.40–17.07] $\times 10^6$ per ejaculate; $P < 0.01$; **Figure 2b**), progressive sperm motility (SMD: 0.36, 95% CI: 0.19–0.53; $P < 0.01$; **Figure 3a**), but not sperm morphology (SMD: 0.38, 95% CI: −0.08–0.83; $P = 0.11$; **Figure 3b**).

Analysis of dose-dependent effects of FSH on the conventional sperm parameters

At low doses, FSH did not improve sperm concentration (MD: 1.88×10^6 ml^{-1}, 95% CI: [−0.64–4.40] $\times 10^6$ ml^{-1}; $P = 0.14$; **Figure 2a**), total sperm count

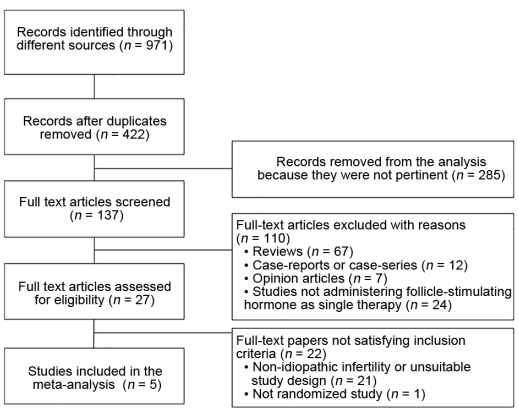

Figure 1: Flowchart of the studies included in the meta-analysis. We identified 971 papers, 27 of which were assessed for eligibility. Based on the inclusion criteria of the present study, five studies were finally included in this meta-analysis.

Table 1: Summary of the studies included and their quality assessment

Source	*Study design*	*Number of patients/ controls*	*Intervention(s)*	*Treatment of the control group*	*FSH serum levels before therapy (mIU ml^{-1}), mean±s.d.*	*Outcomes*	*Grade*
Colacurci *et al*. 2012[21]	RCT	65/64	rhFSH 100 IU on alternate days	Nonantioxidant vitamin supplements	5.9±1.3	Sperm concentration and count, sperm forward motility, sperm morphology	Moderate
Ding *et al*. 2015[22]	RCT	272/82	hpFSH 50 IU on alternate days; hpFSH 100 IU on alternate days; hpFSH 200 IU on alternate days; hpFSH 300 IU on alternate days	Placebo	4.8±1.9	Sperm concentration and count, sperm forward motility, sperm morphology	High
Foresta *et al*. 1998[18]	RCT	60/30	hpFSH 75 IU on alternate days	Placebo	3.4±1.1	Sperm concentration and count, sperm forward motility, sperm morphology	High
Foresta *et al*. 2002[19]	RCT	30/15	rhFSH 50 IU on alternate days; rhFSH 100 IU on alternate days	No treatment	4.1±2.2	Sperm concentration, sperm forward motility, sperm morphology	High
Foresta *et al*. 2005[20]	RCT	62/50	rhFSH 100 IU on alternate days	No treatment	4.6±1.2	Sperm concentration	High

FSH: follicle-stimulating hormone; GRADE: Grading of Recommendations, Assessment, Development and Evaluation; hpFSH: highly purified FSH; RCT: randomized controlled trial; rhFSH: recombinant FSH; s.d.: standard deviation

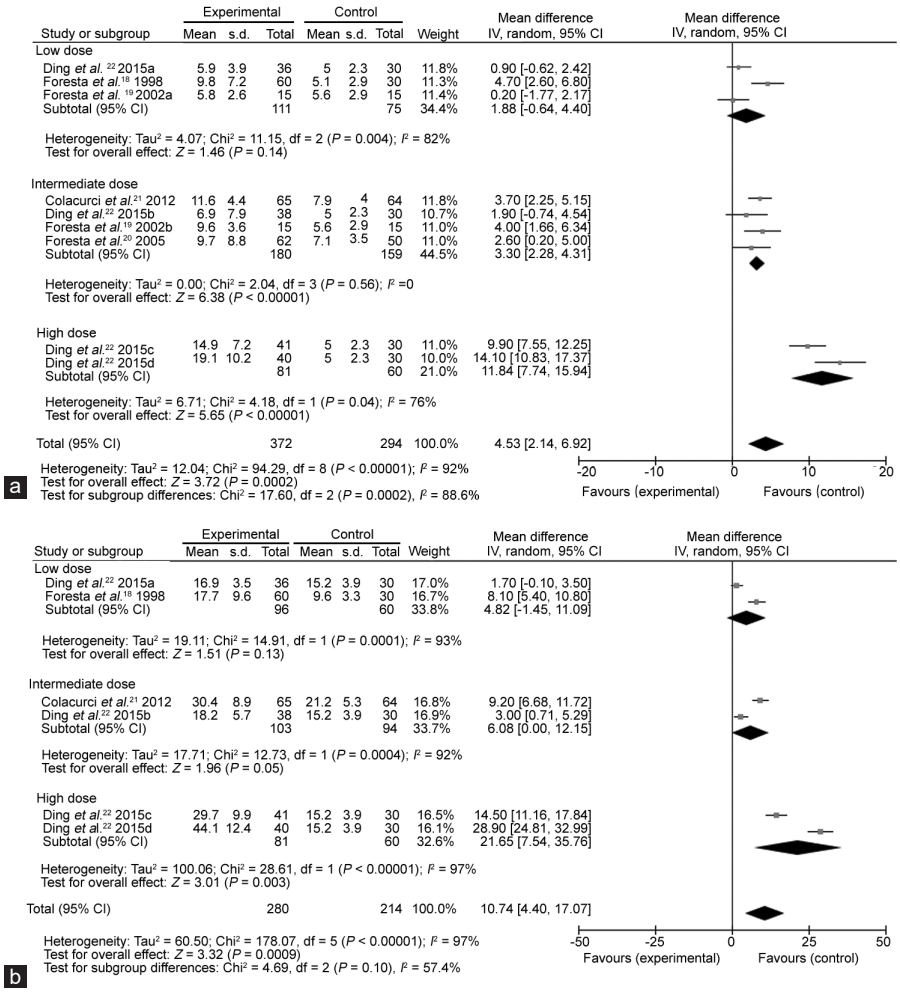

Figure 2: Dose-dependent effect of FSH therapy on sperm concentration and total count. Treatment with FSH improved the (**a**) sperm concentration and (**b**) total sperm count in men with idiopathic oligozoospermia compared with controls. (**a**) At low doses, FSH therapy did not ameliorate the sperm concentration; at intermediate and high doses, FSH administration increased the sperm concentration in a dose-dependent manner. (**b**) At low doses, FSH did not improve the total sperm count; at intermediate doses, the total sperm count showed a trend toward the increase; and at high doses, FSH ameliorated the total sperm count. FSH: follicle-stimulating hormone; CI: confidence interval; s.d.: standard deviation; a–d: study subgroups; IV: Inverse Variance methods; df: degree of freedom.

(MD: 4.82×10^6 per ejaculate, 95% CI: $[-1.45–11.09] \times 10^6$ per ejaculate; $P = 0.13$; **Figure 2b**), sperm morphology (SMD: 0.34, 95% CI: −1.21–1.88; $P = 0.67$; **Figure 3b**), but ameliorated progressive sperm motility (SMD: 0.43, 95% CI: 0.13–0.73; $P < 0.01$; **Figure 3a**).

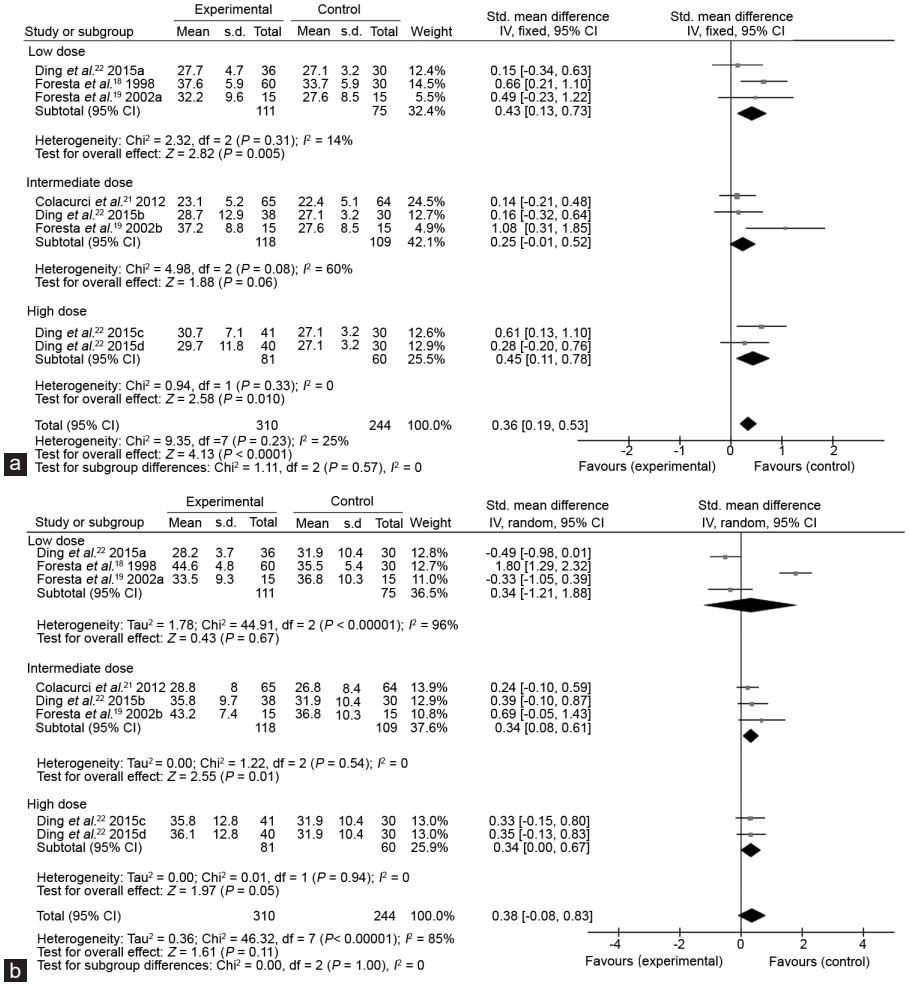

Figure 3: Dose-dependent effect of FSH therapy on progressive sperm motility and morphology. Treatment with FSH improved (**a**) the progressive sperm motility but not (**b**) the sperm morphology in men with idiopathic oligozoospermia compared with controls. (**a**) At low and high doses, FSH therapy ameliorated the progressive sperm motility; at intermediate doses, FSH administration did not increase this parameter. (**b**) At low doses, FSH did not improve the sperm morphology; at intermediate doses, it was ameliorated; and at high doses, the sperm morphology showed a trend toward the increase. FSH: follicle-stimulating hormone; CI: confidence interval; s.d.: standard deviation; std: standard; a–d: study subgroups; IV: Inverse Variance methods; df: degree of freedom.

At intermediate doses, FSH improved sperm concentration (MD: 3.30×10^6 ml^{-1}, 95% CI: [2.28–4.31] $\times 10^6$ ml^{-1}; $P < 0.01$; **Figure 2a**) and morphology (SMD: 0.34, 95% CI: 0.08–0.61; $P = 0.01$; **Figure 3b**). The total sperm count (MD: 6.08×10^6 per ejaculate, 95% CI: [0–12.15] $\times 10^6$ per ejaculate; $P = 0.05$; **Figure 2b**) and the progressive sperm motility (SMD: 0.25, 95% CI: −0.01–0.52; $P = 0.06$; **Figure 3a**) showed a trend toward the increase.

At high doses, FSH ameliorated the sperm concentration (MD: 11.84×10^6 ml^{-1}, 95% CI: [7.74–15.94] $\times 10^6$ ml^{-1}; $P < 0.01$; **Figure 2a**), total sperm count (MD: 21.65×10^6 per ejaculate, 95% CI: [7.54–35.76] $\times 10^6$ per ejaculate; $P < 0.01$; **Figure 2b**), and progressive sperm motility (SMD: 0.45, 95% CI: 0.11–0.78; $P = 0.01$; **Figure 3a**). Sperm morphology showed a trend toward an increase (SMD: 0.34, 95% CI: 0–0.67; $P = 0.05$; **Figure 3b**).

Figure 2 clearly shows the dose-dependent efficacy to the FSH therapy on the sperm concentration and the total sperm count. The higher the dose used the higher is the increase observed.

Analysis of FSH preparation effects on the conventional sperm parameters

hpFSH improved sperm concentration (MD: 5.71×10^6 ml^{-1}, 95% CI: [1.32–10.10] $\times 10^6$ ml^{-1}; $P = 0.01$; **Figure 4a**), total sperm count (MD: 11.09×10^6 per ejaculate; 95% CI: [3.30–18.88] $\times 10^6$ per ejaculate; $P < 0.01$; **Figure 4b**), progressive sperm motility (SMD: 0.38, 95% CI: 0.17–0.59; $P < 0.01$; **Figure 5a**), but not sperm morphology (SMD: 0.47, 95% CI: −0.23–1.17; $P = 0.18$; **Figure 5b**).

Similarly, rhFSH ameliorated the sperm concentration (MD: 2.63×10^6 ml^{-1}; 95% CI: [0.88–4.38] $\times 10^6$ ml^{-1}; $P < 0.01$; **Figure 4a**), total sperm count (MD: 9.20×10^6 per ejaculate, 95% CI: [6.68–11.72] $\times 10^6$ per ejaculate; $P < 0.001$; **Figure 4b**), progressive sperm motility (SMD: 0.32, 95% CI: 0.03–0.61; $P = 0.03$; **Figure 5a**), but not sperm morphology (SMD: 0.21, 95% CI: −0.25–0.67; $P = 0.38$; **Figure 5b**).

Owing to the lack of studies, the combined dose-dependent and FSH preparation-related efficacy on sperm conventional parameters could not be evaluated.

DISCUSSION

FSH is a dimeric glycoprotein that consists of an α and a β chain. Currently, it is administered to patients with hypogonadotropic hypogonadism as well as those with oligozoospermia and serum FSH within the normal range; it is also prescribed for controlled ovarian hyperstimulation protocols. It can be extracted from the purified urine

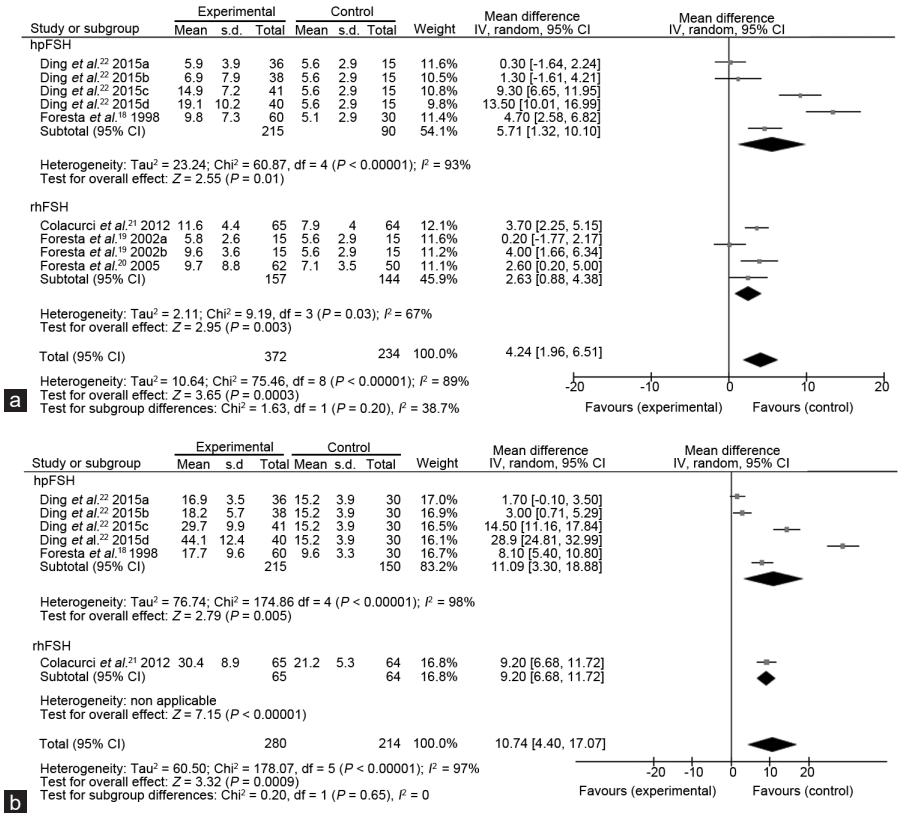

Figure 4: Effects of different FSH preparations on sperm concentration and total sperm count. Both hpFSH and rhFSH improved (**a**) the sperm concentration and (**b**) the total sperm count. FSH: follicle-stimulating hormone; hpFSH: highly purified FSH; rhFSH: recombinant human FSH; CI: confidence interval; s.d.: standard deviation; a–d: study subgroups; IV: Inverse Variance methods; df: degree of freedom.

of postmenopausal women (hpFSH) or synthetized using *in vitro* recombinant technology (rhFSH).[12,13]

The pubertal increase in FSH serum levels induces spermatogenesis in men. FSH receptors are expressed in Sertoli cells. From the molecular point of view, the activation of FSH receptors in mature and differentiated postpubertal Sertoli cells induces the secretion of glial cell-derived neurotrophic factor (GDNF) and fibroblast growth factor 2 (FGF2), both of which promote the self-renewal of stem cell niches and affect the production of inhibin A, which is involved in the proliferation of type A spermatogonia and spermatogenesis.[23-25] These effects represent the rational basis for prescribing FSH in oligozoospermic patients.

Several studies have reported improvement in conventional sperm parameters,[7,9,19–22,26–30] decreased sperm DNA fragmentation index,[21,31] and improved pregnancy rates[32] after FSH administration to patients with idiopathic infertility. Accordingly, the most recent Cochrane review on the use of gonadotropins for idiopathic male factor subfertility showed the effectiveness of FSH treatment on both pregnancy and live birth rates.[33] Despite this, the evidence also indicates that at least a portion of patients are poor FSH responders.[3] Therefore, different possible predictors of responsiveness to FSH treatment have been suggested, such as FSH and inhibin B serum concentrations, testicular histological features, and FSHβ and FSHR polymorphisms.[3] The Italian Society of Andrology and Sexual Medicine (SIAMS) recommends FSH administration to patients with FSH serum levels within the normal range.[29] In fact, studies indicate that the higher this value is the greater is the change of unresponsiveness.[29] Concerning testicular histology, the last SIAMS position statement[29] suggested avoiding FSH prescription to patients with maturation arrest at the spermatid level, because patients with this maturation arrest are generally poor FSH responders.[29] The results of the research investigating the role of inhibin B and FSHβ or FSHR polymorphisms as predictors of responsiveness to FSH treatment are, at the moment, inconclusive. Accordingly, FSHβ or FSHR polymorphisms are currently requested only for research purposes and no role for inhibin B has been recognized in the clinical practice before FSH prescription.[29]

Many different therapeutic schemes have been adopted over the years, including different weekly doses, molecules (hpFSH or rhFSH), and therapy duration. The possible role of the dose dependence and FSH preparation relationship in the unresponsiveness to FSH is not known. Some countries restrict the dose and duration of treatment, and, therefore, it is unclear whether the lack of response may be addressed to an insufficient stimulation. To the best of our knowledge, no conclusive study evaluating the impact of different FSH dosages and different types of FSH (hpFSH *vs* rhFSH) on conventional sperm parameters in patients with idiopathic oligozoospermia has been performed. We analyzed the possible dose-dependent and FSH preparation-related effectiveness on conventional sperm parameters after 3 months of therapy through a meta-analysis of randomized controlled trials.

The dose-dependent analysis provides evidence in favor of the high doses. This is supported by the dose-dependent amelioration of the sperm concentration and total sperm count. Indeed, the higher the doses were the greater was the increase reported by the studies evaluated. At high doses, FSH was effective in increasing both the sperm concentration and the total sperm count. At intermediate doses, only the sperm concentration ameliorated, and none of these parameters

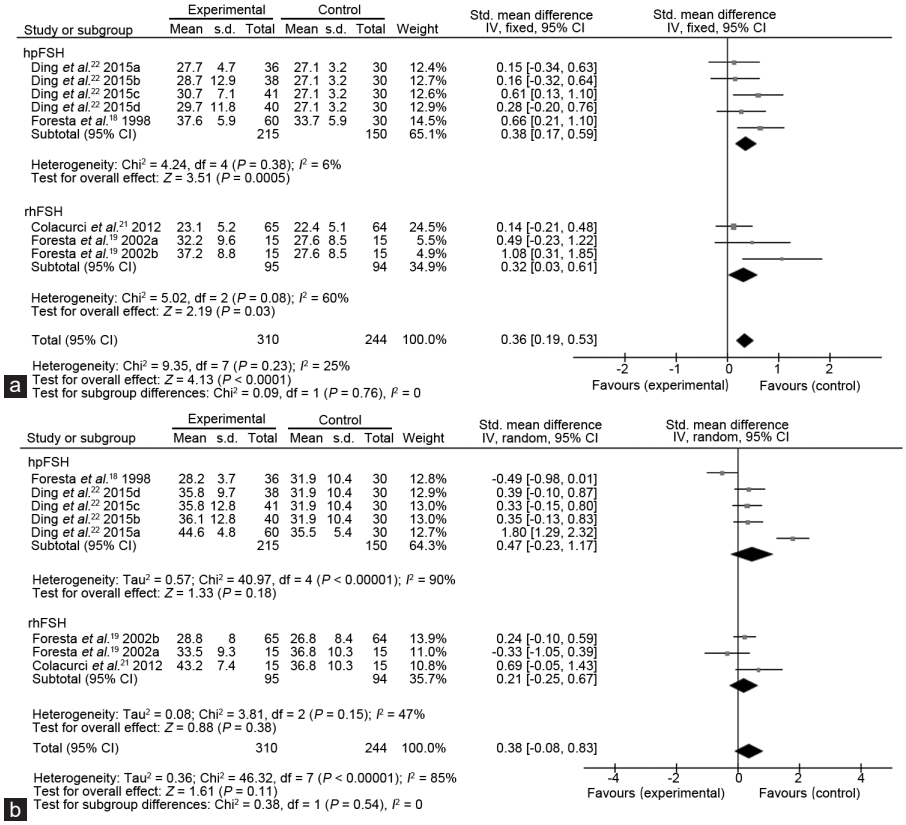

Figure 5: Effects of different FSH preparations on progressive sperm motility and morphology. (**a**) Both hpFSH and rhFSH increased the progressive sperm motility. (**b**) Neither of them improved the sperm morphology. FSH: follicle-stimulating hormone; hpFSH: highly purified FSH; rhFSH: recombinant human FSH; CI: confidence interval; s.d.: standard deviation; std: standard; a–d: study subgroups; IV: Inverse Variance methods; df: degree of freedom.

benefited from the low doses. Concerning the sperm quality, at high and low doses, FSH improved progressive sperm motility, but not morphology. On the contrary, at intermediate doses, it increased only the sperm morphology, but not progressive sperm motility. These results suggest that high doses could better stimulate Sertoli cells, which, as a consequence, provide a better nourishment for a greater number of spermatogonia, thus increasing the final sperm number. The high doses could lead to the improvement of sperm parameters also in poor FSH-responders by the enhanced stimulation, and, therefore, they may be preferred, as already suggested.[30] No side effects and manifestations of FSH overdose have been reported in none of the randomized controlled trial included in this study, as well as in all the studies administering FSH at high doses reported in literature. This fully confirms the safety of high FSH administration in male patients, in contrast to female ones, where ovarian hyperstimulation might occur. The reason why the high and low doses are effective in increasing the progressive sperm motility and the intermediate ones are not is not clear. Similarly, why only intermediate doses improve sperm morphology is not easily understandable. Despite our efforts in the study selection and the restrictive inclusion criteria, selection biases could not be excluded. In contrast with our findings, previous studies observed an improved sperm ultrastructure (meaning a higher percentage of morphologically normal spermatozoa) using transmission electron microscopy after FSH treatment administered at high doses (150 IU daily) compared with baseline[7] or to the placebo-controlled group.[8] The amelioration of the sperm number and motility obtained at high doses may positively impact on the pregnancy rate. One of the included studies[22] showed an increased spontaneous and assisted reproductive technique (ART) pregnancy rate in the group treated with 300 IU on alternate days compared with placebo group. Patients receiving 200 IU on alternate days showed a higher ART pregnancy rate compared with placebo group. Patients treated with lower doses (100 IU or 50 IU on alternate days) did not show different pregnancy rates compared with those of the placebo group.[22] These findings have been confirmed also elsewhere.[30]

hpFSH is extracted from urine, and, therefore, it is rich in hyperglycosylated and acidic isoforms. By contrast, rhFSH is produced by cultured Chinese hamster ovary cells, which possess enzymes with lower protein glycosylation efficiency than the human pool. Despite rhFSH has a lower concentration of hyperglycosylated and acidic isoforms compared with the physiological molecule,[34] the drug-dependent analysis does not provide evidence in favor of either hpFSH or rhFSH. Indeed, both drugs ameliorated the sperm concentration, total number and progressive sperm motility, but not the sperm morphology.

Concerning the duration of FSH treatment, on the basis of the available data, we could meta-analyze the outcomes reported only at the 3rd treatment month. However, the study of Ding and coworkers[22] provides evidence for the effectiveness of therapeutic schemes of longer duration. In fact, using hpFSH at a dosage of 1050 IU per week, these authors observed further increases in sperm concentration, total sperm count, progressive sperm motility, and morphology. Indeed, compared with the values observed in the 3rd month, the sperm concentration ($[30.8 \pm 10.4] \times 10^6$ ml^{-1} *vs* $[15.8 \pm 9.8] \times 10^6$ ml^{-1}) and total sperm count

([62.9 ± 21.2] × 10^6 per ejaculate *vs* [32.1 ± 10.3] × 10^6 per ejaculate) doubled and progressive sperm motility and morphology were further ameliorated (41.7% ± 12.1% *vs* 29.7% ± 11.8% and 51.8% ± 13.5% *vs* 32.1% ± 12.8%, respectively) at the 5th month of therapy.[22] These parameters increased slightly 3 months after the discontinuation of therapy.[22] Similarly, hpFSH administration doubled the sperm concentration after 6 months of treatment compared with the results obtained after 3 months.[24] This is supported by the prior duration of FSH administration required to induce spermatogenesis or testicular growth in patients with hypogonadotropic hypogonadism. Different schemes are adopted, but none of them has a short duration. The last report dealing with this topic described 6–7 months of FSH administration alone, followed by the combined administration of FSH plus human chorionic gonadotropin (hCG) for 33–34 months for sperm to appear in the ejaculate.[35] Similarly, another study reported a 2-month long FSH administration showing a slight increase in testicular volume but not sperm recovery, followed by the addition of hCG. After 12 months from the beginning of therapy, testicular volume was visibly increased, but sperm recovery occurred only after 18 months of therapy.[36] Indeed, according to the physiology of pubertal development, spermatozoa appear in the seminal fluid after 1.5 years from the beginning of puberty defined by the achievement of a testicular volume >4 ml and increased FSH serum concentrations.[37,38] These data indicate that patients with idiopathic oligozoospermia may likely benefit from long-duration protocols.[22,24] Furthermore, the lack of side effects makes long-standing protocols largely safe.

The results of this meta-analysis are somewhat limited by the relatively small number of studies and by their heterogeneity, which includes failure to provide information regarding cigarette smoking, drug consumption, and alcohol abuse in all of the included studies, and the adoption of different editions of the WHO criteria for sperm analysis. Furthermore, the presence of male accessory gland infection was assessed by a simple sperm culture, and other important laboratory (sperm leucocyte concentration, *etc.*) and instrumental (testicular-epididymal and prostate-vesicular ultrasound scans) diagnostic tools were not even mentioned in many of the studies.[39] To partially overcome this drawback, we strictly followed the previously mentioned inclusion criteria. The reasons for the exclusion of some articles from the analysis are reported in **Supplementary Table 3**. Finally, because of the numbers of trials and participants are small, evidence is insufficient to allow final conclusions. Large multicentric trials with adequate numbers of participants are needed.

CONCLUSION

Our evidence-based analysis provides indications in favor of the use of high doses of FSH to improve the conventional sperm parameters. Indeed, we found that FSH administration is efficacious in increasing the sperm concentration, total sperm count, and progressive sperm motility. The dose-dependent analysis showed a greater effectiveness of high FSH doses on sperm concentration and total sperm count. Progressive sperm motility benefited from both low and the high doses. High FSH doses show a greater efficacy compared with intermediate and low ones on conventional sperm parameters. The efficacy of FSH therapy seems to be unrelated to FSH preparation type. Longer duration protocols (>3 months) may likely ensure a greater effectiveness of the treatment.[22,24] Large multicentric trials with adequate numbers of participants investigating the effectiveness of long-standing and high doses protocols on both sperm parameters and pregnancy rate are needed.

AUTHOR CONTRIBUTIONS

RC and AEC conceived the study. SLV, RAC, and LMM carried out the literature search and participated in the study design. RC meta-analyzed the included studies and wrote the paper. AEC and SLV critically analyzed the final version of the paper. All authors read and approved the final manuscript and agreed with the order of presentation of the authors.

COMPETING INTERESTS

All authors declared no competing interests.

Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Therapeutic schemes and weekly follicle-stimulating hormone dosages administered to oligozoospermic men

Therapeutic scheme	*Reference*	*Weekly dosage (IU)*
hpFSH, 50 IU on alternate days for 3 months	Ding *et al*. 2015[22]	175
rhFSH, 50 IU on alternate days for 3 months	Foresta *et al*. 2002[19]	
hpFSH, 75 IU 3 times a week for 3 months	Radicioni and Schwarzenberg, 1999[40]; Merino *et al*. 1996[52]	225
hpFSH, 75 IU on alternate days for 3 months	Zarilli *et al*. 2000[41]; Foresta *et al*. 1998[18]; Foresta *et al*. 2000[42]; Casamonti *et al*. 2017[10]	262.5
hpFSH, 100 IU on alternate days for 3 months	Ding *et al*. 2015[22]	350
rhFSH, 100 IU on alternate days for 3 months	Foresta *et al*. 2002[19]; Foresta *et al*. 2005[20]	
hpFSH, 150 IU 3 times a week for 3–6 months	Acosta *et al*. 1992[43]; Iacono *et al*. 1996[44]; Baccetti *et al*. 2004[8]; Arnaldi *et al*. 2000[27]; Garolla *et al*. 2017[11]; Condorelli *et al*. 2014[9]	450
rhFSH, 150 IU 3 times a week for 3–4 months	Caroppo *et al*. 2003[45]; Condorelli *et al*. 2014[9]	
hpFSH, 75 IU daily for 3 months	Dirnfeld *et al*. 2000[46]; Foresta *et al*. 2000[42]; Fernández-Arjona *et al*. 2003[28]	525
hpFSH, 150 IU on alternate days for 3 months	Palomba *et al*. 2011[29]	
rhFSH, 150 IU on alternate days for 3 months	Colacurci *et al*. 2012[21]	
hpFSH, 200 IU on alternate days for 3 months	Ding *et al*. 2015[22]	700
hpFSH, 150 IU daily for 3 months	Strehler *et al*. 1997[7]; Baccetti *et al*. 2004[8]	1050
hpFSH, 300 IU on alternate days for 3 months	Ding *et al*. 2015[22]	
rhFSH, 150 IU daily for 3 months	Kamischke *et al*. 1998[26]	
rhFSH, 300 IU on alternate days for ≥4 months	Paradisi *et al*. 2006[47]; Paradisi *et al*. 2014[30]	

FSH: follicle-stimulating hormone; hpFSH: highly purified FSH; IU: international units; rhFSH: recombinant human FSH

Supplementary Table 2: Selection criteria for the inclusion of studies (population intervention comparison outcome)

	Included	*Excluded*
Population	Normogonadotropic (1.5 mIU ml^{-1} <FSH ≤12 mIU ml^{-1}) patients with idiopathic oligo, astheno- and/or teratozoospermia and a history of infertility	Varicocele Y chromosome microdeletions Male accessory gland infection (evaluated by spermioculture) History of cryptorchidism Testicular torsion or trauma Azoospermia Hypogonadotropic (FSH <1.5 mmIU ml^{-1}) hypogonadism Hypergonadotropic (FSH ≥12 IU ml^{-1}) hypogonadism Isolated gonadotropin deficiency Hyperprolactinemia Other risk factors for impaired semen quality Normozoospermic fertile men
Intervention(s)	FSH therapy administered for at least 3 and not more than 4 months	
Comparison	Semen analysis performed according to the WHO guidelines (any edition) after the beginning of FSH administration (group of patients) or placebo/ no treatment/ no antioxidant vitamin supplements (control group)	Studies where data of the control group were not provided
Outcomes	Sperm concentration (mil ml^{-1}) Sperm count (mil/ejaculate) Progressive sperm motility (%) Sperm total motility (%) Sperm morphology (%)	Total motile sperm count
Study type	Randomized controlled trials	Not-randomized trials, cross-sectional, case-control and cohort studies, case reports, case series, reviews

WHO: World Health Organization; FSH: follicle-stimulating hormone

Supplementary Table 3: Reasons for exclusion of studies from the analysis

Author	*Reasons*
Acosta *et al*. 1992[43]	Baseline conventional sperm parameters of the treated group were not described
Bartoov *et al*. 1994[48]	Prospective study FSH was administered for 30 days Not all infertile patients had oligozoospermia
Iacono *et al*. 1996[44]	Not-controlled and not-randomized study
Merino *et al*. 1996[52]	Not-controlled and not-randomized study
Matorras *et al*. 1997[49]	Not all infertile patients had oligozoospermia Idiopathic infertility was not an inclusion criterion This study also included patients with a known cause of infertility
Strehler *et al*. 1997[7]	Observational study
Kamischke *et al*. 1998[26]	Hypogonadism was not among the inclusion criteria and some patients underwent to testosterone esters before FSH treatment 10/67 patients underwent to testicular biopsies whose results are not detailed. The selection of patients with maturation arrest may represent a study bias
Radicioni *et al*. 1999[40]	Prospective study The study was conducted in patients with left varicocele Patients did not have oligozoospermia
Arnaldi *et al*. 2000[27]	Observational study
Dirnfeld *et al*. 2000[46]	Retrospective study FSH and hCG were concomitantly administered Idiopathic infertility was not an inclusion criterion This study also included patients with a known cause of infertility
Foresta *et al*. 2000[42]	Not-controlled and not-randomized study Idiopathic infertility was not an inclusion criterion This study also included patients with a known cause of infertility
Zarilli *et al*. 2000[41]	This study was conducted on varicocelectomized patients
Caroppo *et al*. 2003[45]	This study included patients with high FSH levels. Indeed, they ranged from 1.6 to 27 mIU ml^{-1}
Fernández-Arjona *et al*. 2003[28]	Observational study
Baccetti *et al*. 200[48]	The data are not meta-analyzable (the results are shown neither as the mean±s.d. nor as the mean±s.e.m.)
Paradisi *et al*. 2006[47]	This cohort of patients was included in the study by Paradisi *et al*. 2014
Efesoy *et al*. 2009[50]	Not-controlled and not-randomized study Total motile sperm count was the outcome Sperm concentration, total sperm count, sperm motility, and morphology were not detailed
Piomboni *et al*. 2009[51]	Not-controlled and not-randomized study Not all infertile patients had oligozoospermia Idiopathic infertility was not an inclusion criterion This study also included patients with a known cause of infertility The outcome was sperm aneuploidy rate
Palomba *et al*. 2011[29]	Baseline-controlled observational study
Condorelli *et al*. 2014[9]	Observational study
Paradisi *et al*. 2014[30]	Not-randomized placebo-controlled trial
Casamonti *et al*. 2017[10]	Not-controlled and not-randomized study The outcome was hyaluronic acid binding rate Sperm concentration, total sperm count, sperm motility, and morphology were not detailed
Garolla *et al*. 2017[11]	Observational study Varicocele was not among the exclusion criteria. Therefore, its presence or absence in the included patients could not be ascertained

s.d.: standard deviation; s.e.m.: standard error of the mean; FSH: follicle-stimulating hormone; hCG: human chorionic gonadotropin