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

Male infertility is characterized by abnormal conventional sperm parameters (concentration, motility, and morphology), resulting in oligozoospermia (sperm count <15 × 10⁶ ml⁻¹ and total sperm count <39 × 10⁶ 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.

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. 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. FSH serum concentrations are considered predictive of responsiveness to FSH treatment: the lower they are the higher is the probability of a positive response. A definitive cutoff value has not been specified. Previous studies considered 12 mU ml⁻¹ as a threshold. However, recent Italian studies included patients with FSH serum concentration <8 mU ml⁻¹ 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]) or synthesized using recombinant in vitro technology (rhFSH).

Although several studies reported FSH effectiveness in increasing sperm count, a portion of oligozoospermic patients are unresponsive to this treatment. Indeed, it is thought that the treatment should be given to the selected patients. Therefore, effort has been made to identify predictors of the efficacy of FSH therapy, such as inhibin B serum levels as well as FSHβ or FSH receptor polymorphisms. 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
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. 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).

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 F statistics were used in the assessment of statistical heterogeneity. Specifically, statistical heterogeneity was tested using the Chi-square test. If \( F \leq 50\% \), the variation of the studies was considered to be homogenous, and the fixed effects model was adopted. If \( F > 50\% \), 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\(^{19-22} \) 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\(^{19} \)); 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\(^{19,21} \)); 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] × 10^6 ml\(^{-1}\); \( P < 0.01 \; \text{Figure 2a} \)), total sperm count (MD: 10.74 × 10^6 per ejaculate, 95% CI: [4.40–17.07] × 10^6 per ejaculate; \( P < 0.01 \; \text{Figure 2b} \)), progressive sperm motility (SMD: 0.36, 95% CI: 0.19–0.53; \( P < 0.01 \; \text{Figure 2a} \)), 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

Allow doses, FSH did not improve sperm concentration (MD: 1.88 × 10^6 ml\(^{-1}\), 95% CI: [−0.64–4.40] × 10^6 ml\(^{-1}\); \( P = 0.14 \; \text{Figure 2a} \)), total sperm count...
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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. | 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 | 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 | 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 | 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 | 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 × 10⁶ per ejaculate, 95% CI: [−1.45–11.09] × 10⁶ 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).
At intermediate doses, FSH improved sperm concentration (MD: 3.30 × 10^6 ml⁻¹, 95% CI: [2.28–4.31] × 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] × 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] × 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] × 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**

hFSH improved sperm concentration (MD: 5.71 × 10^6 ml⁻¹, 95% CI: [1.32–10.10] × 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] × 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] × 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] × 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...
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of postmenopausal women (hpFSH) or synthetized using in vitro recombinant technology (rhFSH).

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.

These effects represent the rational basis for prescribing FSH in oligozoospermic patients.

Several studies have reported improvement in conventional sperm parameters, decreased sperm DNA fragmentation index, and improved pregnancy rates 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.

Concerning testicular histology, the last SIAMS position statement suggested avoiding FSH prescription to patients with maturation arrest at the spermatid level, because patients with this maturation arrest are generally poor FSH responders. 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.

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 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
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.

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 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 or to the placebo-controlled group. The amelioration of the sperm number and motility obtained at high doses may positively impact on the pregnancy rate. One of the included studies 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. These findings have been confirmed also elsewhere.

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, 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 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 ± 10.4] × 10⁶ ml⁻¹ vs [15.8 ± 9.8] × 10⁶ ml⁻¹) and total sperm count

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.
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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.

REFERENCES
1 World Health Organization. Report of the Meeting on the Prevention of Infertility at the Primary Health Care Levels. Geneva: World Health Organization; 1983.
2 Agarwai A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility and the globe. Reprod Med Endocrinol 2015; 13: 37.
3 Valent D, La Vignera S, Condorelli RA, Rago R, Barone N, et al. Follicle-stimulating hormone treatment in normogonadotrophic infertile men. Nat Rev Urol 2013; 10: 55–62.
4 Duca Y, Calogero AE, Cannarella R, Condorelli RA, La Vignera S. Current and emerging medical therapeutic agents for idiopathic male infertility. Expert Opin Pharmacother 2018; 8: 1–13.
5 Barbottini A, Calogero AE, Balercia G, Garolla A, Krausz G, et al. The use of follicle stimulating hormone (FSH) for the treatment of the infertile man: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS). J Endocrinol Invest 2018; 41: 1107–22.
6 Baccetti B, Strehler E, Capitani S, Collode G, De Santo M, et al. The effect of follicle-stimulating hormone therapy on human sperm structure. Hum Reprod 1997; 12: 1955–68.
7 Strehler E, Sterzik K, De Santo M, Abt M, Wiedemann R, et al. The effect of follicle-stimulating hormone therapy on sperm quality: an ultrastructural mathematical evaluation. J Androl 1997; 18: 439–47.
8 Baccetti B, Piomboni P, Brunì E, Capitani S, Gambara L, et al. Effect of follicle stimulating hormone on sperm quality and pregnancy rate. Asian J Androl 2004; 6: 133–7.
9 Condorelli RA, Calogero AE, Vicari E, Mordio G, Burgio G, et al. Reduced seminal concentration of CD45pos cells after follicle-stimulating hormone treatment in selected patients with idiopathic oligoasthenoteratozoospermia. Int J Androl 2014; 2014: 372060.
10 Casamonti E, Vinci S, Serra E, Fino MG, Brillì S, et al. Short-term FSH treatment and sperm maturation: a prospective study in idiopathic infertile men. Andrology 2017; 5: 414–22.
11 Garolla A, Ghetti M, Così I, Sartini B, Bottacin A, et al. FSH treatment in infertile males candidate to assisted reproduction improved sperm DNA fragmentation and pregnancy rate. Endocrine 2017; 56: 416–25.
12 van Rijikom J, Leufkens H, Crommelin D, Rutten F, Broekmans A. Assessment of biotechnology drugs: what are the issues? Health Policy 1999; 47: 255–74.
13 Zwart-van Rijikom JE, Broekmans FJ, Leufkens HG. From HMG through purified urinary FSH preparations to recombinant FSH: a substitution study. Hum Reprod 2002; 17: 857–65.
14 Ferlin A, Vinanzi C, Selice R, Garolla A, Frigo AC, et al. Toward a pharmacogenetic approach to male infertility: polymorphism of follicle-stimulating hormone beta-subunit promoter. Fertilit Steril 2011; 96: 1344–9.
15 Selice R, Garolla A, Pengo M, Caretta N, Ferlin A, et al. The response to FSH treatment in oligoasthenoteratozoospermic men depends on FSH receptor gene polymorphisms. Int J Androl 2011; 34: 306–12.
16 Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. PLoS Med 2009; 6: e1000100.
17 Swiglo BA, Murad MH, Schünemann HJ, Kunz R, Vigersky RA, et al. A case for clarity, consistency, and helpfulness: state-of-the-art clinical practice guidelines in endocrinology using the grading of recommendations, assessment, development, and evaluation system. J Clin Endocrinol Metab 2008; 93: 666–73.
18 Foresta C, Bettella A, Ferlin A, Garolla A, Rossato M. Evidence for a stimulatory role of follicle-stimulating hormone on the spermatogenic population in adult males. Fertil Steril 1998; 69: 636–42.
19 Foresta C, Bettella A, Merico M, Garolla A, Ferlin A, et al. Use of recombinant human follicle-stimulating hormone in the treatment of male factor infertility. Fertil Steril 2002; 77: 238–44.
20 Foresta C, Bettella A, Garolla A, Ambrosini G, Ferlin A. Treatment of male idiopathic infertility with recombinant human follicle-stimulating hormone: a prospective,
controlled, randomized clinical study. Fertil Steril 2005; 84: 654–61.
21. Colacurci N, Monti MG, Fornaro F, Izzo G, Izzo P, et al. Recombinant human FSH reduces sperm DNA fragmentation in men with idiopathic oligoasthenoteratozoospermia. J Androl 2012; 33: 988–93.
22. Ding YM, Zhang XJ, Li JP, Chen SS, Zhang RT, et al. Treatment of idiopathic oligozoospermia with recombinant human follicle-stimulating hormone: a prospective, randomized, double-blind, placebo-controlled clinical study in Chinese population. Clin Endocrinol (Oxf) 2015; 83: 866–71.
23. van Dissel-Emiliani FM, Grootenhs AJ, de Jong FH, de Rooy-DG. Inhibin reduces spermatogonial numbers in testes of adult mice and Chinese hamsters. Endocrinology 1989; 125: 1899–903.
24. Tadokoro Y, Yomogida K, Ohta H, Tohda A, Nishimune Y. Homeostatic regulation of germinal stem cell proliferation by the GDNF/FSH pathway. Mech Dev 2002; 113: 29–39.
25. Mulleray BP, Skinner MK. Basic fibroblast growth-factor (bFGF) gene-expression and protein-production during puberty development of the semiferous tubule – follicle stimulating hormone-induced sertoli-cell bFGF expression. Endocrinology 1992; 131: 292834.
26. Kamischke A, Behre HM, Bergmann M, Simoni M, Schafter T, et al. Recombinant human follicle stimulating hormone for treatment of male idiopathic infertility: a randomized, double-blind, placebo-controlled, clinical trial. Hum Reprod 1998; 13: 596–603.
27. Arvaldi G, Balercia G, Garbassi G, Mantero F. Effects of long-term treatment with human pure follicle-stimulating hormone on semen parameters and sperm-cell ultrastructure in idiopathic oligoasthenoteratozoospermia. Andrologia 2000; 32: 155–61.
28. Fernandez-Arjonca M, Diaz J, Cortes I, Gonzalez J, Rodriguez JM, et al. Relationship between gonadotrophin secretion, inhibin B and spermatogenesis in oligozoospermic men treated with highly purified urinary follicle-stimulating hormone (uFSH-HP): a preliminary report. Eur J Obstet Gynecol Reprod Biol 2003; 107: 47–51.
29. Palomba S, Falbo A, Espinola S, Rocca M, Capasso S, et al. Effects of highly purified follicle-stimulating hormone on sperm DNA damage in men with male idiopathic subfertility: a pilot study. J Endocrinol Invest 2011; 34: 747–52.
30. Paradisi R, Natale F, Fabbri R, Battaglia C, Seracchioli R, et al. Evidence for a stimulatory role of high doses of recombinant human follicle-stimulating hormone in the treatment of male-factor infertility. Andrologia 2014; 46: 1067–72.
31. Ruvolo G, Roccheri MC, Brucoliemi AM, Longobardi S, Cittadini E, et al. Lower sperm DNA fragmentation after r-FSH administration in functional hypogonadotrophic hypogonadism. J Assist Reprod Genet 2013; 30: 497–503.
32. Santi D, Granata AR, Simoni M. FSH treatment of male idiopathic infertility improves pregnancy rate: a meta-analysis. Endocr Connect 2015; 4: R46–58.
33. Atiya AM, Abou-Setta AM, Al-Imary HG. Gonadotrophins for idiopathic male factor subfertility. Cochrane Database Syst Rev 2013; 8: CDD005701.
34. Wang H, Chen X, Zhang X, Zhang W, Li Y, et al. Comparative assessment of glycosylation of a recombinant human FSH and a highly purified FSH extracted from human urine. J Proteome Res 2016; 15: 923–32.
35. Kohwa E, Hupio H, Hero M, Mettinen PJ, Vaaralahti K, et al. Recombinant human FSH treatment outcomes in five boys with severe congenital hypogonadotrophic hypogonadism. J Endocr Soc 2018; 2: 1345–56.
36. Prior M, Stewart J, McClinty K, Dwyer AA, Quinton R. Fertility induction in hypogonadotrophic hypogonadal men. Clin Endocrinol (Oxf) 2018; 89: 712–8.
37. Dabaja AA, Wosnitza MS, Bolyakov A, Schlegel PN, Paduch DA. When to ask male adolescents to provide semen sample for fertility preservation? Transl Androl Urol 2014; 3: 2–8.
38. Condorelli RA, Cannarella R, Calogero AE, La Vignera S. Evaluation of testicular function in prepubertal children. Endocrine 2018; 62: 274–80.
39. La Vignera S, Calogero AE, Arancio A, Castiglione R, De Grande G, et al. Transrectal ultrasonography in fertile patients with persistently elevated bacteriospermia. Asian J Androl 2008; 10: 731–40.
40. Radicini A, Schwarzenberg TL. The use of FSH in adolescents and young adults with idiopathic, unilateral, left varicocele not undergoing surgical intervention. Preliminary study. Minerva Endocrinol 1999; 24: 63–8.
41. Zarrilli S, Paesano L, Colao A, Mirona V, Lombardi G, et al. FSH treatment improves sperm function in patients after varicocelectomy. J Endocrinol Invest 2000; 23: 68–73.
42. Foresta C, Bettella A, Merico M, Garolla A, Plebani M, et al. FSH in the treatment of idiopathic male infertility. J Androl 2014; 35: 2–10.
43. Ruvolo G, Roccheri MC, Brucculeri AM, Longobardi S, Cittadini E, et al. Recombinant human follicle-stimulating hormone as a pretreatment for idiopathic oligoasthenoteratozoospermic patients undergoing intracytoplasmic sperm injection. Fertil Steril 2003; 80: 1398–403.
44. Dirnfeld M, Katz G, Calderon I, Abramovici H, Bider D. Pure follicle-stimulating hormone as an adjuvant therapy for selected cases in male infertility during in vitro fertilization is beneficial. Eur J Obstet Gynecol Reprod Biol 2000; 95: 105–8.
45. Paradisi R, Busacchi P, Seracchioli R, Porcu E, Venturini S. Effects of high doses of recombinant human follicle-stimulating hormone in the treatment of male factor infertility: results of a pilot study. Fertil Steril 2006; 86: 728–31.
46. Bartov B, Eltes F, Lunenfeld E, Har-Even D, Lederman H, et al. Sperm quality of subfertile males before and after treatment with human follicle-stimulating hormone. Fertil Steril 1994; 61: 727–34.
47. Matorras R, Perez C, Corcostegui B, Pijoan JL, Ramon O, et al. Treatment of the male with follicle-stimulating hormone in intrauterine insemination with husband’s spermatozoa: a randomized study. Hum Reprod 1999; 12: 24–8.
48. Etesoy O, Cayan S, Akbay E. The efficacy of recombinant human follicle-stimulating hormone in the treatment of various types of male-factor infertility at a single university hospital. J Androl 2009; 30: 679–84.
49. Piomboni P, Serafini F, Gambera L, Musacchio C, Collodel G, et al. Sperm aneuploidies after human recombinant follicle stimulating hormone therapy in infertile males. Reprod Biomed Online 2009; 8: 622–9.
50. Merino G, Carranza-Lira S, Martinez-Chéquer JC, Barahona E, Morán C, et al. Sperm characteristics and hormonal profile before and after treatment with follicle-stimulating hormone in infertile patients. Arch Androl 1996; 37: 197–200.
**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. 201522                            | 175               |
| rhFSH, 50 IU on alternate days for 3 months| Foresta et al. 200219                         |                    |
| hpFSH, 75 IU 3 times a week for 3 months   | Radicioni and Schwarzenberg, 199992; Merino et al. 199619 | 225               |
| hpFSH, 75 IU on alternate days for 3 months| Zarilli et al. 200044; Foresta et al. 199846; Foresta et al. 200042; Casamonti et al. 201750 | 262.5             |
| hpFSH, 100 IU on alternate days for 3 months| Ping et al. 201512                            | 350               |
| rhFSH, 100 IU 3 times a week for 3–6 months| Foresta et al. 200215; Foresta et al. 200540 | 450               |
| hpFSH, 75 IU on alternate days for 3 months| Acosta et al. 199245; Iacono et al. 199649; Baccetti et al. 20049; Arnaldi et al. 200047; Casamonti et al. 201750 | 525               |
| rhFSH, 150 IU 3 times a week for 3–4 months| Colacurci et al. 2013121                      | 700               |
| hpFSH, 150 IU daily for 3 months           | Palomba et al. 201129                         | 1050              |
| rhFSH, 150 IU on alternate days for 3 months| Ding et al. 201512                            |                    |
| rhFSH, 200 IU on alternate days for 3 months| Colacurci et al. 201211                      |                    |
| rhFSH, 150 IU daily for 3 months           | Strehler et al. 199772; Baccetti et al. 20049 |                    |
| hpFSH, 300 IU on alternate days for 3 months| Ding et al. 201512                            |                    |
| rhFSH, 150 IU daily for 3 months           | Kamischke et al. 199888                      |                    |
| rhFSH, 300 IU on alternate days for ≥4 months| Paradisi et al. 200642; Paradisi et al. 201490 |                    |

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<sup>−1</sup> < FSH ≤ 12 mIU ml<sup>−1</sup>) patients with idiopathic oligo, astheno- and/or teratozoospermia and a history of infertility | Varicocele                                                               |
|                                                                       | Male accessory gland infection (evaluated by spermioculture)             |
|                                                                       | History of cryptorchidism                                                |
|                                                                       | Testicular torsion or trauma                                             |
|                                                                       | Azospermia                                                              |
|                                                                       | Hypogonadotropic (FSH < 1.5 mIU ml<sup>−1</sup>) hypogonadism            |
|                                                                       | Hypergonadotropic (FSH ≥ 12 IU ml<sup>−1</sup>) 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     | Studies where data of the control group were not provided               |
| 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)       |                                                                         |
| Outcomes                                                               |                                                                         |
| Sperm concentration (mil ml<sup>−1</sup>)                               | Total motile sperm count                                                |
| Sperm count (mil ejaculate)                                            |                                                                         |
| Progressive sperm motility (%)                                         |                                                                         |
| Sperm total motility (%)                                               |                                                                         |
| Sperm morphology (%)                                                   |                                                                         |
| Study type                                                             |                                                                         |
| Randomized controlled trials                                          |                                                                         |

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      | Baseline conventional sperm parameters of the treated group were not described |
| Bartoo et al. 1994      | Prospective study                                                      |
|                         | FSH was administered for 30 days                                       |
|                         | Not all infertile patients had oligozoospermia                         |
| Iacono et al. 1996     | Not-controlled and not-randomized study                                |
| Merino et al. 1996     | Not-controlled and not-randomized study                                |
| Matorras et al. 1997   | 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   | Observational study                                                   |
| Kamischke et al. 1998  | FSH 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  | Prospective study                                                      |
|                         | The study was conducted in patients with left varicocele               |
|                         | Patients did not have oligozoospermia                                   |
| Arnaldi et al. 2000    | Observational study                                                   |
| Dinfield et al. 2000   | 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    | 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    | This study was conducted on varicocelectomized patients                |
| Caroppo et al. 2003    | This study included patients with high FSH levels. Indeed, they ranged from 1.6 to 27 mIU ml⁻¹ |
| Fernández-Arjona et al. 2003 | Observational study                                               |
| Baccetti et al. 2004   | 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   | This cohort of patients was included in the study by Paradisi et al. 2014 |
| Efeso et al. 2009      | 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   | 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    | Baseline-controlled observational study                                |
| Condorelli et al. 2014 | Observational study                                                   |
| Paradisi et al. 2014   | Not-randomized placebo-controlled trial                                |
| Casamonti et al. 2017  | 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    | 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