Journal of Endocrinological Investigation
https://doi.org/10.1007/s40618-018-0843-y

CONSENSUS STATEMENT

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)

A. Barbonetti[1] · A. E. Calogero[2] · G. Balercia[3] · A. Garolla[4] · C. Krausz[5] · S. La Vignera[2] · F. Lombardo[6] · E. A. Jannini[7] · M. Maggi[5] · A. Lenzi[6] · C. Foresta[4] · A. Ferlin[4,8]

Received: 28 November 2017 / Accepted: 27 January 2018

Abbreviations

ART	Assisted reproductive technologies
FSH	Follicle stimulating hormone
FSHR	FSH receptor
GnRH-a	Gonadotropin-releasing hormone agonist
hCG	Human chorionic gonadotropin
hMG	Human menopausal gonadotropin
hpFSH	Highly purified follicle stimulating hormone
ICSI	Intracytoplasmic sperm injection
IUI	Intrauterine insemination
IVF	In vitro fertilization
IVF-ET	In vitro fertilization-embryo transfer
OAT	Oligo/astheno/teratozoospermia
pFSH	Purified follicle stimulating hormone
RCT	Randomized controlled trial
rhFSH	Recombinant human follicle stimulating hormone

✉ A. Ferlin
alberto.ferlin@unibs.it

1 Casa di Cura San Raffaele Sulmona, Sulmona, AQ, Italy

2 Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy

3 Division of Endocrinology, Department of Clinical and Molecular Sciences, Umberto I Hospital, Ancona, Polytechnic University of Marche, Ancona, Italy

4 Unit of Andrology and Reproductive Medicine, Department of Medicine, University of Padova, Padua, Italy

5 Department of Biomedical, Experimental and Clinical Sciences 'Mario Serio', University of Florence, Florence, Italy

6 Laboratory of Seminology-Sperm Bank "Loredana Gandini", Department of Experimental Medicine, University of Rome "La Sapienza", Rome, Italy

7 Department of Systems Medicine, Chair of Endocrinology and Medical Sexology (ENDOSEX), University of Rome Tor Vergata, Rome, Italy

8 Present Address: Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy

Introduction

Infertility refers to the inability of a couple to conceive after 12 months of regular unprotected sexual intercourse [1, 2] and affects about 10–15% of couples of reproductive age [3–5]. Male factor alone is responsible for approximately 30% of cases of infertility, while a combination of male and female factors affects another 20%. Therefore, overall, the male factor would be involved in 50% of infertile couples [2, 3, 6]. In about 30% of cases of male infertility, no obvious cause for subnormal semen parameters can be found after a careful diagnostic workup (2, 3). Indeed, this condition, which is referred to as "idiopathic infertility", represents the most commonly observed form of infertility in clinical practice, but unfortunately, rational treatment are lacking. Although intracytoplasmic sperm injection (ICSI) is regarded as an appropriate treatment for infertile men with severe oligo/astheno/teratozoospermia (OAT), sperm structure and quality may affect its outcome [7]. Therefore, the justified enthusiasm for ICSI as the treatment of choice for severe male factor infertility should not discourage attempts to better understand pathophysiology, to provide when possible an etiological diagnosis, and to improve sperm quality.

As gonadotropins are needed for testis physiology and represent a successful treatment in hypogonadotropic hypogonadism [8], they have been also offered to men with idiopathic infertility based on the hypothesis that spermatogenesis could be stimulated by increasing gonadotropin levels. Available follicle stimulating hormone (FSH) preparations are those extracted and purified from the urine of postmenopausal women, the so-called purified FSH (pFSH) and highly purified FSH (hpFSH) [9], as well as those obtained

Published online: 01 February 2018

from recombinant in vitro technology (rhFSH) [10]. Combination strategies with human chorionic gonadotropin (hCG) can be used in men with hypogonadotropic hypogonadism to restore intratesticular testosterone concentrations and induce spermatogenesis [11]. The addition of rhFSH to hCG treatment protocols results in normal testicular growth and hastens the induction of spermatogenesis in young men with hypogonadotropic hypogonadism [12]. These strategies seem to be effective even in men with late-onset hypogonadism [13]. On the contrary, FSH administration as single therapy is usually considered in men with idiopathic infertility and gonadotropins within the normal range.

Actually, results obtained in idiopathic infertility seem to be still controversial. In fact, the effectiveness of FSH therapy in improving semen parameters in idiopathic male-factor infertility has not been demonstrated by some authors [7, 14–24], whereas others have reported significant improvements in sperm quality and/or pregnancy rates after FSH treatment [15, 16, 20, 21, 24–41]. A meta-analysis by the Cochrane Collaboration [42], only including randomized controlled trials (RCTs), showed that infertile men who received FSH had a significant increase in spontaneous pregnancy rate per couple with respect to patients receiving placebo or no treatment, whereas no significant difference between the two groups in terms of pregnancy rate after assisted reproductive technologies (ART) was observed. More recently, in another meta-analysis [43], including all available controlled clinical trials, when compared to placebo or untreated controls, men receiving FSH showed a significant improvement in sperm concentration and quality and exhibited a significant increase in spontaneous and ART-related pregnancy rate.

The aim of the present article was to analyse the state of the art regarding the clinical evidence on the effectiveness of FSH therapy in male infertility and to provide a position statement on its use from the Italian Society of Andrology and Sexual Medicine (SIAMS). In particular, three major outcomes were assessed: improvement of conventional sperm parameters, improvement of sperm DNA integrity, and improvement of pregnancy rate. The suitability of available possible predictors of response to FSH treatment has been also assessed according to the Evidence-Based Medicine (EBM) criteria. The task force used the following internationally shared coding system [44]: (1) "we recommend" indicates a strong recommendation; (2) "we suggest" denotes a weak recommendation. As far as the evidence grading is concerned: ØOOO denotes very low-quality evidence; ØØOO, low quality; ØØØO, moderate quality; and ØØØØ, high quality.

Effects of FSH on conventional sperm parameters

Recommendations

1. We recommend not prescribing FSH treatment for improving sperm parameters in all infertile men before a specific diagnostic workup (1 ØØØØ).
2. We recommend not using FSH treatment in azoospermic men and in men with obstructive/sub-obstructive forms of infertility (1 ØØØØ).
3. We suggest the use of FSH (either purified or recombinant) to increase sperm concentration and motility in infertile normogonadotropic men with idiopathic oligozoospermia or OAT (2 ØØØO).

Evidence

Several studies have shown that FSH treatment improves conventional sperm parameters in oligozoospermic men with gonadotropin levels within the normal range (generally 1–8 IU/l) [36]. A meta-analysis showed a significant improvement of sperm concentration after FSH administration, with a mean improvement of 2.66×10^6/ml (95% CI 0.47, 4.84; $p = 0.02$, $n = 520$) and a non-significant improvement of concentration of sperm with progressive motility, with a mean raise of 1.22×10^6/ml (95% CI − 0.07, 2.52; $p = 0.06$, $n = 332$) [43]. The efficacy of FSH treatment has been associated with FSH dose and duration of the treatment [24] (Table 1), and with the molecule specifically used (e.g. rhFSH, pFSH, hpFSH) (Table 2).

Concerning the dosage, the evidence from the literature survey suggests that it depends on the type of FSH prescribed. Several observational studies described the efficacy of hpFSH administered at weekly cumulative doses ≤ 450 IU (e.g. 150 IU three times a week, 75 IU on alternate days, etc.) for 3 months (Table 2). With this type and dosage of FSH, a significant improvement of the sperm concentration was showed in all the studies and most of them reported a beneficial effect on sperm motility as well [29–32, 34, 40, 41, 45]. Data on the efficacy of hpFSH on sperm morphology are less consistent [20]. In contrast to hpFSH, rhFSH, administered at a weekly cumulative dose ≤ 450 IU, resulted to be less effective. In more detail, one randomized controlled trial (RCT) [23] and one observational study [33] reported contrasting results on sperm concentration, motility and morphology following treatment with αfollitropin. In the study by Colacurci et al. [23], the administration of 150 IU on alternate days for 3 months did not significantly improve conventional sperm parameters, whereas the administration of 150 IU three times a week for 3 months was able to improve sperm concentration, motility and morphology [33]. According to one RCT [46] and one

Table 1 Effects of FSH treatment on conventional sperm parameters in normogonadotropic infertile men

Author	Cohort	Dosage	Outcome
Low-dose therapy (*weekly cumulative dose* ≤ 450 IU)			
Acosta et al. [15] Observational study	24 men with OAT vs 26 matched controls with OAT	pFSH; 150 IU three times a week for 3 months	No significant change in sperm concentration, motility and morphology
Bartoov et al. [16] Observational study	31 men with teratozoospermia	pFSH; 75 IU daily for 1 month	No significant change in sperm concentration, motility and morphology
Iacono et al. [82] Observational study	33 men with idiopathic oligozoospermia vs 28 controls	pFSH; 150 IU three times per week for 3 months ($n = 17$)	No significant change in sperm concentration, motility and morphology
Baccetti et al. [7] Observational study	81 men with idiopathic infertility	pFSH; 150 IU three times per week for 3 months for test group ($n = 66$); saline for placebo group ($n = 15$)	No significant change in sperm concentration, motility and morphology
Foresta et al. [68] RCT	90 men with oligozoospermia	pFSH; 75 IU three times per week for 3 months for test group ($n = 60$); saline for placebo group ($n = 30$)	Increase in sperm concentration in responding patients ($n = 20$; $p < 0.01$)
Radicioni et al. [29] Observational study	20 men with left-side varicocele	hpFSH; 75 IU three times per week for 3 months	Significant improvement in sperm concentration, progressive motility and morphology ($p < 0.05$)
Dirnfeld et al. [83] Observational study	76 men with OAT vs 102 matched controls	pFSH; 75 IU daily for 3 months for treated group; no treatment for controls	Significant increase in sperm motility ($p < 0.05$)
Arnaldi et al. [30] Observational study	10 men with OAT	hpFSH; 150 IU three times per week for 6 months	Increase in sperm concentration ($p < 0.05$) and motility ($p < 0.05$)
Foresta et al. [31] Observational study	135 men with OAT	hpFSH; Group 1 ($n = 78$): 75 IU three times per week for 3 months; Group 2 ($n = 57$): 75 IU daily for 3 moths	Significant increase in sperm concentration in responding patients of both groups ($p < 0.001$)
Zarilli et al. [32] Observational study	56 men with oligozoospermic vs 20 controls	hpFSH;75 IU on alternate days for 3 months	Significant improvement in sperm concentration ($p < 0.05$), motility ($p < 0.01$)
Foresta et al. [46] RCT	30 men with OAT vs 15 matched controls	rhFSH; Group 1 ($n = 15$): 50 IU three times per week for 3 months; Group 2 ($n = 15$): 100 IU three times per week for 3 months. No treatment for controls	Significant increase in sperm concentration in 11 men in Group 2 ($p < 0.05$)
Caroppo et al. [33] Observational study	33 men with OAT	rhFSH; 150 IU three times per week for 3 months for test group ($n = 23$); no treatment for control group ($n = 10$)	Significant improvement in sperm concentration ($p < 0.05$), motility ($p < 0.05$) and morphology ($p < 0.001$)
Fernandez-Arjona et al. [34] Observational study	29 men with oligozoospermia	hpFSH; 75 daily for 3 months	Significant improvement in sperm concentration, motility and morphology ($p < 0.05$)
Foresta et al. [21] RCT	112 men with idiopathic oligozoospermia	rhFSH; treated group ($n = 62$): 100 IU on alternate days for 3 months; no treatment for control group ($n = 50$)	No significant difference among conventional sperm parameters
Efesoy et al. [22] Observational study	16 men with idiopathic oligoasthenozoospermia	rhFSH; Group 1 ($n =$ NR): 100 IU twice per week for 3 months; Group 2 ($n =$ NR): 150 IU three times per week for 3 months	No significant change in sperm motility
Palomba et al. [45] Observational study	36 men with oligozoospermia	hpFSH; 150 IU three times per week for 3 months	Significant improvement in sperm concentration ($p = 0.047$), motility ($p = 0.041$) and morphology ($p = 0.023$)

Table 1 (continued)

Author	Cohort	Dosage	Outcome
Low-dose therapy (weekly cumulative dose ≤ 450 IU)			
Colacurci et al. [23] RCT	129 men with OA	rFSH; treated group ($n = 65$): 150 IU on alternate days for 3 months; control group ($n = 64$): non-antioxidant vitamin supplements	No significant difference among conventional sperm parameters
Condorelli et al. [37] Observational study	54 men with idiopathic oligoasthenozoospermia	rhFSH; Group A ($n = 20$) (αfollitropin): 150 IU three times per week for 3 months; Group B ($n = 20$) (βfollitropin): 150 IU three times per week for 3 months; hpFSH; Group C ($n = 14$) (urofollitropin): 150 IU three times per week for 3 months	No significant difference among sperm concentration and motility. Significant improvement in sperm morphology ($p < 0.05$)
Ding et al. [24] RCT	354 men with idiopathic oligozoospermia	hpFSH; Group 1 (n = NR): 50 IU on alternate days for three months; Group 2 (n = NR): 100 IU on alternate days for three months	No significant change in sperm concentration, motility, morphology
Casamonti et al. [40] Observational study	40 oligo- and/or astheno- and/or teratozoospermic patients	hpFSH (75 IU on alternate days for 3 months)	Significant improvement in total sperm number ($p = 0.002$), motility ($p = 0.000$) and morphology ($p = 0.008$)
Garolla et al. [41] no treatment for control group ($n = 50$)	166 men with idiopathic oligozoospermia	hpFSH (urofollitropin); treated group ($n = 84$):150 IU three times per week for 3 months; no treatment for control group ($n = 82$)	Significant improvement in sperm concentration, total sperm count and forward motility (all $p < 0.05$), with a doubling of sperm count with respect to T0 and controls
High-dose therapy (weekly cumulative dose ≥ 450 IU)			
Iacono et al. [82] Observational study	33 men with idiopathic oligozoospermia vs 28 matched controls	pFSH; 150 IU daily for 3 months ($n = 16$)	Significant improvement in sperm motility ($p < 0.0001$) and morphology ($p < 0.007$)
Strehler et al. [27] Observational study	46 men with OAT	hpFSH; 150 IU daily for three months	Significant improvement in sperm morphology ($p < 0.001$)
Kamischke et al. [18] RCT	67 men with OAT	rhFSH (α-follitropin); treated group ($n = 34$): 150 IU daily for 3 months and saccharose 30 mg three times per week for 3 months; control group ($n = 33$): saccharose 30 mg three times per week for 3 months	Slight improvement in progressive sperm motility in saccharose-only group ($p > 0.05$)
Baccetti et al. [20] Observational study	44 men with OAT	hpFSH; treated group ($n = 24$): 150 IU daily for 3 months; no treatment for control group ($n = 20$)	No significant change in sperm concentration, motility, morphology
Paradisi et al. [35] RCT	30 men with OAT	rhFSH; treated group ($n = 15$): 300 IU on alternate days and saccharose 30 mg 3 times per week for 4 months; control group ($n = 15$): saccharose 30 mg 3 times per week for 4 months	Increase in sperm concentration and motility
Paradisi et al. [38] Observational study	60 infertile men with moderate/severe oligoasthenozoospermia	rhFSH; treated group ($n = 45$): 300 IU on alternate days for ≥ 4 months; control group ($n = 15$): placebo	Marked increase in sperm count and no change in sperm motility, morphology and viability

Table 1 (continued)

Author	Cohort	Dosage	Outcome
Low-dose therapy (weekly cumulative dose ≤ 450 IU)			
Ding et al. [24] RCT	354 men with idiopathic oligozoospermia	hpFSH; Group 1 (n = NR): 200 IU on alternate days for three months; Group 2 (n = NR): 300 IU on alternate days for 3 and 5 months	Sperm number was significantly increased; this result was observed beginning at the 3rd months. A significant increase in sperm morphology and motility was observed with a dose of 300 IU, respectively, at the 4th and 5th month

FSH follicle stimulating hormone, *hp* highly purified FSH, *OAT* oligoasthenoteratozoospermia, *pFSH* purified FSH, *RCT* randomized controlled trial, *rhFSH* recombinant FSH

observational study [22], βfollitropin, administered at a weekly cumulative dose ≤ 450 IU, does not improve sperm motility and morphology, in a 3-month-long trial. Finally, in another study, the treatment with rhFSH did not seem to be more effective than treatment with hpFSH at the same weekly cumulative dose of 450 IU for 3 months [37].

Few studies reported the effects of rhFSH and hpFSH administered at a weekly cumulative dose > 450 IU on conventional sperm parameters. In particular, two observational studies [20, 27], using hpFSH at a daily dose of 150 IU, found no positive effect on sperm concentration and morphology after a 3-month-long therapy. Such studies reported contrasting results for sperm motility. On the contrary, an RCT reported positive effect on sperm concentration after a 3-month-long therapy with 200 IU administered on alternate days. In addition, a significant increase in sperm motility and morphology was recorded using 300 IU on alternate days for 5 months [24]. Two RCTs [18, 35] and one observational study [38] investigated the effects of rhFSH at the doses of 150 IU daily [18] or 300 IU on alternate days [35, 38] on conventional sperm parameters. Apart from one RCT [18], the others reported positive effects on sperm concentration. Treatment with pFSH, both at low- (150 IU three times a week, 75 IU daily, 75 IU three times a week for 3 months) and at high doses (150 daily for 3 months) showed mild effect on sperm concentration, motility and morphology, according to an RCT and several observational studies (Table 2). No study on biosimilar FSH has been performed.

As far as the duration of FSH therapy is concerned, in the majority of the previously discussed studies the FSH administration was 3 months long. Only four studies [24, 30, 35, 38] evaluated the effects on conventional sperm parameters after 4-month-long therapies (two studies administered rhFSH, two hpFSH) and all of them reported a significant improvement of the sperm concentration. A significant increase in sperm motility was registered by three studies [24, 30, 35]. Furthermore, 4-month-long therapies did not improve sperm morphology, whereas it was significantly increased at the fifth month [24]. Although supported by relatively few studies, these data may suggest a higher efficacy for long-term FSH administration.

Value

Evidence supporting the use of FSH in infertile normogonadotropic men with idiopathic oligozoospermia or OAT is of moderate quality.

Remarks

The evidence reported above derives from RCTs and observational studies. The literature lacks meta-analysis of RCTs. Since human spermatogenesis takes about 72 days

Table 2 Effects of FSH treatment on conventional sperm parameters in normogonadotropic infertile men according with the specific FSH molecule and the dose used

Type of study	Dosage	Concentration	Motility	Morphology
Low dose-therapy (weekly cumulative dose ≤ 450 IU)				
pFSH				
Acosta et al. [15] Ob.S. urofollitropin Group 1: FSH 5–15 mIU/ml; Group 2: FSH 16–25 mIU/ml; Group 3: FSH > 25 mIU/ml	150 IU three times a week for 3 months	–	–	–
Bartoov et al. [16] Ob.S. urofollitropin FSH within the normal range	75 IU daily for 1 month	–	–	–
Iacono et al. [82] Ob.S.	150 IU three times a week for 3 months	–	–	–
Baccetti et al. [7] Ob.S. urofollitropin [< 12 UI/l]	150 IU three times a week for 3 months	–	–	–
Foresta et al. [68] RCT urofollitropin [3.4 ± 1.1 UI/l]	75 IU three times a week for 3 months	– (+ in 33% of patients)	–	–
Dirnfeld et al. [83] Ob.S. urofollitropin + hCG; (Chorigon) 5000 UI every 5 days; FSH within the normal range	75 IU daily for 3 months	–	+	–
hpFSH				
Radicioni et al. [29] Ob.S. urofollitropin [2.3–12.3 UI/l]	75 IU three times a week for 3 months	+	+	+
Arnaldi et al. [30] Ob.S. urofollitropin FSH within the normal range	150 IU three times a week for 6 months	+	+	–
Foresta et al. [31] Ob.S. urofollitropin [Group A: 3.5 ± 1.8 IU/l, Group B: 12.5 ± 5.8 IU/ml, Group C: 18.9 ± 10.7 IU/ml, according to the histology]	75 IU daily for 3 months	+	–	–
	75 IU three times a week for 3 months	+	–	–
Zarilli et al. [32] Ob.S.[4.9 ± 0.5 UI/l]	75 IU on alternate days for 3 months	+	+	NR
Fernandez−Arjona et al. [34] Ob.S.urofollitropin [3.8 ± 1.35 UI/l]	75 IU daily for 3 months	+	+	+
Palomba et al. [45] Ob.S.urofollitropin [7.0 ± 1.1 UI/l]	150 IU three times a week for 3 months	+	+	+
Condorelli et al. [37] Ob.S. urofollitropin [3.1 ± 4.6 UI/l]	150 IU three times a week for 3 months	–	–	+
Casamonti et al. [40] Ob.S. urofollitropin [< 8 UI/l]	75 IU on alternate days for 3 months	+	+	+
Garolla et al. [41] Ob.S. urofollitropin [< 8 UI/l]	150 IU three times a week for 3 months	+	+	–
Ding et al. [24] RCT urofollitropin [4.8 ± 1.9 UI/l]	50 IU on alternate days for 3 months	–	–	–
	100 IU on alternate days for 3 months	–	–	–
rhFSH				
Foresta et al. [46] RCTβfollitropin [4.1 ± 2.2 (1.6–27) UI/l]	50 IU three times a week for 3 months	–	–	–
	100 IU three times a week for 3 months	+	–	–
Caroppo et al. [33] Ob.S.αfollitropin [9.68 ± 6.05 (1.6–27) UI/ml]	150 IU three times a week for 3 months	+	+	+
Foresta et al. [21] RCT [4.6 ± 1.2 UI/l]	100 IU on alternate days for 3 months	– (+ in 48.4% of patients)	–	–
Efesoy et al. [22] Ob.S.βfollitropin [7.18 ± 0.68 UI/l]	100 IU twice a week for 3 months	–	–	–
	150 IU three times a week for 3 months	–	–	–
Colacurci et al. [23] (RCT)αfollitropin [5.9 ± 1.3 UI/l]	100 IU on alternate days for 3 months	–	–	–
Condorelli et al. [37] Ob.S. αfollitropin [2.6 ± 1.1 UI/l]	150 IU three times a week for 3 months	–	–	+
High dose−therapy (weekly cumulative dose ≥ 450 IU)				
pFSH				
Iacono et al. [82] Ob.S.	150 IU daily for 3 months	–	+	+

Table 2 (continued)

Type of study	Dosage	Concentration	Motility	Morphology
hpFSH				
Strehler et al. [27] Ob.S. urofollitropin [< 12 UI/l]	150 IU daily for 3 months	−	+	−
Baccetti et al. [20] Ob.S. urofollitropin [< 12 UI/l]	150 IU daily for 3 months	−	−	−
Ding et al. [24] RCT urofollitropin [4.8 ± 1.9 UI/l]	200 IU on alternate days for 3 months	+	−	−
	300 IU on alternate days for 5 months	+	+	+
rhFSH				
Kamischke et al. [18] RCT αfollitropin [5.0 ± 0.4 UI/l]	150 IU daily for 3 months	−	−	−
Paradisi et al. [35] RCTαfollitropin [4.1 ± 1.6 UI/l]	300 IU on alternate days for 4 months	+	+	−
Paradisi et al. [38] Ob.S.αfollitropin [3.9 ± 1.4 UI/l]	300 IU on alternate days for ≥4 months	+	−	−

FSH follicle stimulating hormone, *hp* highly purified FSH, *NR* not reported, *Ob.S.* observational study, *pFSH* purified FSH, *RCT* randomized controlled trial *rhFSH* recombinant FSH

[36], positive effects on all conventional sperm parameters are likely to be observed in ≥ 4-month-long therapies [24]. Unfortunately, only few studies evaluated the effects of FSH therapy administered for longer than 4 months [24, 30, 35, 38].

Concerning the FSH plasma concentration before therapy, most of the studies included patients with FSH serum levels within the normal range, but not all the studies specified the exact FSH mean value of the treated group (Table 2).

Effects of FSH on pregnancy rate

Recommendations

1. We recommend not prescribing FSH treatment for improving pregnancy rate before a specific couple-oriented diagnostic workup (1 ØØØØ).
2. We suggest the use of FSH (either purified or recombinant) in normogonadotropic male partners of couples with idiopathic male factor infertility for improving spontaneous pregnancy rate (2 ØØOO).
3. We suggest the use of FSH (either purified or recombinant) in normogonadotropic male partners of couples with idiopathic male factor infertility for improving pregnancy rate after ART (2 ØOOO).

Evidence

To date, eight randomized studies have reported spontaneous pregnancy rate after FSH treatment for male idiopathic infertility, compared with placebo [18, 24, 35, 38, 47] or no treatment [17, 21, 48]. Pregnancy rate after ART was evaluated by seven randomized studies [17, 20, 21, 24, 39, 41], only one of which [24] was a randomized, double-blind, placebo-controlled trial.

In the double-blind randomized study by Knuth et al. [47], enrolling men with sperm count between 0.1 and 10×10^6/ml, normal serum FSH and long-lasting male factor idiopathic infertility, two pregnancies were reported in the treatment group ($n = 17$) within 2 months after cessation of treatment with 150 IU human menopausal gonadotropin (hMG) three times a week for 13 weeks in combination with 2.500 IU hCG twice a week. This spontaneous pregnancy rate was within the limits of chance and not significantly different from the zero rate observed in the placebo group ($n = 20$).

Both pFSH and hpFSH (150 IU three times a week) were used by Matorras et al. [17] who enrolled couples undergoing intrauterine insemination (IUI) because of male subfertility, reporting a pregnancy rate per woman of 44.38% (26/58) in the FSH group versus 37.18% (29/78) in the untreated group ($p = 0.47$). In this study, indeed, female factor infertility and primary testicular failure were not ruled out, as up to 44% of the couples reported gynaecological disorders and men with FSH levels below 5 IU/l were excluded.

Highly purified FSH was also used in trials by Baccetti et al. [20] and by Garolla et al. [41]. In the former study, 24 men with idiopathic oligo-asthenozoospermia (FSH level < 12 IU/l) were treated with 150 IU/day hpFSH for 12 weeks. They exhibited a significant improvement in sperm quality, as evaluated by transmission electron microscopy, along with a significantly higher pregnancy rate after ICSI with respect to 20 untreated controls (33.0 vs 20.0%). By using hpFSH treatment (150 IU three times a week for

3 months), very similar cumulative pregnancy rates (33.3 vs 23.2%) after ART (IUI or ICSI) have been reported by Garolla et al. [41] on a larger series of couples (86 cases and 82 untreated controls). In this study, the IUI cohort ($n = 35$) comprised only cases; whereas, the ICSI cohort ($n = 131$) included 82 control subjects and 49 cases [41]. The inclusion criteria for the male partner were: sperm count $< 20 \times 10^6$/ml, normal FSH levels (1–8 IU/l) and history of male factor infertility for at least 2 years. Finally, a multicentre placebo-controlled study by Ding et al. [24], representing the trial with the largest study population ($n = 354$), was conducted in the Chinese population to evaluate the efficacy of different doses of hpFSH (ranging from 50 IU to 300 IU on alternate days) with different treatment lengths (up to 5 months). Only high doses of hpFSH (300 IU on alternate days) for 5 months improved significantly both spontaneous pregnancy rate (30.0 vs 6.8%) and ART pregnancy rate (50.0 vs 18.5%) in comparison to placebo.

In all other randomized studies, rhFSH was used. Kamischke et al. [18] conducted a double-blind randomized placebo-controlled trial primarily aiming at assessing the effect of rhFSH (150 IU daily for 12 weeks) in improving semen parameters in men with male factor idiopathic infertility (at least 2 semen parameters below the World Health Organization criteria, along with FSH < 12 IU/l). Two spontaneous pregnancies in the treated group ($n = 31$) and none in the placebo group ($n = 30$) occurred. Further pregnancies were reported by ART more than 3 months after treatment completion, when, however, any benefit derived from FSH therapy was not expected based on spermatogenesis duration.

More recently, in a study by Foresta et al. [21], 112 men affected by idiopathic oligozoospermia (FSH levels 1–7 IU/l), were randomized to receive rhFSH (100 IU on alternate day for 3 months) or no treatment. A significantly higher spontaneous pregnancy rate was observed in the subgroup of men (5/30, 16.7%) exhibiting a significant increase in sperm count (responder group), with respect to non-responder (1/32, 3.1%) and untreated groups (2/50, 4.0%). Furthermore, the improvement in semen parameters allowed the responder patients to undergo less frequently in vitro fertilization-embryo transfer (IVF-ET)/ICSI than IUI. In a subsequent study by the same group [48], the authors' primary end point was to evaluate the response of idiopathic oligozoospermia to rhFSH (150 IU three times a week for 3 months) in terms of improved sperm count on the basis of Ala307Thr-Asn680Ser FSHR gene polymorphisms. In this study, the difference in spontaneous pregnancy rate between treated ($n = 70$) and untreated group ($n = 35$) did not reach statistical significance (14.8 vs 4.6%). The same regimen and dose of rhFSH have been more recently used by Farrag et al. [39], enrolling 82 men undergoing ICSI for idiopathic male factor infertility (FSH levels ≥ 12 IU/l). In the treatment group, the authors reported a clinical pregnancy rate after ICSI significantly higher compared to the untreated control group (42 vs 20%, respectively, $p < 0.02$).

High doses of rhFSH (300 IU every other day) were used in two double-blind randomized placebo-controlled trials [35, 38], where spontaneous pregnancy was considered only as secondary efficacy outcome and only normogonadotropic men with idiopathic oligozoospermia were enrolled. Although the first study [35], reported four and no pregnancies after rhFSH ($n = 15$) and placebo treatment ($n = 15$), respectively, the low number of events did not allow a meaningful statistical analysis. In the subsequent study by the same authors [38], the difference reached statistical significance: spontaneous pregnancy rate in the treatment group ($n = 45$) was 26.7% while no pregnancy was registered in the placebo group ($n = 15$).

Two meta-analyses were performed in order to comprehensively evaluate whether FSH administration to men with idiopathic male factor infertility could improve pregnancy rates spontaneously and/or after ART [42, 43]. The first meta-analysis, published by the Cochrane Collaboration [42], identified only six valid randomized controlled trials, overall including 457 participants. By meta-analysing available data, authors showed that men who received gonadotropin treatment had almost a fivefold increase in spontaneous pregnancy rate per couple with respect to men receiving placebo or no treatment (OR 4.94, 95% CI 2.13–11.44; $p = 0.0002$). The separate analysis of the four studies reporting spontaneous pregnancy rate only in couples with no female factor reached the same results (OR 5.00, 95% CI 1.88–13.34; $p = 0.001$). On the contrary, no significant difference between the two groups in terms of pregnancy rate after ART (IUI or ICSI) was observed. Recently, in another meta-analysis [43] including 15 trials (614 men treated with FSH and 661 men treated with placebo or untreated), concluded that men receiving gonadotropins do not only exhibit a 4.5-fold increase in spontaneous pregnancy rate (95% CI 2.17–9.33, $p < 0.0001$), but also a significantly higher pregnancy rate after ART (OR 1.60, 95% CI 1.08–2.37, $p = 0.02$). This meta-analysis also included non-randomized studies [33, 49], a crossover study by Ben-Rafael et al. [19] and a randomized study by Foresta et al. [50], where rhFSH was administered to patients with severe testiculopathy after gonadotropin suppression by gonadotropin-releasing hormone agonist (GnRH-a).

Value

The quality of the evidence supporting the use of FSH in normogonadotropic male partner of couples with idiopathic male factor infertility to improve spontaneous pregnancy rate was rated as low. The quality of the evidence to

suggest the use of FSH in normogonadotropic male partner of couples with idiopathic male factor infertility for improving pregnancy rate after ART was rated as very low.

Remarks

Although all randomized controlled trials demonstrated a treatment effect in the same direction, favouring gonadotropin therapy, a number of limitations decrease the quality of evidence supporting the recommendations. Firstly, evidence is downgraded by the low number of RCTs, involving few participants and documenting few outcomes with quite large confidence intervals. Indeed, although pooling different studies in the meta-analyses showed positive outcomes in terms of spontaneous pregnancy rate [42, 43] and ART-related pregnancy rate [43] after FSH treatment, the collective sample sizes did not allow to achieve adequate power and precision of the overall estimates. To date, only a double-blind randomized placebo-controlled trial has reported pregnancy rates after ART [24]. Secondly, a methodology limitation arises from a possible attrition bias, as only five studies [17, 18, 21, 24, 39], among the available RCTs, correctly reported the dropout rate and the evaluation of data after dropout. Thirdly, enrolled infertile populations were quite heterogeneous with often unknown female factor and different types of gonadotropins in different unstandardized empirical regimes and doses, with different follow-up lengths were used. In the meta-analysis by Santi et al. [43], sub-dividing studies according to the FSH preparations (purified/recombinant), the improvement in spontaneous pregnancy rate remained significant for each preparation. Nevertheless, as no study compared the efficacy of different FSH preparations, we cannot suggest specific types of gonadotropins. Similarly, clinical efficacy of increasing FSH dosage in cases of unresponsiveness to standard dosage, is still unclear and detailed therapeutic regimes and doses cannot be recommended, as only one study [24] compared the effects of increasing doses of hpFSH (50, 100, 200 and 300 IU on alternate days up to 5 months). In that study, although the sperm number was significantly increased beginning at the third month of FSH treatment at the dose of 200 IU, only the highest doses (300 IU on alternate days) for 5 months significantly improved both spontaneous pregnancy rate and ART pregnancy rate in comparison to placebo.

Effects of FSH on sperm DNA integrity

Recommendation

1. We suggest administering FSH (either recombinant or purified) to idiopathic infertile patients, especially in those with high values of basal sperm DNA fragmentation, to improve sperm chromatin integrity (2 ØØOO).

Evidence

DNA fragmentation consists of single (SSB) and double DNA strand breaks (DSB) and may occur from impaired chromatin condensation and protamination defects, which are consequences of apoptotic process and/or oxidative damage from free radicals. Those DSB, if unrepaired, may induce mutations, genome instability and cellular death. Thus, genome integrity must be controlled by a sophisticated cellular mechanism called DNA Damage Response network, which includes various proteins, able to respond to genotoxic stress in order to protect the organism by repairing DNA damage. Both the amount of damage and the repair capabilities of the cell will influence the outcome: DSB persistence will lead to apoptosis or cellular senescence to prevent the store of DNA mutations [51–54]. Sperm DNA damage has been associated with impaired spermatogenesis and infertility with negative consequences on biological events such as fertilization and embryonic development [55, 56].

In natural conception, 50–70% of spontaneous pregnancy losses can be attributed to aneuploidies, above all trisomies, and the remaining 30–50% are unexplained, but may be due to as-yet unknown epigenetic or genetic factors [57]. Sperm DNA damage, including chromatin fragmentation, has been associated with recurrent pregnancy loss [58]. Moreover, a strong association was found between DNA damage and failure to achieve natural pregnancy, while high levels of DNA damage were also associated with a low pregnancy rate following ART [59].

Numerous methods have been developed to evaluate sperm DNA integrity, with the aim of establishing the degree of chromatin condensation. Aniline blue and Chromomycin A3 are used to evaluate the histone–protamine replacement process. Under normal conditions, optimal chromatin condensation can be considered as a replacement level of 85%. Terminal deoxynucleotidyl transferase UTP-driven nick end labelling (TUNEL) and COMET assay are the most widely used methods for evaluating sperm chromatin integrity. TUNEL detects the presence of endogenous DNA strand breaks in sperm through use the enzyme terminal deoxynucleotidyl transferase (TdT) [60]. The COMET assay enables DNA integrity to be evaluated by visualizing strand breaks in individual cells with single cell

gel electrophoresis. Fluorescence in situ and hybridization (FISH) techniques have been used to evaluate the presence of sperm aneuploidies.

Although the assessment of sperm chromatin integrity is still unstandardized and cannot be routinely used in the evaluation of infertile patients, recent evidence reveals it as a marker of male reproductive potential [61–63].

Few papers have explored the effect of FSH treatment on DNA fragmentation. Colacurci et al. [23] detected an improvement of sperm DNA fragmentation in oligoasthenozoospermic patients treated with rhFSH (150 UI every other day). Ruvolo et al. [64] reported similar results. Garolla et al. [41] confirmed the previous observations, administering hpFSH. All these authors agree that the subset of patients that benefit most from the therapy is that with the highest basal DNA fragmentation index (DFI), indicating DFI > 15% as a possible cut-off. Simoni et al. [65], while confirming DFI improvements after FSH therapy, underline that such improvement was significant only in patients carrying a homozygous wild-type N genotype for the p.N680S allele of the FSH receptor (*FSHR*) gene. Therefore, this observation seems to indicate *FSHR* gene genotype as a possible marker of response to therapy, regardless of pretherapy sperm quality. While results on sperm DNA fragmentation are encouraging, FSH therapy does not seem to be clearly associated with a reduction of chromosomal aneuploidies, detected through FISH. In fact, while Piomboni et al. [66] detected improvements of aneuploidies in a small series of 22 men undergoing rhFSH 150 UI every other day for 3 months, the already cited paper by Garolla et al. [41] could not find significant differences in the percentage of sperm aneuploidies before and after therapy.

Value

Evidence supporting the use of FSH in idiopathic infertile patients to improve sperm DNA integrity is of low quality. The task force places higher value in patients with high degrees of basal sperm DNA fragmentation.

Remarks

In consideration of the limited evidence provided by the few available papers, it is advisable to suggest to idiopathic infertile patients to undergo FSH therapy especially in the presence of high levels of DFI. However, lack of standardization and infrequent employment in a routine clinical setting do not allow identifying a reliable DFI cut-off level predictive of chromatin integrity improvement. Further studies are needed to better define the subgroup of patient who will benefit from therapy, as well as the appropriateness of utilization of techniques evaluating sperm DNA fragmentation.

Predictors of response to FSH treatment

Recommendations

1. We recommend considering FSH treatment in idiopathic oligozoospermic infertile men only when FSH plasma concentrations are in the normal range (1 ØØØØ).
2. We suggest not using FSH treatment in oligozoospermic infertile men with hypospermatogenesis associated with maturational disturbances at the spermatid level (2 ØØOO).
3. We suggest to use the analysis of polymorphisms on the *FSHR* and *FSHB* genes to predict the clinical response to FSH treatment only for research purposes (2 ØØOO).

Evidence

Semen abnormalities may be sustained by various alterations of the seminiferous epithelium, which could explain the failure of FSH therapy reported by some studies [17, 18, 67]. In fact, FSH therapy induces significant improvements only in a proportion of infertile patients. Therefore, a careful and complete diagnostic workup of the infertile male partner is mandatory to derive predictive information on the response to FSH treatment.

In 1968, Baccetti et al. [7] evaluated the effects of FSH treatment on the quality of human spermatozoa and pregnancy by examining the sperm ultrastructure of 81 infertile patients. Using spermatozoa as andrological biomarkers, they showed that the therapeutic effect of FSH depends on the type of sperm defect. Certain alterations regarding the acrosome, the chromatin, the mitochondria, and the axoneme appeared to be sensitive to FSH. All responders had spermatozoa affected by immaturity or apoptosis, suggesting that the success of therapy was predictable. Also Strehler et al. [27] investigated the effect of FSH administration in order to evaluate its potential for improving sperm ultrastructure. Forty-six patients with OAT attending assisted reproduction received 150 IU FSH in daily dosages of over a period of 12 weeks. Using transmission electron microscopy to examine subcellular organelles after the FSH treatment, they observed a higher percentage of integrity leading to a higher number of morphologically normal spermatozoa. These findings suggested that treatment with FSH can be an effective way to improve sperm quality particularly in those cases of OAT associated with ultrastructural sperm alteration.

Recombinant human FSH was tested in a randomized, double-blind, placebo-controlled study by Kamischke et al. [18] to examine its role in male idiopathic infertility. A total of 67 patients were randomized to treatment

(12 weeks with 150 IU rhFSH) or to placebo. In the treated group, testicular volume and DNA condensation were improved compared to placebo and baseline. This suggested looking for parameters that might identify patients who may benefit from FSH treatment.

In a prospective, controlled, randomized clinical study, Foresta et al. [21] evaluated the effects of FSH treatment on seminal parameters and spontaneous pregnancy in 112 infertile men affected by idiopathic oligozoospermia. Sixty-two subjects were treated with 100 IU of rhFSH on alternate days for 3 months, and 50 patients did not receive any treatment. Results showed that FSH therapy does not improve sperm concentration or pregnancy rate when infertile male patients are chosen solely by the clinical criteria of idiopathic oligospermia and normal FSH concentration. Subgroup analysis indicated that selected patients might benefit from medical therapy in terms of better sperm parameters and fertility outcome. Because subjects with high FSH plasma levels do not benefit from FSH treatment, in 2009 Foresta et al. [50] performed a prospective, controlled, randomized clinical study to evaluate the effect of recombinant FSH plus hCG on seminal parameters and pregnancy rate when high FSH plasma concentrations have been suppressed. Eighty-seven men affected by severe testiculopathy were included, 57 were treated with a GnRH-a and then with rhFSH and hCG, and 30 patients did not receive any treatment. Results from this trial showed that FSH therapy improves sperm parameters in severe male factor infertility when endogenous high FSH plasma levels are suppressed.

Three studies performed by the same research group, demonstrated that the knowledge of the tubular status obtained by testicular fine needle aspiration cytology (FNAC), enables to predict the positive response to FSH therapy in oligozoospermic patients [46, 68, 69]. In a placebo-controlled, double-blind randomized clinical study, Foresta et al. [68] evaluated tubular status and semen parameters of 90 oligozoospermic subjects with normal FSH plasma levels before and after FSH treatment. After 3 months of treatment, they reported no improvement of sperm parameters in placebo-treated patients. Among FSH-treated patients, 30% responded to FSH treatment at least doubling sperm count. Among patients who did not respond, the results of pretreatment testicular cytologic examination were consistent with hypospermatogenesis associated with maturational disturbances at the spermatid level. In contrast, patients who responded to treatment with FSH had isolated hypospermatogenesis without maturational disturbances. In a randomized single-blind study published in 2002, Foresta et al. [46] tested the effects of treatment with rhFSH on seminal parameters and seminiferous epithelium in 45 idiopathic patients with oligozoospermia and normal FSH plasma levels. Fifteen subjects underwent 3 months of treatment with 50 IU, 15 with 100 IU on alternate days or no treatment. The findings of this study demonstrated that rhFSH at a dose of 100 IU, increases the spermatogonial population and sperm production in idiopathic oligozoospermic patients with normal FSH and a cytological picture of hypospermatogenesis with no maturation arrest. In another experimental controlled study, Garolla et al. [69] compared the predictive power of spermatid count in semen and FNAC for ART outcome after FSH therapy. A total of 174 men with severe oligozoospermia and normal plasma FSH concentration were included. Ninety-two men with hypospermatogenesis received FSH therapy for 3 months. The authors reported a strong relation between higher spermatid count and hypospermatogenesis with maturative disturbance. FSH therapy showed significant improvement in sperm parameters and natural or assisted fertility in patients with lower spermatid count. This study suggested that spermatid count could represent a new predictor of response to FSH therapy.

It has been shown that some polymorphisms in the *FSHR* gene are able to influence the expression and/or sensitivity of the receptor for the hormone and the reproductive parameters both in men and women [70–73] The two most common SNPs in the coding region occur at nucleotides 919 and 2039 in exon 10, in which A/G transitions cause amino acid exchange from threonine (Thr) to alanine (Ala) at codon 307 and from asparagines (Asn) to serine (Ser) at codon 680, respectively [74, 75]. There is a linkage between these polymorphic sites and the two SNPs resulting in two major, almost equally common allelic variants in the Caucasian population, Thr307-Asn680 (TN) and Ala307-Ser680 (AS), producing two distinct receptor isoforms [76], leading to three genotypes (TN/TN, TN/AS and AS/AS). Moreover, molecular studies on the *FSHB* gene (coding for the β-subunit of FSH) showed that a G/T single-nucleotide polymorphism located in the *FSHB* gene promoter (− 211 bp from the mRNA transcription start site; rs10835638) is responsible for the endogenous FSH level [77, 78]. The combination of G/T SNP can lead to three genotypes: homozygous TT and GG and heterozygous GT.

Based on these findings, some authors suggested that different genetic polymorphisms observed in *FSHR* and *FSHB* genes could influence the response to exogenous FSH administration [40, 48, 65, 79]. In a controlled, randomized, clinical study performed by Selice et al. [48], the authors evaluated the response of recombinant FSH treatment in terms of sperm production based on Ala307Thr-Asn680Ser polymorphisms in the *FSHR* gene in a group of 105 oligozoospermic subjects with hypospermatogenesis and normal FSH levels. Seventy patients were randomized to treatment group (150 IU thrice per week for 3 months) and 35 to non-treatment group. When treated subjects were subdivided based on *FSHR* genotype, only subjects with at least one serine in position 680 (homozygous AS/AS and heterozygous TN/AS) had a significant improvement

of seminal parameters, whereas homozygote subjects for Thr307-Asn680 (TN/TN) showed no difference. This study suggested that the analysis of this gene could represent a valid pharmacogenetic approach to predict the response to FSH treatment.

In order to verify whether an SNP in the *FSHB* gene promoter could be a pharmacogenetic tool for the treatment of male infertility with FSH, Ferlin et al. [79] performed a cross-sectional and prospective study. They evaluated 514 subjects with non-obstructive azoospermia and oligozoospermia and 248 subjects with normozoospermia. FSHB − 211 TT genotype was associated with significantly lower FSH levels compared with GG and GT genotypes. Treatment with FSH induced a significantly higher improvement in sperm count and quality in TT homozygotes regarding carriers of the G allele. This study suggested that *FSHB* − 211 TT genotype might represent a valid pharmacogenetic predictor for identification of potential responders to FSH treatment. Nevertheless, more recently, a prospective study on 40 patients affected by idiopathic OAT did not identify any specific subgroup of "responders" in terms of total sperm count and total motile sperm count based on the *FSHB* and *FSHR* polymorphisms [40].

Value

The evidence supporting the measurement of FSH plasma concentrations (within the normal range) to select infertile men undergoing FSH therapy is of high quality. There is low evidence to advice against FSH treatment in those oligozoospermic men with maturation arrest of spermatogenesis. There is currently not enough quality of evidence to recommend the use of a pharmacogenetic approach to predict the response to the FSH treatment in clinical practice: the analysis of *FSHR* and *FSHB* genes could be suggested for experimental purposes.

Remarks

Randomized controlled trials selecting oligozoospermic patients with normal FSH plasma levels demonstrated positive effects of the standard FSH treatment (150 IU, three times a week, for 3 months) on sperm parameters and natural or assisted fertility.

Although RCTs demonstrated that patients with spermatid arrest (observed both by FNAC and spermatid count in semen) showed absent or limited response to FSH treatment, the major limitation is that all these studies were performed by the same group [46, 68, 69].

Regarding the pharmacogenetic approach to FSH treatment, the four available studies have contradictory results and no RCTs are available [40, 48, 65, 79]. Although this is a new and promising field of medicine, the pharmacogenetic approach in relationship with FSH treatment requires large multicentric studies in order to provide evidence for its clinical use. In fact, while Selice et al. [48] and Ferlin et al. [79] indicated higher responsiveness in patients carrying the polymorphic variants in *FSHR* and *FSHB* genes, the study by Simoni et al. [65] reported a significant positive effect in men with homozygous wild-type genotype of the *FSHR* and Casamonti et al. [40] observed a similar frequency of responders in all *FSHR* and *FSHB* genotypes.

Conclusions and future directions

Both available meta-analyses have demonstrated a significant positive effect of FSH therapy on sperm parameters and pregnancy rate in men with altered sperm parameters and normal FSH levels [42, 43]. However, studies are extremely heterogeneous in many aspects: (i) patient selection criteria; (ii) primary and secondary end-points; (iii) FSH doses and type of FSH; (iv) duration of treatment. It is therefore clear that future large studies are necessary to better define to whom, at which doses and for how long we should prescribe FSH.

In the large majority of studies, FSH administration (both hpFSH and rhFSH) have a significant positive effect both on quantitative and qualitative sperm parameters in about 50% of treated patients. This combined effect is typically observed after 3 months of treatment, which corresponds to the length of spermatogenesis (about 72 days). Based on the hypothesis that FSH acts at the early germ cell stages, some authors speculated on a higher efficacy of a longer therapeutic regimen. However, the few studies, based on 4 or 5 months treatments, did not clearly demonstrate a higher proportion of "responders" in respect to the standard regimen. On the other hand, a recent Italian study [40] has evaluated the effect of FSH treatment on the latest phase of spermatogenesis (called "spermiogenesis"). In this study, a significant improvement of sperm maturation, expressed in terms of higher sperm hyaluronic acid binding capacity, has been observed already after 1 month of hpFSH (with a 300 IU weekly dose) with a further increase after 3 months of treatment. The return of sperm hyaluronic acid binding capacity value to the baseline values, after the washout period, strongly supports that the observed effect is truly due to the administration of FSH.

Based on the clinical trials discussed in the present article, we can speculate that FSH has a double action on both spermatogonia and round spermatids. This double action can be used in different clinical contexts; for instance, a long-term (at least 3 months) treatment will increase sperm parameters with an ultimate potential benefit on spontaneous pregnancy rate. On the other hand, a 1-month therapy could be indicated prior to ART, with

the aim to increase the proportion of functionally mature spermatozoa with consequent higher likelihood of assisted pregnancy [80]. In order to verify the clinical value of the short-term regimen, a placebo-controlled study with the primary end-point of assisted pregnancy is urgently needed. In addition, basic research, aimed at unrevealing the physiological mechanisms of FSH action on germ cell proliferation and maturation, is needed to help clinicians to define the most appropriate treatment length.

Idiopathic infertility is a heterogeneous etiologic category in which many different, yet unknown genetic/epigenetic factors are likely to be involved [2, 3]. Since these etiologic factors are not necessary related to hormonal regulation of spermatogenesis, it is plausible that only a portion of idiopathic OAT men respond to FSH therapy. One of the most challenging aspects of FSH treatment consists in our ability to predict responsiveness prior therapy. In this respect, pharmacogenetics [40, 48, 65, 79] has been proposed as a potential predictive tool. The hypothesis behind the pharmacogenetic approach is based on genetic polymorphisms, able to modulate FSH synthesis and action. Hence, one of the most likely "responsive" subgroup is expected to include those individuals who have a genetically determined low capacity either to synthesize (polymorphism in the promoter region of *FSHB* gene) and/or to respond to FSH (polymorphisms in the *FSHR* gene) [81]. Such a "functional central hypogonadism" could be responsible for the inappropriately normal FSH levels in the presence of oligozoospermia and the administration of exogenous FSH could overcome this functional defect. Clearly, such a pharmacogenetic approach could convert FSH treatment in idiopathic infertile men from empiric into rational therapy. Unfortunately, data in the literature are scarce and inconclusive, underlying the need for future clinical trials on large study populations, with the possibility to analyse the combined effect of the above polymorphisms. Apart from "functional central hypogonadism", we can also expect that a "reinforced" FSH action may be efficient also in oligozoospermia caused by mutations in FSH responsive genes. Again, basic research and future genetic studies in infertile men will likely to provide a more rational basis for the selection of the best candidates for FSH therapy.

Our medical armamentarium in idiopathic OAT is frustratingly scarce and FSH treatment represents a glimmer of hope for these patients. Although more clinical and basic studies are needed to better define the best therapeutic regimen, current data are encouraging and suggest that a 3-month FSH therapy with a minimum weekly dose of 300 IU can be attempted in idiopathic OAT with the aim to improve both quantitative and qualitative sperm parameters and pregnancy rate.

Funding No specific funding was used in relation to this manuscript

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Does not apply to this kind of paper.

References

1. Isaksson R, Tiitinen A (2004) Present concept of unexplained infertility. Gynecol Endocrinol 18(5):278–290
2. Krausz C (2011) Male infertility: pathogenesis and clinical diagnosis. Best Pract Res Clin Endocrinol Metab 25(2):271–285
3. Tournaye H, Krausz C, Oates RD (2017) Novel concepts in the aetiology of male reproductive impairment. Lancet Diabetes Endocrinol 5(7):544–553
4. Wang SX (2011) The past, present, and future of embryo selection in in vitro fertilization: frontiers in reproduction conference. Yale J Biol Med 84(4):487–490
5. Singh K, Jaiswal D (2011) Human male infertility: a complex multifactorial phenotype. Reprod Sci 18(5):418–425
6. Hirsh A (2003) Male subfertility. BMJ 327(7416):669–672
7. Baccetti B, Strehler E, Capitani S, Collodel G, De Santo M, Moretti E et al (1997) The effect of follicle-stimulating hormone therapy on human sperm structure (Notulae seminologicae). Hum Reprod 12(9):1955–1968
8. Kliesch S, Behre HM, Nieschlag E (1995) Recombinant human follicle stimulating hormone and human chorionic gonadotropin for induction of spermatogenesis in a hypogonadal male. Fertil Steril 63(6):1326–1328
9. van Rijkom J, Leufkens H, Crommelin D, Rutten F, Broekmans A (1999) Assessment of biotechnology drugs: what are the issues? Health Policy 47(3):255–274
10. Zwart-van Rijkom JE, Broekmans FJ, Leufkens HG (2002) From HMG through purified urinary FSH preparations to recombinant FSH: a substitution study. Hum Reprod 17(4):857–865
11. Vicari E, Mongioì A, Calogero AE, Moncada ML, Sidoti G, Polosa P, D'Agata R (1992) Therapy with human chorionic gonadotrophin alone induces spermatogenesis in men with isolated hypogonadotrophic hypogonadism-long-term follow-up. Int J Androl 15(4):320–329
12. Zacharin M, Sabin MA, Nair VV, Dabadghao P (2012) Addition of recombinant follicle-stimulating hormone to human chorionic gonadotropin treatment in adolescents and young adults with hypogonadotropic hypogonadism promotes normal testicular growth and may promote early spermatogenesis. Fertil Steril 98(4):836–842
13. La Vignera S, Condorelli RA, Cimino L, Russo GI, Morgia G, Calogero AE (2016) Late-onset hypogonadism: the advantages of treatment with human chorionic gonadotropin rather than testosterone. Aging Male 19(1):34–39
14. Acosta AA, Oehninger S, Ertunc H, Philput C (1991) Possible role of pure human follicle-stimulating hormone in the treatment of severe male infertility by assisted reproduction: preliminary report. Fertil Steril 55(6):1150–1156
15. Acosta AA, Khalifa E, Oehninger S (1992) Pure human follicle stimulating hormone has a role in the treatment of severe male

infertility by assisted reproduction: Norfolk's total experience. Hum Reprod 7(8):1067–1072
16. Bartoov B, Eltes F, Lunenfeld E, Har-Even D, Lederman H, Lunenfeld B (1994) Sperm quality of subfertile males before and after treatment with human follicle-stimulating hormone. Fertil Steril 61(4):727–734
17. Matorras R, Perez C, Corcostegui B, Pijoan JI, Ramon O, Delgado P et al (1997) Treatment of the male with follicle-stimulating hormone in intrauterine insemination with husband's spermatozoa: a randomized study. Hum Reprod 12(1):24–28
18. Kamischke A, Behre HM, Bergmann M, Simoni M, Schäfer T, Nieschlag E (1998) Recombinant human follicle stimulating hormone for treatment of male idiopathic infertility: a randomized, double-blind, placebo-controlled, clinical trial. Hum Reprod 13(3):596–603
19. Ben-Rafael Z, Farhi J, Feldberg D, Bartoov B, Kovo M, Eltes F et al (2000) Follicle-stimulating hormone treatment for men with idiopathic oligoteratoasthenozoospermia before in vitro fertilization: the impact on sperm microstructure and fertilization potential. Fertil Steril 73(1):24–30
20. Baccetti B, Piomboni P, Bruni E, Capitani S, Gambera L, Moretti E et al (2004) Effect of follicle-stimulating hormone on sperm quality and pregnancy rate. Asian J Androl 6(2):133–137
21. Foresta C, Bettella A, Garolla A, Ambrosini G, Ferlin A (2005) Treatment of male idiopathic infertility with recombinant human follicle-stimulating hormone: a prospective, controlled, randomized clinical study. Fertil Steril 84(3):654–661
22. Efesoy O, Cayan S, Akbay E (2009) 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 30(6):679–684
23. Colacurci N, Monti MG, Fornaro F, Izzo G, Izzo P, Trotta C et al (2012) Recombinant human FSH reduces sperm DNA fragmentation in men with idiopathic oligoasthenoteratozoospermia. J Androl 33(4):588–593
24. Ding YM, Zhang XJ, Li JP, Chen SS, Zhang RT, Tan WL et al (2015) 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) 83(6):866–871
25. Sigg C, Baclu M (1994) Improvement of sperm concentration and bovine mucus penetration in idiopathic oligozoospermia by administration of pure FSH. Hum Reprod 9(Suppl 4):47
26. Merino G, Carranza-Lira S, Martinez-Chequer JC (1996) Sperm characteristics and hormonal profile before and after treatment with follicle-stimulating hormone in infertile patients. Arch Androl 37(3):197–200
27. Strehler E, Sterzik K, De Santo M, Abt M, Wiedemann R, Bellati U et al (1997) The effect of follicle-stimulating hormone therapy on sperm quality: an ultrastructural mathematical evaluation. J Androl 18(4):439–447
28. Glander HJ, Kratzsch J (1997) Effects of pure human follicle stimulating hormone (pFSH) on sperm quality correlate with the hypophyseal response to gonadotropin-releasing hormone (GnRH). Andrologia 29(1):23–28
29. Radicioni A, Schwarzenberg TL (1999) The use of FSH in adolescents and young adults with idiopathic, unilateral, left varicocele not undergoing surgical intervention. Preliminary study. Minerva Endocrinol 24(2):63–68
30. Arnaldi G, Balercia G, Barbatelli G, Mantero F (2000) Effects of long-term treatment with human pure follicle-stimulating hormone on semen parameters and sperm-cell ultrastructure in idiopathic oligoteratoasthenozoospermia. Andrologia 32(3):155–161
31. Foresta C, Bettella A, Merico M, Garolla A, Plebani M, Ferlin A et al (2000) FSH in the treatment of oligozoospermia. Mol Cell Endocrinol 161(1–2):89–97
32. Zarrilli S, Paesano L, Colao A, Mirone V, Lombardi G, De Rosa M (2000) FSH treatment improves sperm function in patients after varicocelectomy. J Endocrinol Invest 23(2):68–73
33. Caroppo E, Niederberger C, Vizziello GM, D'Amato G (2003) Recombinant human follicle-stimulating hormone as a pretreatment for idiopathic oligoasthenoteratozoospermic patients undergoing intracytoplasmic sperm injection. Fertil Steril 80(6):1398–1403
34. Fernández-Arjona M, Díaz J, Cortes I, González J, Rodriguez JM, Alvarez E (2003) 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 107(1):47–51
35. Paradisi R, Busacchi P, Seracchioli R, Porcu E, Venturoli S (2006) Effects of high doses of recombinant human follicle-stimulating hormone in the treatment of male factor infertility: results of a pilot study. Fertil Steril 86(3):728–731
36. Valenti D, La Vignera S, Condorelli RA, Rago R, Barone N, Vicari E et al (2013) Follicle-stimulating hormone treatment in normogonadotropic infertile men. Nat Rev Urol 10(1):55–62
37. Condorelli RA, Calogero AE, Vicari E, Mongioi' L, Burgio G, Cannarella R et al (2014) Reduced seminal concentration of CD45pos cells after follicle-stimulating hormone treatment in selected patients with idiopathic oligoasthenoteratozoospermia. Int J Endocrinol 2014:372060
38. Paradisi R, Natali F, Fabbri R, Battaglia C, Seracchioli R, Venturoli S (2014) Evidence for a stimulatory role of high doses of recombinant human follicle-stimulating hormone in the treatment of male-factor infertility. Andrologia 46(9):1067–1072
39. Farrag A, Sagnella F, Pappalardo S, Costantini A, Lisi F, Carfagna P et al (2015) The use of r-hFSH in treatment of idiopathic male factor infertility before ICSI. Eur Rev Med Pharmacol Sci 19(12):2162–2167
40. Casamonti E, Vinci S, Serra E, Fino MG, Brilli S, Lotti F et al (2017) Short-term FSH treatment and sperm maturation: a prospective study in idiopathic infertile men. Andrology 5(3):414–422
41. Garolla A, Ghezzi M, Cosci I, Sartini B, Bottacin A, Engl B et al (2017) FSH treatment in infertile males candidate to assisted reproduction improved sperm DNA fragmentation and pregnancy rate. Endocrine 56(2):416–425
42. Attia AM, Abou-Setta AM, Al-Inany HG (2013) Gonadotrophins for idiopathic male factor subfertility. Cochrane Database Syst Rev 23(8):CD005071
43. Santi D, Granata AR, Simoni M (2015) FSH treatment of male idiopathic infertility improves pregnancy rate: a meta-analysis. Endocr Connect 4(3):R46–R58
44. Swiglo BA, Murad MH, Schünemann HJ, Kunz R, Vigersky RA, Guyatt GH et al (2008) 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 93(3):666–673
45. Palomba S, Falbo A, Espinola S, Rocca M, Capasso S, Cappiello F et al (2011) Effects of highly purified follicle-stimulating hormone on sperm DNA damage in men with male idiopathic subfertility: a pilot study. J Endocrinol Invest 34(10):747–752
46. Foresta C, Bettella A, Merico M, Garolla A, Ferlin A, Rossato M (2002) Use of recombinant human follicle-stimulating hormone in the treatment of male factor infertility. Fertil Steril 77(2):238–244
47. Knuth UA, Honigl W, Bals-Pratsch M, Schleicher G, Nieschlag E (1987) Treatment of severe oligospermia with human chorionic gonadotropin/human menopausal gonadotropin: a placebo-controlled, double blind trial. J Clin Endocrinol Metab 65(6):1081–1087

48. Selice R, Garolla A, Pengo M, Caretta N, Ferlin A, Foresta C (2011) The response to FSH treatment in oligozoospermic men depends on FSH receptor gene polymorphisms. Int J Androl 34(4):306–312
49. Ashkenazi J, Bar-Hava I, Farhi J, Levy T, Feldberg D, Orvieto R et al (1999) The role of purified follicle stimulating hormone therapy in the male partner before intracytoplasmic sperm injection. Fertil Steril 72(4):670–673
50. Foresta C, Selice R, Moretti A, Pati MA, Carraro M, Engl B et al (2009) Gonadotropin administration after gonadotropin-releasing-hormone agonist: a therapeutic option in severe testiculopathies. Fertil Steril 92(4):1326–1332
51. Zini A (2011) Are sperm chromatin and DNA defects relevant in the clinic? Syst Biol Reprod Med 57(1–2):78–85
52. Smit M, van Casteren NJ, Wildhagen MF, Romijn JC, Dohle GR (2010) Sperm DNA integrity in cancer patients before and after cytotoxic treatment. Hum Reprod 25(8):1877–1883
53. Ribas-Maynou J, Gawecka JE, Benet J, Ward WS (2014) Double-stranded DNA breaks hidden in the neutral Comet assay suggest a role of the sperm nuclear matrix in DNA integrity maintenance. Mol Hum Reprod 20(4):330–340
54. Muratori M, Tamburrino L, Marchiani S, Cambi M, Olivito B, Azzari C et al (2015) Investigation on the origin of sperm DNA Fragmentation: role of apoptosis, immaturity and oxidative stress. Mol Med 21:109–122
55. Zini A, Bielecki R, Phang D, Zenzes MT (2001) Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril 75(4):674–677
56. Zini A, Boman JM, Belzile E, Ciampi A (2008) Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 23(12):2663–2668
57. Carrell DT, Wilcox AL, Lowy L, Peterson CM, Jones KP, Erickson L et al (2003) Elevated sperm chromosome aneuploidy and apoptosis in patients with unexplained recurrent pregnancy loss. Obstet Gynecol 101(6):1229–1235
58. Carlini T, Paoli D, Pelloni M, Faja F, Dal Lago A, Lombardo F et al (2017) Sperm DNA fragmentation in Italian couples with recurrent pregnancy loss. Reprod Biomed Online 34(1):58–65
59. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S et al (2012) The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. Hum Reprod 27(10):2908–2917
60. Muratori M, Forti G, Baldi E (2008) Comparing flow cytometry and fluorescence microscopy for analyzing human sperm DNA fragmentation by TUNEL labeling. Cytom A 73(9):785–787
61. Evenson DP, Wixon R (2006) Clinical aspects of sperm DNA fragmentation detection and male infertility. Theriogenology 65(5):979–991
62. Castillo J, Estanyol JM, Ballesca JL, Oliva R (2015) Human sperm chromatin epigenetic potential: genomics, proteomics, and male infertility. Asian J Androl 17(4):601–609
63. Simon L, Zini A, Dyachenko A, Ciampi A, Carrell DT (2017) A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. Asian J Androl 19(1):80–90
64. Ruvolo G, Roccheri MC, Brucculeri AM, Longobardi S, Cittadini E, Bosco L (2013) Lower sperm DNA fragmentation after r-FSH administration in functional hypogonadotropic hypogonadism. J Assist Reprod Genet 30(4):497–503
65. Simoni M, Santi D, Negri L, Hoffmann I, Muratori M, Baldi E et al (2016) Treatment with human, recombinant FSH improves sperm DNA fragmentation in idiopathic infertile men depending on the FSH receptor polymorphism p. N680S: a pharmacogenetic study. Hum Reprod 31(9):1960–1969
66. Piomboni P, Serafini F, Gambera L, Musacchio C, Collodel G, Morgante G et al (2009) Sperm aneuploidies after human recombinant follicle stimulating hormone therapy in infertile males. Reprod Biomed Online 18(5):622–629
67. Attia AM, Al-Inany HG, Farquhar C, Proctor M (2007) Gonadotrophins for idiopathic male factor subfertility. Cochrane Database Syst Rev 17:CD005071
68. Foresta C, Bettella A, Ferlin A, Garolla A, Rossato M (1998) Evidence for a stimulatory role of follicle-stimulating hormone on the spermatogonial population in adult males. Fertil Steril 69(4):636–642
69. Garolla A, Selice R, Engl B, Bertoldo A, Menegazzo M, Finos L et al (2014) Spermatid count as a predictor of response to FSH therapy. Reprod Biomed Online 29(1):102–112
70. Aittomaki K, Lucena JL, Pakarinen P, Sistonen P, Tapanainen J, Gromoll J et al (1995) Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. Cell 82(6):959–968
71. Behre HM, Greb RR, Mempel A, Sonntag B, Kiesel L, Kaltwasser P et al (2005) Significance of a common single nucleotide polymorphism in exon 10 of the follicle-stimulating hormone (FSH) receptor gene for the ovarian response to FSH: a pharmacogenetic approach to controlled ovarian hyperstimulation. Pharmacogenetics Genomics 15(7):451–456
72. Greb RR, Grieshaber K, Gromoll J, Sonntag B, Nieschlag E, Kiesel L et al (2005) A common single nucleotide polymorphism in exon 10 of the human follicle stimulating hormone receptor is a major determinant of length and hormonal dynamics of the menstrual cycle. J Clin Endocrinol Metab 90(8):4866–4872
73. Tuttelmann F, Laan M, Grigorova M, Punab M, Sober S, Gromoll J (2012) Combined effects of the variants FSHB-211G > T and FSHR 2039A > G on male reproductive parameters. J Clin Endocrinol Metab 97(10):3639–3647
74. Simoni M, Gromoll J, Nieschlag E (1997) The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology and pathophysiology. Endocr Rev 18(6):739–773
75. Liu JY, Gromoll J, Cedars MI, La Barbera AR (1998) Identification of allelic variants in the follicle-stimulating hormone receptor genes of females with or without hypergonadotropic amenorrhea. Fertil Steril 70(2):326–331
76. Simoni M, Gromoll J, Hoppner W, Kamischke A, Krafft T, Stahle D et al (1999) Mutational analysis of the follicle-stimulating hormone (FSH) receptor in normal and infertile men: identification and characterization of two discrete FSH receptor isoforms. J Clin Endocrinol Metab 84(2):751–755
77. Grigorova M, Rull K, Laan M (2007) Haplotype structure of FSHB, the beta-subunit gene for fertility-associated follicle-stimulating hormone: possible influence of balancing selection. Ann Hum Genet 71(Pt 1):18–28
78. Grigorova M, Punab M, Ausmees K, Laan M (2008) FSHB promoter polymorphism within evolutionary conserved element is associated with serum FSH level in men. Hum Reprod 23(9):2160–2166
79. Ferlin A, Vinanzi C, Selice R, Garolla A, Frigo AC, Foresta C (2011) Toward a pharmacogenetic approach to male infertility: polymorphism of follicle-stimulating hormone beta-subunit promoter. Fertil Steril 96(6):1344–1349
80. Worrilow KC, Eid S, Woodhouse D, Perloe M, Smith S, Witmyer J et al (2013) Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): significant improvement in clinical outcomes-multicenter, double-blinded and randomized controlledtrial. Hum Reprod 28(2):306–314
81. Grigorova M, Punab M, Punab AM, Poolamets O, Vihljajev V, Zilaitienė B et al (2014) Reproductive physiology in young men is cumulatively affected by FSH-action modulating genetic

variants: FSHR -29G/A and c.2039 A/G, FSHB -211G/T. PLoS ONE 9(4):e94244
82. Iacono F, Barra S, Montano L, Lotti T (1996) Value of high-dose pure FSH in the treatment of idiopathic male infertility. J Urol (Paris) 102(2):81–84
83. Dirnfeld M, Katz G, Calderon I, Abramovici H, Bider D (2000) 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 93(1):105–108