Follicle-stimulating hormone treatment for male factor infertility

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Follicle-stimulating hormone (FSH) treatment has been proven effective in stimulating spermatogenesis and improving the reproductive ability of men with hypogonadotropic hypogonadism, while the usefulness of such a treatment in infertile patients with normal pituitary function is restricted to a subgroup of responders that, however, cannot be identified by the current diagnostic tools before treatment. In this review we summarize the role played by FSH in the modulation of spermatogenesis, the effect of FSH treatment at a standard replacement dose and at higher dose on sperm parameters, spontaneous and in vitro fertilization pregnancy rates, and the efforts made to identify possible responders to FSH treatment. (Fertil Steril® 2022;■:■–■. ©2022 by American Society for Reproductive Medicine.)

Male infertility accounts for 40%–50% of total infertility causes. Although the advent of assisted reproductive technology has allowed many men with infertility to father children, who are genetically their own, hormonal treatments may still improve the reproductive chances of patients with oligozoospermia, who normally are candidates for intracytoplasmic sperm injection, but may have their sperm parameters improved after treatment to a range more suitable for intrauterine insemination or even natural conception. Additionally, hormonal treatments may lead to the return of sperm in the ejaculate of patients with azoospermia resulting from spermatogenic dysfunction, avoiding more invasive surgical treatments, or increase the probability of successful sperm retrieval by means of microdissection and testicular sperm extraction.

The classical hypothesis that hormonal treatment should be proposed only to patients with hormonal dysfunction, i.e., patients with hypogonadotropic hypogonadism, has been challenged by studies demonstrating the use of stimulating spermatogenesis in patients with normal pituitary function. In particular, treatment with follicle-stimulating hormone (FSH) has been able to improve sperm parameters and pregnancy rates, albeit in a subset of male patients with infertility.

The scope of this review is to update the evidence for FSH treatment in the male patients and discuss what future developments might allow the identification of possible responders and provide the best dose and duration of FSH treatment to improve treatment outcomes.

MECHANISM OF FSH ACTION

Follicle-stimulating hormone is a glycoprotein composed of a common α subunit and a β subunit that confers biologic specificity to the hormone, associated with noncovalent interactions, with cysteine residues involved in disulfide bonds within the subunits. The intrinsic bioactivity of FSH is determined by the variations in carbohydrate content (glycosylation, terminal sialylation, and sulfonation within α and β subunits): these variations give rise to the FSH isoforms of variable in vivo bioactivity, receptor binding ability, and metabolic clearance. A high number of sialic acid residues increases the half-life of circulating human FSH and can reduce receptor affinity at the target organ (1); on the other hand, hypo glycosylated FSH ($hFSH^{21/18}$) isoforms seem to be more active than fully glycosylated isoforms in terms of cAMP production, cAMP response element binding protein phosphorylation, and protein kinase A (PKA activity), probably owing to the availability of more binding sites at the receptor level (2). However, totally deglycosylated gonadotropins are still able to interact with their cognate receptors but cannot evoke the generation of second messenger signals (3). Interestingly, young healthy men compared with healthy women have significantly more sialylated circulating FSH isoforms (1); the onset of puberty is accompanied by a significant increment in the proportion of more sialylated FSH isoforms as well as in

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variation in oligosaccharide complexity (4). This suggests that promotion of spermatogenesis requires long-acting, fully bioactive FSH isoforms.

The secretion of FSH from the pituitary gland under the control of feed-forward stimulation of pulsatile gonadotropin-releasing hormone (GnRH) secretion, of the activin-follistatin-inhibin B loop, with inhibin B playing a key role unless seminiferous tubule function is severely compromised (5), and of testosterone that modulates its oligosaccharide complexity. Castration in prepubertal and adult rats or administration of the non–steroidal-antiandrogen flutamide results in the predominance of FSH isoforms with incomplete oligosaccharides, whereas the administration of dihydrotestosterone, a nonaromatizable androgen, restores the FSH isoform profile (4). Therefore, it may be inferred that the disorders of testicular function, which are accompanied by decreased inhibin B and/or testosterone production, may affect FSH oligosaccharide complexity and have an impact on the overall FSH biologic efficiency.

In addition, FSH interacts with its cognate receptor (FSHR), a G-protein-coupled receptor expressed exclusively on Sertoli cells: binding of FSH to the extracellular component of the receptor on the Sertoli cells activates both the cAMP/PKA pathway, the ERK 1/2 pathway and the PI3K/Akt/MTORC1 signaling pathway, leading to alterations in gene transcription through modification in the expression and activity of a number of transcription factors, including cAMP response element binding protein and many others (6). In addition to these pathways, others, such as intracellular calcium flux, activation of protein kinase B-, and epidermal growth factor receptor, have been also postulated as mediators of FSH action.

ROLE OF FSH IN THE MODULATION OF SPERMATOGENESIS

A crucial role in the hormonal control of spermatogenesis in humans is played by FSH. A study of 5 men with a putatively inactivating mutation of the *FSHR* gene indicated that none had azoospermia and 2 had children, leading to questioning of the importance of FSH in the modulation of spermatogenesis (7). However it was subsequently demonstrated that the mutant *FSHR* was not completely inactive and that the residual FSH action was still able to stimulate spermatogenesis (8). Moreover, FSH β subunit gene mutations are more severe than those of the FSHR: all men with inactivating mutations in FSH β subunit, in fact, have complete azoospermia (9).

Further, FSH determines the number of Sertoli cells at neonatal and peripubertal ages: in the absence of FSH or its cognate receptor, Sertoli cell number decreases by 30%–45% in comparison to normal testicular development (10). Moreover, FSH modulates the expression of a great number of Sertoli cell genes: Sertoli cell proliferation and maturation is reduced in GnRH-deficient hypogonadal mice, in whom FSH administration activates the expression of many transcripts involved in cellular differentiation and proliferation for which the abundance was decreased (11). Sertoli cells differentiate and proliferate until a finite number, and because they may support the development of a limited number of germ cells, their final number determines the quantity of sperm produced. In addition, FSH is thought to play a role as a survival factor for spermatogonia because acute FSH suppression induces spermatogonial apoptosis, and may increase spermatogonial differentiation and proliferation (12, 13). Lack of FSH function, as experimentally provoked in mice knockout for the FSH β subunit (FSHKO) or for its receptor (FSHRKO), is associated with a reduced number of Sertoli cells and of spermatogonia and spermatocytes (approximately 50% lower compared with wild type animals) (14). Conversely, FSH treatment increases spermatogonial and spermatocyte number in hypophysectomized or GnRH-immunized adult rats (15).

Additionally, FSH regulates the responsiveness of Sertoli cells to androgens by inducing the expression of the androgen receptor and seems to be involved in the pubertal onset of spermatogenesis because it promotes the decline in microRNA 92a-3p expression during Sertoli cells maturation, whose higher expression is associated with functionally compromised Sertoli cells, impaired blood-testis barrier formation, and apoptosis of premeiotic germ cells (16, 17). The action of FSH on spermatogenesis seems to be limited to the progression of meiosis until the pachytene spermatocyte stage, whereas testosterone is required for further steps; however, it is believed that FSH may also influence the process of spermiation, a stage of spermatogenesis particularly sensitive to hormone regulation during which elongated spermatids are released from the Sertoli cells. In vitro suppression of both FSH and testosterone action in rats led to a significant up regulation of 23 microRNAs, 2 of which (miR-23b and miR-217) were complimentary to the phosphatase and tensin homolog (PTEN), which is actively involved in the cellular processes associated with the adhesion of spermatids to Sertoli cells (18). Because PTEN expression is massively enhanced after FSH in vitro stimulation of Sertoli cells, FSH is required for the inhibition of PTEN-directed miRNA expression at the posttranscriptional level, to stabilize the PTEN messenger ribonucleic acid expression, and regulate cell adhesion (19). Finally, experimental studies in mice and human and nonhuman primates suggest that FSH may play a role in the modulation of the sperm chromatin condensation process: FSH-immunized men and male bonnet monkeys were found to display defective chromatin packaging (20), whereas FSHRKO mice showed impaired expression of transition proteins and protamine 2 at an early stage of postnatal development, and retention of mono-ubiquitylated histone 2A in adulthood (21). Selective FSH deficiency induced by fluphenazine decanoate led to core/ubiquitylated histone persistence and chromatin condensation defects in mice (22).

FSH AS TREATMENT OF MALE FACTOR INFERTILITY

There is a great consensus about the effectiveness of FSH treatment in promoting spermatogenesis and inducing fertility in patients with FSH deficiency. According to a recent review evaluating studies performed from the early 1970s to 2019 on a total of 1,118 patients with hypogonadotropic hypogonadism, sperm in the ejaculate were found in 64%–95%

of patients after 12 months of treatment on an average, with extremely variable final sperm concentrations among cases (0.1–68 million/mL, weighted mean sperm concentration 10.8 million/mL) (23). Pregnancies were recorded in 17%–57% of patients, most of these were by natural conception, regardless of the final sperm concentrations obtained: low sperm concentrations did not prevent the possibility of fathering a child. Patients with high testicular volume recorded at baseline and no history of cryptorchidism were more likely to have sperm in the ejaculate after treatment. Pretreatment with FSH alone for 4 months before pulsatile GnRH treatment was associated with the appearance of sperm in the ejaculate in all participants, whereas sperm was found in 67% patients undergoing the same treatment but without FSH pretreatment (24). Treatment with FSH was also effective in men with isolated FSH deficiency without FSH β gene mutation (25, 26). Although no consensus has so far been reached on the optimal strategy to induce fertility in these patients, treatment with FSH has been approved in most countries for male patients with infertility with overt FSH deficit.

The demonstration that exogenous FSH administration could be effective in stimulating spermatogenesis in men with infertility with FSH deficit, as well as the alleged discovery of functional alterations in the pulsatile release of gonadotropins in men with idiopathic oligozoospermia because of altered feedback signal from the testes with impaired spermatogenesis, prompted the experimental use of hormonal stimulation of spermatogenesis with FSH in patients with infertility and normal hypothalamic and pituitary function, despite their normal or high serum FSH levels, with the belief that proper FSH stimulation could improve spermatogenesis (27–29). In 1991, Acosta et al. (30) administered purified urinary FSH (150 IU 3 times a week intramuscularly for at least 3 months) to men with infertility with previous fertilization failure in in vitro fertilization (IVF) cycles or with severe oligoasthenoteratozoospermia (sperm concentration $<$ 5 million/mL, motility $<$10%, morphology $<$4%): treatment was effective in improving IVF fertilization rate, regardless of the patients' basal serum FSH level, which ranged between 5 and above 25 mIU/mL, and an IVF pregnancy rate of 19.4% was achieved. A few years later, Bartoov et al. (31) demonstrated that FSH treatment of men with teratozoospermia led to significant improvement in sperm structure as observed by transmission electron microscopy, a finding further confirmed by other independent teams (32, 33).

From the late 1990s to date, several studies were designed to evaluate the effect of FSH treatment on sperm parameters, as well as on spontaneous and IVF or intracytoplasmic sperm injection pregnancy rates. The latest Cochrane systematic review on this matter, evaluating data from 5 randomized controlled trials with 412 participants receiving FSH compared with placebo or no treatment, reports that FSH treatment significantly improves spontaneous pregnancy rate per couple (16% vs. 7%; Peto odds ratio [OR], 4.94; 95% confidence interval [CI], 2.13–11.44; I2 = 0, moderate quality evidence), while it does not affect the intrauterine insemination or IVF pregnancy rate (34). However, a more recent meta-analysis that included both randomized and non–randomized-controlled clinical trials, found that FSH treatment was able to improve the IVF pregnancy rate compared with no treatment (8 studies, 597 participants, OR, 1.6; 95% CI, 1.08–2.37; P=.002); however, the number needed to treat was high (10 patients to be treated to achieve 1 spontaneous pregnancy and 18 to achieve an IVF pregnancy) (35). With the obvious limitation of the different study designs, inclusion criteria, and FSH dosage as well as treatment schemes among studies, these results suggest that FSH treatment is not a suitable treatment for all men with infertility and oligozoospermia. Some men respond to the treatment with a significant improvement in sperm concentration, but seldom does the final concentration approach the threshold for natural conception. Moreover, no improvement in sperm concentration is observed in approximately 50%–70% of cases (36).

Evidence about the effect of FSH treatment in patients with nonobstructive azoospermia (NOA) is scantier because most of the studies evaluating the effect of hormonal treatment on sperm retrieval rates used a combination of FSH plus clomiphene citrate or human chorionic gonadotropin (hCG) in an effort to optimize the production of testosterone by Leydig cells (37). In the only study using FSH as monotherapy, a cohort of 108 men with NOA received FSH treatment (N = 63), 75 IU thrice a week, or no treatment (N = 45): sperm retrieval rate was significantly high in FSH-treated compared with controls (64% vs. 33%, P<.0001), with hypospermatogenesis being the testicular histopathological subcategory most responsive to treatment (38). However, the results of studies evaluating other outcome measures besides sperm retrieval suggest that the role played by exogenous FSH coadministration in patients with NOA may be of relevance in promoting the proliferation of spermatogonia, as demonstrated by increased spermatogonial proliferating cell nuclear antigen expression observed only in patients receiving FSH plus hCG compared with those treated with hCG alone (39). In addition, FSH increased the androgen receptor expression on Sertoli cells, which was associated with high chances of successful sperm retrieval in patients with previous sperm retrieval failure (40). Further studies are warranted to confirm such a beneficial effect of FSH on the residual spermatogenesis in patients with NOA.

Ferring Pharmaceuticals is initiating a 400 patient trial in the United States and Europe to investigate whether men with idiopathic infertility (unexplained reduction of semen quality) show improvement in the likelihood of spontaneous pregnancy observed in their female partners after treatment with recombinant FSH compared with placebo (ClinicalTrials.gov Identifier: NCT05403476). The trial will evaluate daily injections of follitropin delta at a dose of 12 μg for 6 months with a primary endpoint of spontaneous pregnancy (documentation of at least 1 intrauterine gestational sac with fetal heartbeat) observed in the female partner within 9 months after randomization of male subject).

The trial will include male subjects aged 18–50 years with a history of infertility for 12–60 months with their current partner, total sperm count of 5–16 million, total motile sperm count of 5–16 million, and serum: FSH levels of 1.5–8.0 IU/L,

luteinizing hormone levels of 1.2–7.5 IU/L, and testosterone levels of $\geq$300 ng/dL. The trial is expected to provide robust evidence as to whether therapeutic modulation of FSH activity can benefit this patient population.

HOW TO SELECT THE IDEAL CANDIDATES FOR FSH TREATMENT?

Moderate to severe spermatogenic dysfunction is usually reflected by increased serum FSH levels, decreased inhibin B secretion, and reduced testicular volume; however, in a subset of these men with infertility normal or subnormal serum FSH level are found despite reduced inhibin B levels and testicular volume. This has prompted some investigators to speculate that this subgroup of patients represents the ideal candidates for FSH treatment, and therefore many studies were designed to include only normogonadotropic patients to whom to administer FSH: nevertheless, FSH serum levels alone were insufficient to predict response in idiopathic men with infertility. On the contrary, as reported above, Acosta et al. (30) demonstrated that FSH treatment was effective in improving IVF fertilization rate regardless of the patients' basal serum FSH. Furthermore, as summarized above, exogenous FSH may stimulate spermatogenesis even in patients with NOA despite high or very high serum FSH levels. It is interesting to note that an experimental study, by demonstrating that receptor recycling promotes the maintenance of gonadotropin receptors at the cell surface, thus preserving hormonal responsiveness even under continuous hormonal stimulation, laid the physiological bases for the use of exogenous FSH administration even in men with high serum FSH levels (41).

The discovery of single nucleotide polymorphisms (SNPs) affecting the transcriptional activity of FSH β subunit and of the sensitivity of the FSHR has prompted some investigators to administer FSH to patients with infertility carrying variations in FSH β subunit or FSHR genes: this has been termed the "pharmacogenetic approach to male infertility." A single SNP in the promoter region of the FSH β gene, c-211G>T (rs10835638), was experimentally shown, using the mouse gonadotropic cell line LβT2, to regulate transcription of the FSH β subunit. Particularly, the presence of a T allele was found to reduce the transcriptional activity of this gene, by approximately 50%, because of the impaired binding of the homeodomain transcription factor LHX3 (42). Similarly, the impact of the SNPs in exon 10 in FSHR was found to affect female fertility in vitro and in vivo; however, in vitro studies using Sertoli cells to evaluate the molecular impact of these SNPs on FSHR sensitivity are not available (43). Clinical studies provided conflicting results about the realistic impact of FSHB and/or FSHR SNPs on male infertility: a study on a German cohort of men with infertility demonstrated that subjects carrying one or 2 FSHB−211 T alleles had reduced FSH levels, together with testicular volume and sperm count, compared with GG homozygous men, whereas FSHR 2039 A>G polymorphism had an impact on the same parameters only if combined with FSHB polymorphism (44). In a more recent study including 2,020 men from the general Danish population, no clear association was found between both polymorphisms and sperm parameters (45). The pharmacogenetic approach to FSH treatment was tested by 3 studies, albeit with different endpoints. A subanalysis of a large population based study showed that, among the 67 subjects who received FSH, those homozygous for FSHB -211 TT genotype showed significantly high improvement in sperm parameters compared with the remaining patients (46). The same group performed another study to evaluate the effect of FSH treatment in 105 men with oligozoospermia and carriers of the FSHR N680S polymorphisms: 70 received FSH whereas 35 received no treatment. Sperm parameters improved after treatment only in patients with at least one serine in position 680, but FSH treatment did not affect the pregnancy rate (47). These latter results were challenged by those of a further multicenter Italian study, which found that the FSHR N680S homozygous N polymorphism was responsive to FSH treatment, in terms of improvement of the sperm fragmentation index after treatment (48). Owing to these conflicting results and to insufficient data, the clinical utility of pharmacogenetic approaches to FSH treatment remains to be demonstrated.

Recently we have developed a molecular analysis, using genome wide alterations in sperm deoxyribonucleic acid (DNA) methylation, to identify epigenetic signatures that could identify possible responders to FSH treatment among patients with idiopathic male infertility (49). We recruited 12 men with infertility, 25–45 years of age, with a total sperm concentration of 1–10 million, serum FSH level between 2 and 12, and normal testosterone level as well as 9 fertile donors, all of whom had had a child within the last 5 years, with a sperm concentration and motility above the 50th percentile according to the parameters set forth in the 5th edition of the *World Health Organization guidelines*. The infertile group received 150 IU of urinary or recombinant FSH 3 times per week for 12 weeks, whereas the fertile donors did not receive any treatment. An initial semen sample was collected on enrollment, a second at the start of treatment, and a third after 3 months of treatment (for patients) or no treatment (for fertile donors), for semen analysis and for epigenetic analysis: DNA was extracted from the sperm, then fragmented for a methylated DNA immunoprecipitation analysis that evaluates 95% of the genome, comprising low density CpG regions, to identify differential DNA methylated regions (DMRs). Two hundred seventeen DMRs were identified at a $P <$ 1e-05 from the comparison of infertile and fertile subjects; there was an efficient separation between the patient population with vs. without infertility with minimal overlap, confirmed also by a validation test performed in some fertile and infertile patients not used in the initial test owing to exclusions (mostly because of smoking and drinking habits). Most of the DNA methylation change involved an increase in DNA methylation (hypermethylation). Patients with infertility who showed a twofold to threefold increase in sperm concentration and/or motility after FSH treatment were considered responders: these subjects showed distinct epigenetic biomarkers (56 DMRs at p<1e-05) compared with nonresponders, with an equal distribution of hypermethylation and hypomethylation changes. By demonstrating that epigenetic biomarkers may efficiently identify possible responders to FSH treatment, this study provides the proof of concept that epigenetic

diagnostics can be developed and applied to the therapeutic treatment of male patients with infertility. Further studies are, of course, needed to support these preliminary findings.

SPERMATOGENESIS (HYPER)STIMULATION WITH HIGH DOSE FSH: A NEW FRONTIER FOR THE TREATMENT OF MALE FACTOR INFERTILITY?

In 1957, Fowler and Edwards (50) treated mice with pregnant mare serum gonadotropin and hCG as well as demonstrated that exogenous gonadotropins could induce superovulation and pregnancy, with the degree of superovulation dependent on the amount of pregnant mare serum injected. This work overturned the dogma that the adult ovary was resistant to gonadotropins and paved the way to the use, from the eighties on, of urinary and recombinant gonadotropins for ovarian stimulation and controlled ovarian hyperstimulation for IVF (51). Many advances have been made in this field since then, thanks to dose-finding studies and to the testing of innovative stimulation protocols, such that well-defined protocols for controlled ovarian hyperstimulation are now available. Notably, ovarian hyperstimulation is commonly attempted in women with diminished ovarian reserve, despite their high serum FSH level.

A similar scientific prejudice persists today about the unfeasibility of stimulating spermatogenesis outside of the case of overt or plausible FSH deficit. Such a point of view, indeed, conflicts with the evidence that spermatogenesis may be stimulated in adult men with normal or even increased FSH secretion: hemicastration is followed by a compensatory increase in the surviving the testicular volume, supported by increased FSH secretion, in animal models as well as in men (52, 53). In the male rhesus monkey, the rise in FSH stimulates the proliferation of B spermatogonia, providing evidence for the possibility that increased FSH may stimulate spermatogenesis in the adult (54). Similarly, men with pituitary tumors secreting biologically active gonadotropins, with resulting elevated serum FSH levels, display testicular enlargement due to increased length of the seminiferous tubules and, in some cases, increased sperm count (55).

Most studies aimed at determining the effect of exogenous FSH stimulation on spermatogenesis, and those that have demonstrated that such stimulation may, to some extent, improve spermatogenesis in a quantitative and qualitative manner, were designed to use an FSH replacement dose (75–150 IU) that resembled those used in studies of patients with FSH deficit. However, when a high FSH dose was used, results were even more encouraging. Paradisi et al. (56) administered FSH 300 IU every other day for 4 months to 45 men with oligozoospermia (sperm concentration 7.2 $\pm$ 3.6 x 10^6/mL), whereas 15 age-matched men with oligozoospermia (sperm concentration 7.4 $\pm$ 4.1 x10^6/ml) were placebo treated: a threefold improvement in sperm concentration was recorded in FSH-treated patients and 12 (26.7%) spontaneous pregnancies were achieved (56). One year later, Ding et al. (57) designed a prospective, randomized, double-blind, placebo-controlled trial with increasing FSH dosage (from 50 to 300 IU on alternate days for 3 months), and found that only those patients receiving the highest FSH dose achieved significantly higher spontaneous (15% vs. 6.6%) and IVF (41.1% vs. 25%) pregnancy rates compared with placebo-treated patients; this high dose FSH treatment promoted a threefold increase in sperm concentration, similar to what was recorded in the other study using the same high FSH dose (56).

It has been proposed that the patients who did not respond to FSH treatment in previous studies probably did not receive a sufficiently high FSH dose for a sufficient time to stimulate spermatogenesis (36). Such an interesting hypothesis merits further investigation.

CONCLUSIONS

Sperm parameters and spontaneous as well as IVF pregnancy rates in a subgroup of patients with oligozoospermia can be improved by FSH administration, which seems to exert a stimulatory effect on spermatogenesis even in patients with severe spermatogenic dysfunction, such as patients with NOA. However, the studies performed in the past 30 years, owing to the different study designs, treatment formulations, and primary outcome measures, are not sufficient to provide conclusive evidence about how to identify the possible responders to treatment. The ideal dosage and duration for FSH administration to promote effective stimulation of spermatogenesis in men with infertility requires ongoing and future well-designed clinical trials.

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