PROSPECTS FOR FSH TREATMENT OF MALE INFERTILITY

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ABSTRACT

Context. Despite the new opportunities provided by assisted reproduction techniques, male infertility treatment is far from being optimized. One possibility, based on pathophysiological evidence, is to stimulate spermatogenesis with gonadotropins.

Evidence Acquisition. We conducted a comprehensive systematic PubMed literature review, up to January 2020, of studies evaluating the genetic basis of FSH action, the role of FSH in spermatogenesis, and the effects of its administration in male infertility. Manuscripts evaluating the role of genetic polymorphisms and FSH administration in women undergoing assisted reproduction were considered whenever relevant.

Evidence Synthesis. FSH treatment has been successfully used in hypogonadotropic hypogonadism, but with questionable results in idiopathic male infertility. A limitation of this approach is that schemes for male infertility have been borrowed from hypogonadism, without daring to overstimulate, as is done in women undergoing assisted reproduction. FSH effectiveness does not depend only on its serum levels, but also on individual, genetic variants able to determine hormonal levels, activity and receptor response. Single-nucleotide polymorphisms (SNPs) in *FSHB* and *FSHR* genes have been described, some of them impacting testicular volume and sperm output. The *FSHR* p.N680S and the *FSHB* -211G>T variants could be genetic marker to predict FSH response.

Conclusions. FSH may be helpful to increase sperm production in infertile men, even if the evidence to recommend the use of FSH in this setting is weak. Placebo-controlled clinical trials, considering *FSHB-FSHR* haplotype, are needed to define the most effective dosage, the best treatment length and the criteria to select candidate responder patients

Keywords: FSH, infertility, spermatogenesis, LH

1. Introduction

Male infertility is emerging as a major medical problem with impressive socio-demographic consequences, influencing family planning and demography and, possibly, general male health (1). In 1998, the United States (US) Supreme Court included infertility among disabilities under the Americans with Disabilities Act and male infertility is now codified in the International Classification of Disease Tenth Revision (2020 ICD-10-CM, Diagnosis Code N46). However, the definition of male infertility is not clear-cut. The WHO and the International Committee for Monitoring Assisted Reproductive Technology define couple infertility as failure to reach pregnancy despite 12 or more months of unprotected intercourses (2) but other, extended definitions have been proposed (3-5). It is estimated that 15-20% of young couples are infertile and a male factor is present in about half of them, with sole male responsibility in 30% and a co-contributing female factor in 20% of cases (6). Epidemiological data, however, are incomplete, since the clinical work-up on the male is missing or is sketchy in the majority of infertile couples (7).

Even if a diagnosis of male infertility is made, the cause cannot be identified in up to 40% of cases, bringing to a diagnosis of male idiopathic infertility (8) for which, by definition, no etiologic cure exists. Under these circumstances, pregnancy being the final goal, the therapeutic approach does not address the affected man but his (generally fertile) partner via assisted reproduction technologies (ART) and intracytoplasmic sperm injection (ICSI), a remarkable example of gender inequity in which women bear the burden of ovarian stimulation and its side effects/complications. Although the advent of ICSI opened concrete possibilities for paternity to infertile men, the overall pregnancy rate remains suboptimal (9) and some concerns about its long-term safety exist (9-11).

ART is a remunerative business, not expected to decline in the upcoming years. For a number of reasons, extending beyond male infertility, a steadily increasing number of couples are turning to doctors to procreate and the booming fertility business is predicted to rake globally USD 41bn in sales in 2026 (12). Unfortunately, this commodization of infertility treatment is related to a

worrisome decrease in ART effectiveness (13). More scientific approaches to male (idiopathic) infertility treatment are urgently needed.

Since spermatogenesis is regulated by hormones, the question arises whether gonadotropins can be used to improve sperm output and function in male idiopathic infertility; and a new approach was recently proposed (14). In this review, we summarize the state-of-the-art, pinpointing the critical issues and suggesting possible future strategies concerning the use of gonadotropins, focusing on factors that one should carefully consider before planning future studies in this field.

2. Use of gonadotropins for male infertility

The endocrine regulation of spermatogenesis by gonadotropins is a well-established topic, extensively reviewed (15-17). Gonadotropins are effectively used to stimulate sperm production in patients with hypogonadotropic hypogonadism (HH). In this setting, the administration of exogenous gonadotropins or pulsatile gonadotropin-releasing hormone (GnRH) induces the increase of intratesticular and serum testosterone, the development of secondary sexual characteristics, and the appearance of sperm in ejaculate (18,19). Pulsatile GnRH therapy is the physiological approach, restoring endogenous gonadotropin secretion by pituitary (20-22), but its use has been almost abandoned. More often, testicular stimulation in HH is obtained by the administration of exogenous gonadotropins, using human chorionic gonadotropin (hCG) either alone or in combination with follicle-stimulating hormone (FSH) (19). hCG alone is able to stimulate spermatogenesis (23), although FSH addition increases final sperm number (24-26). This synergic action results in sperm production in the vast majority of HH men (69-81%) (24,26), although the time required is quite variable, ranging from 3 to 19 months, and semen parameters rarely achieve WHO normal ranges (24). Although treatment of HH by gonadotropins is pathophysiologically solid, it remains only

empirical, and no evidence-based clinical guidelines, reporting the most appropriate scheme regimen and duration of the replacement therapy, have been produced so far. Reviews and opinion papers suggest the use of hCG 1000-2500 IU twice a week and of FSH 75-225 IU three times a week (19,27) but no randomized prospective trials to find the best regimen have been carried out so far . In addition, even if hCG is able to induce the necessary intratesticular testosterone increase, it does not constitute the physiological hormone in human adults. hCG is used for practical convenience, due to its relatively long half-life and to the low number of injections required for sustaining the therapy, as well as to the lack of pharmacological LH preparations registered for this indication. Currently, recombinant LH is available but no clinical experience in males exists. Given that LH and hCG interact with the same receptor (the LHCGR) but activate different molecular pathways (28-30), and that hCG was shown to be pro-inflammatory in the testis (31), the effect of clinical treatments with LH in HH men would be worthy of investigation. On the other hand, the use of urinary and recombinant FSH molecules in HH men have led to similar improvement of semen parameters and pregnancy rate, although evidence is limited (32-35).

Starting from its efficacy in restoring sperm production in HH, gonadotropins have been repeatedly proposed as an empirical approach to treat idiopathic infertile men. FSH administration was attempted in the context of idiopathic oligo-astheno-teratozoospermia (OAT) to increase sperm output/quality without increasing serum testosterone. In HH, the missing LH action is the crucial factor, while both LH and FSH levels are apparently within the physiological range or even increased in idiopathic infertility. Therefore, the rationale for using FSH is different in the two cases outlined above. In the context of idiopathic infertility, FSH is proposed to increase sperm output and/or improve sperm quality (36). Yet the regimens used so far have been inherited from the experience in HH treatment without attempting any real hyperstimulation, especially given that most of the applied dosages were in the substitution range (14). Only seventeen clinical trials and four meta-analyses (36-39) evaluated such a clinical approach and, *de facto*, we do not have sufficient, evidence-based information for coming to conclusions on the effectiveness of this therapy. Overall,

the published controlled studies, not all placebo-controlled and rarely blind, have demonstrated modest beneficial effects for pregnancy rate and sperm number (37-39). However, the number-needed-to-treat calculated on combined results ranges between 10 and 18, suggesting that more than 15 men with idiopathic infertility should be treated to obtain at least one pregnancy (14,39). This limited efficacy may depend on the heterogeneity of FSH therapeutic schemes, involving variable but moderate dosages and durations (36), and on the unclear aetiology of OAT. It is possible that FSH treatment might be effective only in a subset of patients, who, however, cannot be selected *a priori*. Thus, the real efficacy of FSH administration in idiopathic infertility remains controversial and difficult to prove (14,36). The position statements of some scientific societies suggest that the cautious, potential FSH application in this clinical condition might help in selected subjects (40,41) but the selection criteria of patients who might benefit from this treatment are lacking.

To understand whether and in which patients FSH treatment should be tested, we need to consider FSH, FSH deficiency and the FSH receptor (FSHR) comprehensively, including the molecular and pharmacogenetics aspects of their action in the testis.

3. Pathophysiological rationale of FSH treatment of male idiopathic infertility

3.1 The concept of gonadotropin efficacy

Gonadotropins are released in a pulsatile fashion and their concentration in serum varies during the day. The pulsatile pattern is more evident for LH than FSH, probably because the secretion of the latter is less influenced by GnRH (42). A reduction of gonadotropin pulse frequency and/or amplitude may result in the decrease of their effect, independently from the measured serum levels. Therefore, in men affected by congenital HH serum gonadotropins may be undetectable, low or normal (43,44). On the other hand, in fertile, eugonadal men, normal testosterone serum levels and a normal sperm count may be associated with very low serum levels

of gonadotropins. Finally, low or undetectable LH and FSH serum levels could coexist with normal testosterone levels in androgen abuse (45), thus the diagnosis of gonadotropin deficiency cannot be established solely by basal serum levels below the normal range but needs to be coupled with other clinical or biochemical findings.

While the evaluation of serum testosterone and LH levels allow to diagnose LH deficiency, the definition of FSH deficiency remains problematic outside the context of documented, congenital HH. A subset of men with OAT and low-to-normal serum FSH could be FSH-deficient as a consequence of reduced FSH activity, which depends on several factors including the amount of circulating FSH, its glycosylation, (46) as well as the expression levels and the function of the FSHR (see below). Thus, a condition of clinical deficiency may occur whenever FSH is unable to attain its clinical effect, independently of its circulating levels. Overall, the efficacy of FSH depends on individual, genetic variants determining not only secretion and circulating amount but also biological activity (29,47,48) and gonadal response (47,49-51). On this ground, FSH treatment of men with OAT might become rational in a personalized, genotype-dependent fashion.

3.2 FSH is a highly heterogeneous hormone

To understand the therapeutic potential of FSH in males, it is essential to consider some molecular aspects. FSH is a complex, highly heterogeneous glycoprotein hormone, resulting from N-glycosylation of the alfa and beta subunit via the oligosaccharyltransferase enzyme complex of the gonadotrope cells in the pituitary, which occurs during protein synthesis (52). The specific glycosylation sites of the beta FSH subunit are the amino acid positions Asn^{7} and Asn^{24}. These amino acids can be fully glycosylated, giving rise to the glycoform called FSH^{24}, migrating as 24 kDa band on immunoblots, or to partially glycosylated (only Asn^{7} or only Asn^{24}) FSH, denominated FSH^{21} and FSH^{18}, respectively (53). This is the basis of FSH macroheterogeneity. A further level of heterogeneity

is provided by the so-called microheterogeneity, which results from oligosaccharide structure variation of the glycosylation branches. The hypoglycosylated glycoform FSH^{21} is more abundant in women in their fertile years, while FSH^{24} is predominant in the perimenopausal and postmenopausal age. Although changes of FSH sialylation and complex oligosaccharides were demonstrated during sexual development (54), the physiological pattern in males is largely unknown.

Overall, more glycosylated FSH shows an apparently lower receptor binding activity and reduced signal transduction, which may be related to reduced receptor occupancy and/or biased signaling. Experimentally, FSH^{24} and FSH^{21} have a partially different biological action both in *Fshb* KO mice (55) and on isolated mouse follicles (56). Both urinary-derived and recombinant therapeutic FSH preparations maintain heterogeneity akin to natural, pituitary FSH, with some differences related to cell source (52). Overall, however, they show the same biological effects *in vitro* (48), so that major differences in therapeutic results that depend on the type of FSH preparation used are unlikely. No clinical trials have ever been performed by testing different FSH glycoforms, since they are not available for this purpose. Recombinant and urinary preparations differ in the FSH glycosylation pattern but not in their efficacy (48,57).

3.3 Mechanism of FSH action

The mechanism of action of FSH is far more complex than what was assumed until a couple of decades ago (58) and is still the object of intense investigation (59). FSH binds to the FSHR (53), which is a G protein-coupled receptor with a large extracellular domain (ECD) with leucine-rich repeats and a hinge region, connecting the ECD to the seven transmembrane stretches, ending intracellularly with the C-terminal portion (58) . Although extragonadal expression was proposed (60), the *FSHR* is primarily expressed in Sertoli and in granulosa cells. The existing transgenic mouse models demonstrate that FSH is essential for follicular growth and oocyte maturation but not for

spermatogenesis (61-64). Crystal structure of FSH complexed with its receptor gave hints about FSH binding (65), which would impose a conformational change to the extracellular domain, shifting from its putative function as inverse agonist to the stimulation of intracellular signaling cascade activation (66). This mechanism involves the hinge region and the extracellular loops of the receptor (67,68), as well as the carbohydrate branches bound to specific FSH residues (69), most likely providing a further regulatory mode of signal transduction. However, molecular pharmacology of the FSHR is still in its dawning age and more research on this topic is needed.

The study of FSH action in an *in vitro* setting is particularly challenging because all attempts to obtain consistent Sertoli and granulosa cell lines permanently expressing the *FSHR* have failed so far. Experimental evidence indicates that overexpressing the *FSHR* in such cells induces cyclic adenosine monophosphate (cAMP)-mediated apoptosis (70), a negative finding which suffers from a publication bias and which is rarely reported in literature. Therefore, other cell systems are used (primary cells, transiently transfected cells, non-gonadal cell lines), identifying the cAMP/protein kinase A (PKA) pathway as the best-known signal transduction mechanism. This pathway involves Gαs protein subunit activation of the enzyme adenylyl cyclase, intracellular cAMP increase, and PKA activation (71) (Fig. 1). The intracellular activity of this enzyme seems to be sexually dimorphic. In granulosa cells, PKA induces the simultaneous activation of the steroidogenic-related transcription factor cAMP-dependent response element binding-protein (CREB), mitogen-activated kinases 1 and 2 (ERK1/2) modulating cell proliferation, and the P38 mitogen-activated protein kinases (P38 MAPK), which is linked to cytoskeletal rearrangements and pro-apoptotic signals (72). In immature Sertoli cells, ERK1/2-mediated cell proliferation may occur also *via* the action of the Gαi protein (73), the Gq protein/phospholipase C (PLC)-pathway (74) or the recruitment of other receptor interactors, such as β-arrestins, which are involved in FSHR internalization (75), desensitization (76,77) and control of target RNA translation (71,78,79). The mitogenic signal is supported also by the phosphoinositide 3-kinases (PI3K)/protein kinase B (AKT)-pathway (80), at least in immature Sertoli cells, while PI3K/AKT-pathway activation dependency is due to paracrine factors at later maturation stages (81).

The mechanism regulating stage-specific activation of signal transduction cascades is not completely understood, but it may be hypothesized that β-arrestins-, proliferation-associated pathways may be preferentially activated at low FSHR density in the cell membrane, instead of the cAMP/PKA-pathway (82). If so, single nucleotide polymorphisms (SNPs) falling within the *FSHR* promoter (rs1394205; -29A>G), modulating expression levels, might influence signal transduction and, possibly, reproductive parameters in men (83), contributing to the phenotypes resulting from the cumulative effect of other *FSHR* and *FSHB* SNPs together (84-87).

New insights in the complexity of FSH-mediated signal transduction were provided by the discovery of heteromeric assembly of FSHR with other G protein-coupled receptors (GPCRs). These studies identified the LHCGR as a possible molecular partner of the FSHR on the granulosa cell surface (88), likely determining the fate of female gonadal cells (30). Although LHCGR is not expressed in human Sertoli cells, other GPCRs, such as the G protein-coupled estrogen receptor 1 (89,90), might potentially interact with the FSHR in men.

In addition to modulating signal transduction, receptor heteromers may act as modulators of GPCRs internalization and trafficking (91). Interest in these functions is increasing, since they could be involved in post-endocytotic signaling, which presents some peculiarities in the case of gonadotropin receptors (92). For instance, in granulosa cells, defective functioning of the endosome-associated RAB5A molecule results in abnormally high FSHR level and cAMP/PKA/CREB-pathway activation likely linked to polycystic ovary syndrome (PCOS) pathogenesis (93). In Sertoli cells, the FSHR was found in endosomal compartments (94), where it is stored according to age-dependent kinetics, thus suggesting a new regulatory mechanism accompanying testicular cell maturation (95).

To sum up, the current *in vitro* evidence shows that the FSHR forms dimers, oligomers and heterodimers, and activates several signal transduction pathways depending on receptor density, stimulation duration and molecular interactions with other receptors, and continues signaling once internalized, with mechanisms that are only partially known (73). The therapeutic implications of

these finding are still uncertain, but they must be kept in mind whenever analyzing the association of mutations and polymorphisms with clinical conditions and/or the pharmacologic effects of FSH.

3.4 Effect of FSH in the primate, adult testis

The current knowledge of FSH action in the adult human testis is limited. Mouse models are indicating that FSH is dispensable for spermatogenesis, however some pleotropism of LH and FSH is emerging, so that, under certain circumstances, FSH can take over the lack of intratesticular testosterone and support spermatogenesis (61,96). Data concerning human subjects derive from pituitary FSH secreting tumors, hemicastration, naturally occurring mutations, as well as from experiments on non-human primates. Patients with pituitary FSH-secreting tumors have enlarged testes (97-99). Activating *FSHR* mutations support spermatogenesis in the putative absence of intratesticular testosterone (100-102). *FSHB* gene mutations are very rare but they are so far invariably associated with azoospermia (103). Finally, hemicastration, as in the case of testis tumor, is associated with enlargement of the remaining testis (104) a sign of tubular activity and germ cell proliferation. In monkeys, hemicastration is associated with a volume increase of the remaining testis, related to abrupt inhibin B decrease resulting in FSH increase and B spermatogonia proliferation (105), and FSH administration stimulates spermatogenesis (106,107). Therefore, FSH is very important for primate spermatogenesis and an increase of its levels/action stimulates spermatogenesis over the baseline, an effect that could be therapeutically exploited (14).

3.5 Pharmacogenetics of FSH action

FSH signaling may be modulated by *FSHB* and *FSHR* gene polymorphisms, associated with various clinical pictures, including seminal and hormonal alterations and male infertility (108). The best studied *FSHR* and *FSHB* genetic variants are the rs6165, rs6165, rs1394205 in the *FSHR* gene and the rs10835638 in the *FSHB* gene (Fig. 2) (Table 1). While effects of these SNPs have been extensively reviewed (108-111), aspects that are relevant for future developments of FSH therapy of male infertility are discussed below.

Few *FSHB* and *FSHR* mutations impairing the molecular interaction of the two molecules have been described (112), while no SNPs affecting the ligand-receptor complex formation are known so far. The common SNP rs6165 (c.2039A>G nucleotide variation; p.T307A amino acid change) falls within the large *FSHR* extracellular portion, likely contributing to ligand binding. Despite being a promising candidate marker of FSH action, it has never been confirmed as causative of, or associated with specific male phenotypes alone. Rather, specific allelic combinations of this *FSHR* SNP with others could impact the reproductive male phenotype (108), especially when considered together with another common *FSHB* gene promoter SNP (rs10835638; -211G>T) (51,113) linked to the mRNA transcript levels of the FSH beta subunit (114-116).

In (prepubertal) Sertoli cells, FSH-induced PKA activation triggers mainly ERK1/2 phosphorylation and downstream proliferative events, reflecting the role of these cells as a supporting, nurturing unit of male gametes. Most importantly, it is established that the *FSHR* SNP rs6166 (c.2039A>G; p.N680S), located at the intracellular tail of the receptor, impacts FSH-induced kinetics of cAMP/CREB and ERK1/2 activation (117) and could thereby affect Sertoli cell proliferation. Homozygous p.N680S S carrier cells displayed weaker ERK1/2 and slower CREB FSH-dependent activation than homozygous N, providing the basis to explain genotype-specific variations in FSH-dependent steroidogenesis and gametogenesis. To date, *FSHR* p.N680S is known to be a genetic determinant of the gonadal response to FSH (70,108). In men, its role was demonstrated by clinical

studies, where the homozygous *FSHR* p.N680S S or N state impacted outcome of FSH treatment (50), susceptibility to testicular germ cell cancer (118) and, weakly, serum FSH levels and testicular volume (119). Taken all together, these data suggest that the *FSHR* p.N680S S impacts proliferative signals at the intracellular level and feedback control of the hypothalamus-pituitary-gonadal axis.

The *FSHR* promoter SNP rs1394205 (-29G>A) affects transcriptional activity of the *FSHR in vitro* (120) and the *FSHR* level of expression in the ovary (121) and could modulate FSH levels in men, alone or in combination with other SNPs (85).

For what concerns post-receptor intracellular events, no genetic variants modulating signal transduction and endosomal functioning associated with male infertility are known. However, it might be speculated that the cumulative effect of different SNPs in genes regulating FSH-FSHR signaling could be relevant in some cases of idiopathic infertility, becoming promising targets for new pharmacological therapies (122).

In summary, there is sufficient evidence to conclude that common SNPs of the *FSHB* and *FSHR* genes influence the amount of circulating hormone, the level of expression and the signal transduction of the FSHR. This, in turn, is expected to have an impact on testicular volume and sperm output.

3.6 Clinical studies with FSH based on pharmacogenetics of FSH action

The first demonstration of the influence of the *FSHR* p.N680S and p.T307A on human reproduction dates back to twenty years ago, concerns women undergoing assisted reproduction (123), and was followed by the first and still unique pharmacogenetic clinical trial evaluating effects of these SNPs in ovarian response (124). *FSHR* -29G>A and *FSHB* -211G>T were then investigated as well. A recent meta-analysis confirmed that the *FSHR* p.N680S is predictive of the FSH dosage required and of the number of oocytes retrieved in women undergoing controlled ovarian

stimulation (125), indicating that a pharmacogenomic approach could provide substantial advances in ART.

In light of the data concerning women, similar results are expected in men. In fact, the modulatory activity of the *FSHR* p.N680S was demonstrated in two different, large cohorts of men (51,119). Lower testicular volume and impaired fertility were reported in p.N680S homozygous S (51,119), consistently with lower activity of the S genotype on proliferative signals *in vitro*. However, the clinical effect was modest, and it was detected thanks to the analysis of large cohorts of patients (over 1000 men enrolled). These data are corroborated by results of a retrospective, combined evaluation of three *FSHR* SNPs, demonstrating that the c.2039A>G A (p.N680S N) allele is more frequent in fertile men than the S allele (87). Most importantly, the p.N680S N allele was investigated in association with the *FSHR* -29G>A G and c.919A>G A alleles, forming a specific haplotype associated with the fertile *status* in men (83). Beside the *FSHR* SNPs, the *FSHB* -211G>T SNP might be involved in male fertility, since it is associated with a reduction in *FSHB* gene transcription and significantly reduced FSH serum levels in infertile men (85) and patients with Klinefelter syndrome (126). In addition, this SNP is predictive of positive sperm retrieval upon ICSI (127) and of age at testicular growth in puberty (128), but it does not affect the number of Sertoli cells (129). Studies in adult men detected reduced testicular volume, sperm count, testosterone, and increased LH serum levels in *FSHB* -211G>T T homozygous men (51,115,130). All these data suggest a functional role for the *FSHB* genotype on spermatogenesis (129). The combination of *FSHR* and *FSHB* SNPs should have a much stronger impact on male fertility than each of them alone can have (51,85,128).

Reasonably, the testicular response to FSH would be mediated by the combinatory effects of *FSHR* expression, its signal transduction, and FSH levels in blood (108), suggesting that the haplotype impacts the efficacy of FSH therapy. However, only few clinical trials assessing this issue have been published so far and provided conflicting results (49,50,131,132). Indicative results were provided by

a study performed in 66 men with idiopathic infertility, which revealed that three months of treatment by recombinant FSH improved sperm quality, measured as DNA fragmentation index (DFI), of *FSHR* p.N680S homozygous N, but not of homozygous S carriers (50). Moreover, when the *FSHB* SNP was included in the analysis *a posteriori*, the improvement of sperm quality was limited to men with *FSHR* p.N680S N and *FSHB* -211G>T G homozygous haplotype (51). So far this study is the only prospective, controlled, registered and properly designed pharmacogenomic trial available in male infertility, suggesting that the *FSHR* p.N680S SNP may be a genetic marker of gonadal response to FSH in males. The role of the *FSHB* -211G>T remains not sufficiently tested by prospective, interventional studies (49,132), and therefores requires verification. It is possible that specific *FSHR-FSHB* haplotypes could be useful to tailor FSH treatment and, consequently, to improve its efficacy in idiopathic male infertility by identifying a subgroup of "responders". Although some strategies have been suggested (113,133), this issue has not yet been evaluated by pharmacogenetic clinical trials.

4. FSH for male idiopathic infertility: Prospects and needs

Overall it appears that FSH treatment of male infertility might have a rationale in the hormone capability to stimulate and increase sperm output. The case is clear in HH. In the case of idiopathic infertility, existing data suggest that FSH may be helpful to improve sperm parameters in some patients but not in all of them. This might depend on the etiology of OAT, unknown by definition, and/or on the genetic background impacting FSH action, but this statement remains speculative in the absence of well powered, controlled studies.

It is astonishing how few placebo-controlled clinical trials on FSH treatment of male idiopathic OAT have been published so far (14) (Table 2). The first placebo-controlled trial dates back to over 30 years ago (134) and did not achieve any improvement of spermatogenesis but it succeeded in halting research in the field for the following decades, until now. With the current

knowledge, the treatment protocol above could not have worked. At that time, the standard treatment of HH was transposed without modifications to idiopathic infertility and did not increase sperm output, possibly due to the overwhelming LH activity administered as human menopausal gonadotropin (hMG) enriched by hCG, with clearly excessive LH-like activity in patients who were not hypogonadal/testosterone deficient (29). However, another placebo-controlled trial based on recombinant FSH alone did not show any improvement in sperm output either (135), clearly showing that this therapy is not indicated for all men, at least at the dosage used and when patients are unselected, idiopathic, infertile males. We recently suggested that a FSH dosage higher than the replacement dose may be used in future trials (14), especially considering that studies using higher dosages demonstrated significant, dose-dependent increases of sperm output (136,137). Nevertheless, the criteria for the selection of men who could benefit from this treatment should be defined (113,126). In addition, the length of the treatment should be increased to cover two spermatogenetic cycles, i.e. at least 5 months (51). Recently, a survey evaluating the empirical use of FSH in male infertility in Italy gave hints about the attitude of infertile males toward such a demanding and long-lasting injection treatment and their wish to contribute to the resolution of couple infertility. The study showed that men are ready to share the burden of infertility treatment (138). Indeed, FSH is used for this purpose in Italy, where, from the regulatory point of view, idiopathic infertility is interpreted as a form of functional and selective hypogonadism due to insufficient/ineffective FSH action. This is, however, an exception, and few clinical data are available to help solve the question of FSH efficacy in the treatment of male idiopathic infertility in a real-world setting.

All available studies did not report adverse events in men after FSH stimulation, differently from women, for whom an excess of FSH may result in ovarian hyperstimulation syndrome, as demonstrated in case of FSH-secreting tumors (139-143), activating *FSHR* mutations (144), and as an adverse event in ART (145). In men, no consequences of FSH hyperstimulation have been described so far, apart from the expected testicular volume increase over the baseline, as happens in men with

FSH-secreting tumors (97). Therefore, a pharmacological FSH hyperstimulation may be proposed with the goal of improving sperm output, should not be expected to produce adverse events, and should be evaluated in properly designed, high-dose clinical trials.

In essence, the questions of whether FSH is effective, at the doses used so far, as an adjuvant therapy to stimulate spermatogenesis in some cases, and of how do we select them, remain unanswered. Is pharmacogenetics of FSH action the key? In order to answer this question, a well powered, placebo-controlled trial is needed. Since we do not know which *FSHB/FSHR* haplotype responds better to treatment, the trial should be large enough to include different dosages and allow the assessment of the haplotype effect on the primary end point. The question of the primary end point has also been considered and it seems that semen characteristics represent the most appropriate end point for the time being (113). The engagement of all stakeholders is necessary to reach this goal (14). In the absence of such a trial, FSH treatment of male idiopathic infertility remains empirical and, most probably, ineffective in the majority of treated men.

5. Conclusions

There is an increasing interest in the treatment of male infertility. The recent advances in the understanding of the molecular mechanism of action of FSH, the transgenic mouse models, and the effects of genetic mutations/polymorphisms of the *FSHB* and *FSHR* genes contributed substantially to our current knowledge about the role of FSH on human spermatogenesis and the impact of the genetic background on FSH levels and action. However, too few clinical studies have been performed so far and the existing meta-analyses are based only on a few hundred patients (37,39). In some patients, FSH might be effective in improving sperm parameters, although clear-cut selection criteria must be established *a priori*. The *FSHB-FSHR* haplotype might be one of such criteria, but this issue should be further tested by statistically powerful clinical trials designed with the aim of attaining a testicular overstimulation by FSH doses which is high enough to improve sperm output (14). Should such an approach work, at least in a subset of patients, it would not matter that the therapy is not etiological, since its rationale is to obtain a boost in spermatogenesis independently of the cause of its fault. This proof-of-principle trial can be implemented using any of the current, registered FSH preparations. If successful, novel biologicals with very long-acting FSH activity could be engineered to reduce the burden of the injections.

Another question is whether FSH is the best hormonal strategy. Given the preponderant role of LHCGR-mediated androgen production in supporting human spermatogenesis, as demonstrated by the efficacy of the treatment of HH with hCG alone, it would be interesting to investigate whether a mild overstimulation of the Leydig cell compartment has any effect on sperm production. A modest but significant reduction of serum testosterone was demonstrated in men with idiopathic infertility for the first time in 2004 (146) and was repeatedly confirmed thereafter (147-149). Although still within the physiological range, testosterone and the testosterone/LH ratio are lower, while estradiol and the estradiol/testosterone ratio are higher in infertile than in fertile men (150-152). This suggests that LH-induced stimulation of aromatase activity or testosterone production (or

secretion into the blood stream) is less efficient in infertile men. On the other hand, no studies evaluated the clinical effect of FSH + LH co-administration, neither those of LH alone, in male idiopathic infertility so far, and therefore this aspect deserves investigation. The short half-life of LH might be advantageous in this case, since the aim would not be to increase serum testosterone levels but, rather, to moderately rise Leydig cell activity. Some studies based on clomiphene citrate and other selective estrogen receptor modulators or aromatase inhibitors would suggest some benefit for a sub-group of patients (153,154), although insufficient data for achieving clear-cut indications exist at this point.

In conclusion, the genetic background influencing FSH action appears to be a very promising tool for tailoring FSH therapy of male idiopathic infertility, an issue which awaits a very much needed *ad hoc* clinical trial.

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Figure legends

Figure 1.

FSHR-mediated intracellular events in human Sertoli cells. Hormone binding to the receptor leads to the activation of multiple signaling pathways triggered by Gα proteins and G βγ complex, as well as β-arrestins and adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1). Gαs protein activation mediates proliferative signals via ERK1/2 and the transcription factor CREB, although the intracellular cAMP increase and PKA activation, occurring upstream, are linked to p38 MAPK-dependent cell death. Its inhibition is due to the action of ERK1/2, that is activated also by other Gα proteins and β-arrestins, which, in turn, leads to FSHR internalization, while APPL1 and the βγ dimer induces cell survival signals.

Figure 2. Location of the most common SNPs in the human *FSHB* and *FSHR* genes and protein. Exons are indicated by numbers, and white and yellow boxes represent the untranslated and translated regions, respectively. Gene location on its chromosome is also indicated (Kb = kilobase), while SNP position is indicated by arrows. A) *FSHB* gene structure (GenBank Gene ID: 2488). B) *FSHR* gene structure (GenBank Gene ID: 2492). C) p.T307A and p.N680S SNPs position in the FSHR protein.

Table 1. Single nucleotide polymorphisms in *FSH* and *FSHR* evaluated in male infertility.

Gene	SNP Code	Allele sequence	Protein sequence
FSHR	rs6165	c.919A>G	p.T307A
FSHR	rs6166	c.2039A>G	p.N680S
FSHR	rs1394205	-29G>A	NA
FSHB	rs10835638	-211G>T	NA

***[Footnote to Table 1]**: FSHB: follicle-stimulating hormone Beta subunit gene; FSHR: follicle-stimulating hormone receptor gene; NA: not applicable; SNP: single nucleotide polymorphism.*

Table 2. Clinical trials available in the literature, considering follicle-stimulating hormone (FSH) administration to men with idiopathic infertility.

Year of study	Citation	Study design	Number of patients (treated /non-treated)	FSH preparations used	FSH scheme
1987	(134)	Controlled trial	19/20	hMG and hCG	75 IU and 2500 IU daily, respectively
1994	(155)	Observational	31/101	Urinary-derived	75 IU daily
1997	(156)	Controlled trial	58/78	Urinary-derived	150 IU on alternate days
1998	(135)	Controlled trial	34/33	Recombinant	150 IU daily
1999	(157)	Observational	39/39	Urinary-derived	75 IU daily
2000	(158)	Observational	77/20	Urinary-derived	75 IU on alternate days
2000	(159)	Controlled trial	20/20	Urinary-derived	75-150 IU daily
2002	(160)	Controlled trial	30/15	Recombinant	50-100 IU on alternate days
2003	(161)	Observational	23/23	Recombinant	150 IU on alternate days
2004	(162)	Controlled trial	24/20	Urinary-derived	150 IU daily
2005	(163)	Controlled trial	62/50	Recombinant	100 IU on alternate days
2006	(137)	Controlled trial	15/15	Recombinant	300 IU on alternate days
2009	(164)	Controlled trial	57/62	Recombinant	150 IU on alternate days
2011	(132)	Controlled trial	70/35	Recombinant	150 IU on alternate days
2012	(165)	Controlled trial	65/63	Recombinant	100 IU on alternate days
2015	(136)	Controlled trial	272/82	Urinary-derived	50-100-200-300 IU on alternate days
2016	(50)	Controlled trial	38/28	Recombinant	150 IU on alternate days

[Footnote to Table 2*]: FSH: follicle-stimulating hormone; hCG: human chorionic gonadotropin; hMG: human menopausal gonadotropin.*

Figure 1

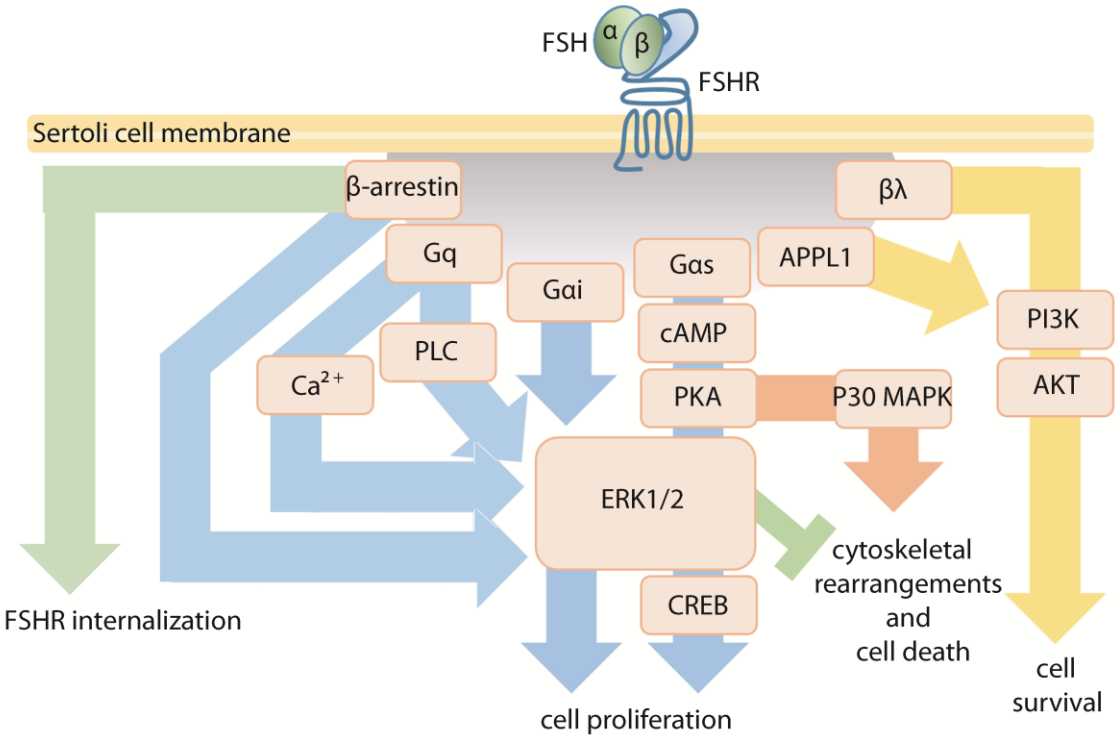

Figure 2