UNIVERSIDADE ESTADUAL DE CAMPINAS
SISTEMA DE BIBLIOTECAS DA UNICAMP
REPOSITÓRIO DA PRODUÇÃO CIENTIFICA E INTELECTUAL DA UNICAMP

Versão do arquivo anexado / Version of attached file:

Versão do Editor / Published Version

Mais informações no site da editora / Further information on publisher's website:
https://www.sciencedirect.com/science/article/pii/S1521693422001912

DOI: https://doi.org/10.1016/j.bpobgyn.2022.102310

Direitos autorais / Publisher's copyright statement:
©2022 by Elsevier. All rights reserved.

DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo
CEP 13083-970 – Campinas SP
Fone: (19) 3521-6493
http://www.repositorio.unicamp.br

Best Practice & Research Clinical Obstetrics and Gynaecology 86 (2023) 102310

Contents lists available at ScienceDirect

Best Practice & Research Clinical Obstetrics and Gynaecology

journal homepage: www.elsevier.com/locate/bpobgyn

2

Male infertility and gonadotropin treatment: What can we learn from real-world data?

Sandro C. Esteves [a, b, c, *], Arnold P.P. Achermann [a, b], Manuela Simoni [d, e, f], Daniele Santi [d, f], Livio Casarini [d, e]

[a] Andrology and Human Reproduction Clinic, Av. Dr. Heitor Penteado, 1464, Campinas, 13075-460, Brazil
[b] Department of Surgery (Division of Urology), University of Campinas (UNICAMP), Campinas, SP, Brazil
[c] Faculty of Health, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[d] Unit of Endocrinology, Department of Biomedical, Metabolic, and Neural Sciences, University of Modena and Reggio Emilia, Modena, 41126, Italy
[e] Center for Genomic Research, University of Modena and Reggio Emilia, Modena, 41125, Italy
[f] Unit of Endocrinology, Department of Medical Specialties, Azienda Ospedaliero-Universitaria of Modena, Ospedale Civile of Baggiovara, Modena, 41126, Italy

Keywords:
Male infertility
Hormonal disorders
Hormonal therapy
Gonadotropins
Assisted reproductive technology
Real-word data

A B S T R A C T

Gonadotropin therapy to treat specific male infertility disorders associated with hypogonadotropic hypogonadism is evidence-based and effective in restoring spermatogenesis and fertility. In contrast, its use to improve fertility in men with idiopathic oligozoospermia or nonobstructive azoospermia remains controversial, despite being widely practiced. The existence of two major inter-related pathways for spermatogenesis, including FSH and intratesticular testosterone, provides a rationale for empiric hormone stimulation therapy in both eugonadal and hypogonadal males with idiopathic oligozoospermia or nonobstructive azoospermia. Real-world data (RWD) on gonadotropin stimulating for these patient subsets, mainly using human chorionic gonadotropin and follicle-stimulating hormone, accumulated gradually, showing a positive therapeutic effect in some patients, translated by increased sperm production, sperm quality, and sperm retrieval rates. Although more evidence is needed, current insights from RWD research indicate that selected male infertility patients might be managed more effectively using gonadotropin therapy, with potential gains for all parties involved.
© 2023 Elsevier Ltd. All rights reserved.

* Corresponding author. ANDROFERT, Andrology and Human Reproduction Clinic, Av. Dr. Heitor Penteado, 1464, Campinas, Brazil.
E-mail address: s.esteves@androfert.com.br (S.C. Esteves).

https://doi.org/10.1016/j.bpobgyn.2022.102310
1521-6934/© 2023 Elsevier Ltd. All rights reserved.

Introduction

Male infertility is a disease of the male reproductive system with various non-mutually exclusive causes and contributory factors, encompassing congenital and genetic factors, anatomical dysfunctions, hormonal disorders, ejaculatory dysfunctions, genital tract infections, immunological abnormalities, chronic diseases, cancer and related treatments, gonadotoxin exposure, inadequate lifestyle, and sexual disorders incompatible with intercourse [1–9].

Global estimates indicate that male factors, alone or combined with female factors, contribute to at least 50% of reported infertility cases and that over 50 million men have infertility worldwide [10]. In many cases, the factor(s) impairing male fertility can be identified through an andrological evaluation and treated or alleviated, positively impacting the couple's pregnancy prospects [11–15]. However, only about half of the men facing infertility seek medical assistance in developed countries [16]. A well-conducted andrological evaluation is critical to achieving these goals and includes a detailed medical and reproductive history of the patient, a physical examination, and at least two routine semen analyses carried out in a specialized andrology laboratory [1,7,17]. The second-line investigations (such as hormonal assessment, sperm functional tests, genetic analysis, and imaging studies) might be necessary and are based on the clinical and semen analysis findings [2,9,15,18–22].

Hormonal therapy has been integral to male infertility treatment options [23]. The rationale of this approach relates to the critical regulatory role of the hypothalamic–pituitary–gonadal (HPG) axis on spermatogenesis and the common knowledge that hormonal abnormalities are potentially treatable causes of male infertility [7,11,23–25]. Indeed, the use of hormonal therapy to treat specific endocrine disorders (such as hypogonadotropic hypogonadism and hyperprolactinemia) is well-established and evidence based [7,24]. The administration of exogenous human chorionic gonadotropin (hCG), alone or combined with exogenous follicle-stimulating hormone (FSH), restores spermatogenesis to varying degrees in up to 90% of patients with hypogonadotropic hypogonadism, with reported pregnancy rates of up to 65% (natural or assisted) [7,26,27]. In these patients, sperm output is usually higher when hCG is combined with FSH (vs. hCG alone), particularly in men with congenital forms of hypogonadotropic hypogonadism [7,26–28]. Despite that, data from randomized controlled trials (RCTs) comparing drugs and regimens in men with hypogonadotropic hypogonadism are lacking.

By contrast, the use of hormonal therapy to improve fertility in men with idiopathic oligozoospermia or nonobstructive azoospermia (NOA) remains controversial [23], despite being widely practiced. Surveys conducted among urologists indicate that 65–87% of practitioners routinely prescribe drugs targeting the HPG axis, including selective estrogen receptor modulators (SERMs), aromatase inhibitors, hCG, FSH, and human menopausal gonadotropin (hMG) [29,30]. Moreover, a multicenter study conducted in Italy—where the National Medicines Agency approves FSH therapy for infertile men with idiopathic infertility and FSH levels <8 IU/L, independently of semen parameters—showed that hormonal therapy is prescribed to 55% of eligible patients [31]. The existence of two major pathways for spermatogenesis, including the supraphysiological FSH stimulation and intratesticular testosterone (ITT) production boosting, has provided a rationale for empiric hormone stimulation therapy in both eugonadal and hypogonadal males with idiopathic oligozoospermia or NOA [28,32].

Notwithstanding these observations, large-scale RCTs on the matter concerned are lacking, and knowledge about side effects and reproductive outcomes after the treatment is scanty, precluding conclusive clinical recommendations. Accordingly, major urological/andrological guidelines do not currently advocate the routine use of empiric hormonal therapy for spermatogenesis stimulation in men with idiopathic oligozoospermia or NOA [23,33]. Despite that, real-world data (RWD) on the use of hormonal stimulation for male infertility treatment, mainly through exogenous gonadotropins, have accumulated gradually.

Data from observational studies may represent a valid study type to acquire knowledge about the potential role of hormonal therapy in specific male infertility conditions [34]. RWD may also present an opportunity for obtaining data on patients with characteristics outside those typically required for trial eligibility [35–37]. RWD allow for assessing how therapy affects a heterogeneous population, generating hypotheses for testing in RCTs. Also, it adds information for evaluating trial feasibility by examining the impact of planned inclusion/exclusion criteria in the relevant population, both within a

geographical area and at a particular trial site [35]. Informing prior probability distributions and identifying prognostic indicators or patient baseline characteristics for enrichment or stratification are other advantages of RWD observational studies [38,39].

In this review, we summarize the evidence concerning the use of hormonal therapy with exogenous gonadotropins for male infertility treatment, focusing on men with idiopathic oligozoospermia and those with NOA. We also review the essential knowledge about spermatogenesis hormonal control, summarize the recent animal and human data on the molecular mechanisms of gonadotropin action, and discuss the biological plausibility of gonadotropin therapy and its potential clinical utility, focusing on semen and pregnancy data. Lastly, we appraise the knowledge gaps and suggest a path for future research.

Spermatogenesis hormonal regulation

Spermatogenesis consists of the diploid spermatogonial proliferation and differentiation into haploid spermatozoa [25,40]. The process occurs in close contact with the Sertoli cells, the male counterpart of granulosa cells, located within testicular seminiferous tubules, and is regulated primarily by FSH and luteinizing hormone (LH)-driven testosterone [41]. The pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the release of pituitary FSH and LH. Inhibin B and estradiol are the primary inhibitors of FSH secretion, but many other pituitary-regulatory proteins, such as activin and follistatin, have been implicated in FSH secretion and action [25,28,40].

Follicle-stimulating hormone

The FSH is a heterodimer displaying an α subunit, common to all gonadotropins and the cognate molecule thyroid-stimulating hormone (TSH), and a β subunit specific for its receptor (FSHR) [42]. The α subunit is coded by the *CGA* gene located in chromosome 6 [43], while the *FSHB* gene codes the β subunit and is in chromosome 11 [44,45]. The gene transcriptional activity is primarily modulated by the presence of a single-nucleotide variation (SNV; formerly single-nucleotide polymorphism [SNP]) falling within the promoter *FSHB* gene [46], 211 base pairs upstream from the transcription start site. It is characterized by the G to T nucleotide change (−211 G/T; rs10835638) and is associated with reduced serum FSH levels, decreased sperm count, and reduced testicular volume; patients carrying the T allele do not adequately upregulate FSH circulating levels to achieve full spermatogenesis [47]. Other SNVs, also located in chromosome 11 and within the *FSHB* gene, might also affect the FSH serum level and its action on spermatogenesis in men with idiopathic or unexplained infertility [48].

The number of spermatogonia modulates pituitary FSH secretion [49]. Normal FSH levels are typically associated with adequate spermatogonial quantity. By contrast, when spermatogonia are absent or their number is markedly reduced, the endogenous FSH levels increase [50]. Furthermore, low FSH levels are typically observed in men with primary pituitary dysfunction (e.g., primary hypogonadotropic hypogonadism) or in those using testosterone replacement therapy [7,49]. Along these lines, glycosylation partially regulates FSH activity, which provides several hormone variants naturally released by the pituitary [51]. In particular, hypoglycosylated FSH molecules have a higher bioactivity than the fully glycosylated variants [52], a feature linked to different glycoprotein–receptor interactions on the cell surface [53].

The FSH provides indirect structural and metabolic support for spermatogenesis via its receptors in the Sertoli cells [28,40,41]. It regulates structural genes involved in the organization of cell–cell junctions and genes required for the metabolism and transport of regulatory and nutritive substances from Sertoli to germ cells [40]. In the testis, endothelial FSHRs mediate FSH transport across the gonadal endothelial barrier [54]. The FSH also has a regulatory role in Sertoli cell number that is critical to maintaining spermatogenesis. Specifically, FSH regulates the mitotic proliferation of Sertoli cells, supports their growth and maturation, and prompts the release of androgen-binding protein [41].

The amplitude of the Sertoli cell response to FSH is modulated by a common *FSHR* SNP, characterized by the A to G nucleotide change at position 2039 of the transcription start site, resulting in the asparagine to serine change at position 680 of the protein chain [54]. In particular, it is known that

homozygous women for the serine receptor phenotype undergoing medically assisted reproduction are less sensitive to ovarian stimulation with exogenous FSH [55], and some evidence is also rising in men [56]. Another *FSHR* SNP likely modulating the response of Sertoli cells to FSH is the G to A nucleotide change at position −29 of the *FSHR* gene [57]. An *in vitro* study suggested that this SNP influences receptor mRNA transcriptional levels [58], although some clinical observations found no association with serum FSH levels and sperm parameters [59]. Reasonably, the pharmacogenomic response to FSH should account for the combined effect of several *FSHB* and *FSHR* SNPs [60].

In summary, the primary role of FSH is to increase sperm quantity in synergy with ITT. Although an adequate FSH level is not mandatory for the completion of spermatogenesis in humans, its deficiency markedly reduces sperm production.

Luteinizing hormone

LH is a glycoprotein released by pituitary gonadotrope cells in a pulsatile fashion upon assembly of the α and LHβ subunits; the molecule carries two glycosylations in the α and one in the β subunits [42,61]. The latter originated from mRNA transcripts coded by the *LHB* gene located in chromosome 19q13.32. The *LHB* gene is embedded in a genetic cluster containing six highly similar genes and pseudogenes (*CGBs*) coding the β subunit of hCG in pregnant women [62]. LHβ and hCGβ coding genes share about 95% of their identities and differ mainly for an additional sequence of *CGBs*, resulting in a 28-amino acid extension of the molecule and five additional glycosylation sites [63].

LH's primary function is to stimulate testosterone production by the Leydig cells. It acts through its transmembrane receptors (LHCGR) located in the Leydig cells [25]. It is common knowledge that, upon hormone binding, the receptor undergoes a conformational change linked to Gαs protein dissociation from the βγ dimer and triggers the activation of multiple signaling pathways. However, several decades ago, pioneering experiments performed with hCG used as an LHCGR ligand demonstrated that treatment with hCG leads to the intracellular increase in the cyclic adenosine monophosphate (cAMP) [64]. The second messenger, cAMP, is one of the critical players triggering steroidogenesis, which occurs as an event involving upstream activation of protein kinase A (PKA), phosphorylation of the transcription factor, cAMP-responsive element-binding protein (CREB), and upregulation of target genes coding steroidogenic enzymes [65].

Furthermore, recent studies have elucidated the role of other LHCGR intracellular interactors, such as Gq and Gαi proteins and β arrestins [66]. These molecules mediate relatively marked LH-induced phosphorylation of protein kinase B (AKT) and extracellular signal-regulated kinase 1/2 (ERK1/2), leading to survival and mitogenic signals [67,68]. Moreover, they support steroidogenesis [69] and are involved in receptor internalization and trafficking [70], inducing the intracellular routing of the receptor and transmitting sustained cAMP signaling [71]. Therefore, LH controls its receptor, inducing the sequestration from the cell membrane and changing its mode of action. Downstream LH-mediated events account for the production of growth factors (e.g., EGF-like) by Leydig cells and play a critical role in spermatogonial proliferation [72].

Testosterone

In males, testosterone is the primary circulating androgen. Over 95% of testosterone is secreted by the Leydig cells in the testes, which produce approximately 6–7 mg of testosterone daily [73]. The remaining circulating testosterone comes from the adrenal gland. The major substrate for testosterone synthesis is cholesterol [25]. Testosterone can be converted to estradiol by aromatase or dihydrotestosterone by 5a-reductase [74]. LH stimulates the transcription of genes that encode the enzymes involved in the steroidogenic pathways.

LH-driven testosterone is critical for spermatogenesis. ITT acts via its intracellular androgen receptors, present in the Sertoli cells, which secrete testosterone-dependent paracrine stimuli for the development of germ cells [73,75,76]. The primary function of ITT relates to the post-meiotic progression of round spermatids to mature sperm (spermiogenesis) [32,40,73,75]. ITT is also needed to transition from type A to type B spermatogonia and androgen receptor up-regulation, ultimately enhancing Sertoli cell function [73,75].

Biological plausibility of gonadotropin therapy for male infertility treatment in nonobstructive azoospermia and idiopathic oligozoospermia

Imbalances of reproductive hormones are common in men with infertility. Primary testicular disorders caused by congenital (cryptorchidism and spermatogenic failure), genetic (Klinefelter syndrome and Y chromosome microdeletions), anatomical (varicocele, testicular trauma, or torsion or infection), and neoplasms (e.g., testicular cancer and related gonadotoxic treatments) are associated with oligozoospermia or azoospermia; these factors can impair testosterone synthesis [2–4,23,77]. Indeed, biochemical hypogonadism, defined by low serum testosterone levels (e.g., <300–350 ng/dL or <10–12 nmol/L) [78], affects up to 50% of males with NOA [79,80]. As a result, the pituitary gland typically responds by increasing FSH and LH secretion to stimulate sperm and androgen production. A hormonal assessment, including FSH and testosterone levels as minimum standards, is recommended for patients presenting with oligozoospermia or azoospermia, erectile dysfunction, hypospermia (ejaculate volume <1 ml), endocrinopathies (current or previous), or testicular hypotrophy [1,4,11,23,77].

Lessons from mouse knockout models

Murine studies have demonstrated differential endocrinological, histopathological, and reproductive outcomes from disrupting the gonadotropins and/or their receptors (Table 1). LH receptor knockout (LuRKO) male mice have high circulating FSH and LH levels, underdeveloped external and internal genitalia, dramatically low circulating testosterone levels, and spermatogenesis arrested at the round spermatid stage [81]. These mice's reproductive phenotype is overall similar to that of human males affected by severe hypergonadotropic hypogonadism caused by inactivating *LHCGR* mutations [82]. LuRKO mice recover spermatogenesis after the administration of exogenous testosterone, indicating that the steroid is a significant player in male gonadal function when FSH levels are adequate [82]. LuRKO mice highlight the relevance of functional LH receptors for testosterone production and spermatogenesis, although the minimum ITT concentration required to maintain spermatogenesis remains largely unknown.

Table 1
Precise mechanisms related to individual roles of LH and FSH using gonadotropin knockout (KO) and receptor KO mice.

Characteristics	Mouse KO model	FSH, LH, and T serum levels	Mechanism	Phenotype and effect on spermatogenesis
Lack LH receptors	LuR	High LH and FSH, and low T	Lack of T action	Underdeveloped external and internal genitalia, and spermatogenesis halted at the round spermatid stage
Lack LH beta chain responsible for LH specific activity	LHbeta	Normal FSH, normal but defective LH, and low T	Lack of T action	Decreased testis size, Leydig cell hypoplasia, and spermatogenic arrest at the round spermatid stage
Lack Sertoli-cell AR	SCAR	Normal T, FSH, and LH	Lack of T action	Normal testis size, maturation arrest, and disrupted Sertoli cell organization
Lack Leydig cell AR	LAR	High FSH and LH, low T	Lack of T action	Normal Leydig cell count and size, and spermatogenesis arrested at the spermatid stage
Lack FSH beta chain responsible for FSH specific activity	FSHbeta	Normal LH and T Normal T Defective FSH	Lack of FSH action	Reduced testis size and seminiferous tubule diameter, but presence of full spermatogenesis, although varying from normal to reduced
Lack FSH receptors	FSHR	Normal FSH, LH and T	Lack of FSH action	Reduced testis size and seminiferous tubule diameter, but presence of full spermatogenesis, although varying from normal to reduced (more pronounced effect than FSHbeta_KO)

T; testosterone, ITT: intratesticular testosterone, AR: androgen receptor, LH: luteinizing hormone, FSH: follicle-stimulating hormone.

Male knockout models lacking the LHβ chain (LHβ-null mice), which is responsible for specific LH-beta activity, exhibit a reproductive phenotype similar to that of LuRKO mice [83]. They have postnatal defects in gonadal development and function, including decreased testis volume, Leydig cell hypoplasia, spermatogenic arrest at the round spermatid stage, low circulating testosterone levels, and infertility. However, FSH levels in LHβ-null mice are normal, and hCG administration can restore spermatogenesis [84], thus making these mice a valuable model for studying LH ligand deficiency in humans. In both LuRKO and LHβ-null models, the defective LH pathway leads to insufficient testosterone production and action, resulting in a spermatogenesis halt at the spermatid stage.

Fshr and Fshb KO male mice are fertile and have similar reproductive phenotypes characterized by reduced testis size and substantially maintained spermatogenesis [85], like their corresponding human phenotype [86]. These mice exhibit reduced seminiferous tubule diameter, possibly reflecting a failure of FSH stimulation to Sertoli cell division during the perinatal period, and sperm production varying from normal to reduced. In a study crossbreeding LurKO and transgenic mice expressing Sertoli cell-specific mutant Fshr (Fshr-CAM) with high constitutive activity [87], the double-mutant mice had minimal testosterone production but, surprisingly, almost normal spermatogenesis that persisted even after administration of the potent anti-androgen flutamide. This study questioned the relevance of relatively high ITT levels in supporting spermatogenesis. Likewise, other studies in rodents have revealed that low testosterone levels influence the localization of androgen receptor expression in male gonadal tissues [88], likely occurring as an adaptative process to maintain the androgenic activity and support spermatogenesis. Interestingly, animal studies have indicated that high FSH levels could be sufficient to maintain spermatogenesis without testosterone [87]. The molecular mechanism explaining this phenomenon is still unclear, but it has been speculated that high FSH-dependent signaling might stimulate Sertoli cells to produce unknown paracrine factors overlapping androgen signaling in Leydig cells [87].

Cell-specific Sertoli-cell androgen receptor (AR) knockout (SCARKO) mice exhibit adequate circulating gonadotropin and testosterone levels and normal testicular size. However, the lack of testosterone action results in maturation arrest and disruption of Sertoli cell organization [89,90]. This model highlights the role of androgens in germ cell progression through meiosis as their spermatocytes exhibit normal expression of meiotic prophase targets but fail to undergo further divisions and remain blocked in the leptotene/zygotene prophase. Furthermore, this model indicates that initiation, but not the subsequent stages of early meiotic prophase, is androgen independent. By contrast, germ cell-, smooth muscle cell-, and peritubular myoid cell-specific AR knockout displayed no major defective reproductive phenotypes.

Leydig cell-specific AR knockout (LARKO) exhibits impaired steroidogenic function, resulting in spermatogenesis arrest at the round spermatid stage [90]. LARKO mice show decreased expression of genes coding steroidogenic enzymes; their testosterone levels are about ten times lower than the wild type, despite high circulating LH and FSH levels. Surprisingly, Leydig cell size and number in LARKO mice are normal, leading to the speculation that differentiation and establishment of steroidogenic function of adult Leydig cells do not require functional AR [90]. The models discussed above substantiate the potential clinical utility of gonadotropin therapy in specific male infertility scenarios.

Gonadotropins' molecular properties, action, and preparations

Effect of FSH on sertoli cells

In Sertoli cells, FSH binding to its receptor triggers the activation of Gαs protein and, in turn, of the adenylyl cyclase enzyme. These events lead to the intracellular increase in the second messenger cAMP [91], achieving a new equilibrium between its production and the degradation rate operated by phosphodiesterases (PDEs) [92]. cAMP binds PKA, resulting in the release of PKA catalytic subunits and indirectly mediating the phosphorylation of the ERK1/2 mitogen-activated protein kinase (MAPK) and the transcription factor CREB [91] (Fig. 1). The activation of these molecules is associated with mitogenic signals at the postnatal age [93]. Thus, FSH is a central regulator of intracellular cAMP concentration and a critical player in promoting Sertoli cell proliferation, synergically complemented by the action of androgens, activin A, and insulin-like growth factor (IGF)-I [94].

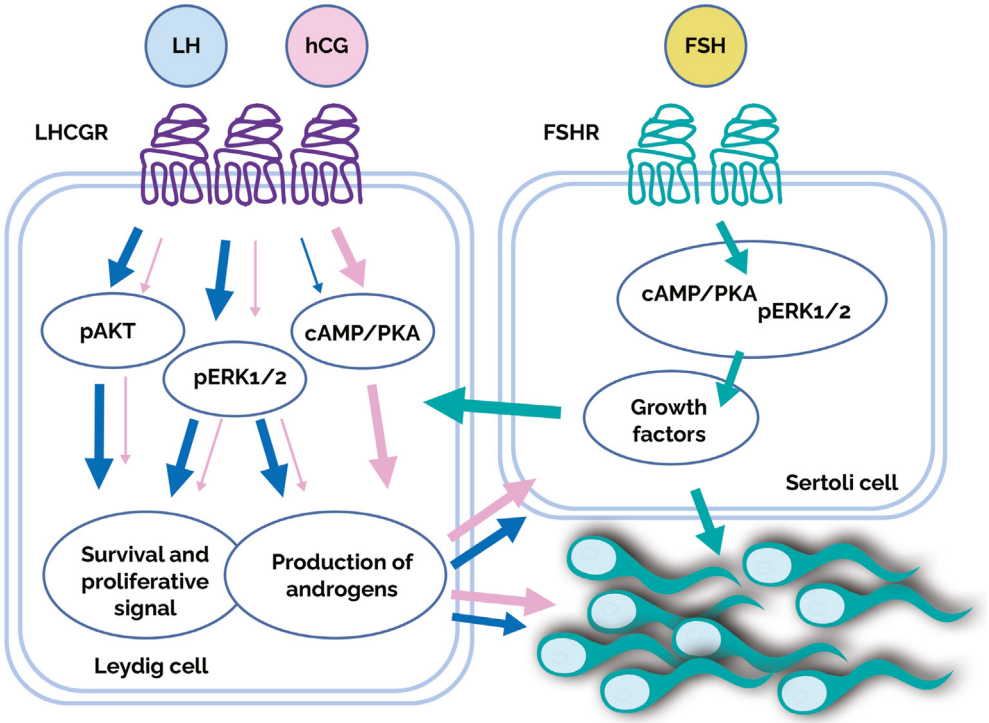

Fig. 1. Gonadotropins' action in the male gonads. LH and hCG target its Leydig cell-specific receptor, activating quantitatively and qualitatively different intracellular signaling pathways. While LH directly mediates the activation of survival and proliferative events, hCG triggers preferentially steroidogenic signals. LH signals are preferentially exerted via phosphorylation of ERK1/2 and AKT after the recruitment of G protein and b-arrestins, resulting in proliferative/antiapoptotic events. hCG is more potent than LH for activating the cAMP/PKA pathway. Still, both molecules have a qualitatively similar balance of stimulatory and inhibitory steroidogenic signals to boost the synthesis of testosterone required for spermatogenesis. The arrows' width indicates the differential activity of LH (blue arrows) and hCG (pink arrows), as described above. FSH binds its receptor expressed in Sertoli cells, stimulating the production of key growth factors and activation of mitogenic signals mainly through the cAMP/PKA pathway.

Interestingly, intracellular levels of cAMP are developmentally stage dependent, because FSH efficacy in inducing second messenger production increases from birth to puberty [93]. The age-related rise of the Sertoli cell dependence from cAMP could be a requirement to maintain the effectiveness of FSH in activating proliferative signals. These events are accompanied by the decline in sensitivity to FSH, over time, inducing the production of other molecules associated with survival signals at the prenatal stage, such as phosphatidyl-inositide-3 kinase (PI3K), AKT, mammalian target of rapamycin (mTOR), and ribosomal protein S6 kinase beta-1 (p70S6K) [95].

Besides the well-known cAMP/PKA pathway, the last two decades provided new insights into the FSH mode of action in Sertoli cells, revealing a complex network of intracellular signaling cascades activated by the hormone. The FSHR coupling to Gαi protein mediates the phosphorylation of ERK1/2 (pERK1/2) independently of cAMP, further supporting the FSH-dependent mitogenic signal [93,96]. Moreover, G protein-dependent pERK1/2 activation would occur very rapidly, within 1–5 min upon hormone-receptor binding, while prolonged ERK1/2 phosphorylation is sustained by other FSHR intracellular interactors, such as β-arrestins [97]. These molecules are scaffold proteins that sequentially dictate the kinetics of the ERK1/2 phosphorylation profile, cooperate with Gαs protein in triggering p70S6K activation, and are involved in the trafficking of the receptor [98]. Therefore, β-arrestins exert several actions related to FSH functioning, thus playing an essential role in supporting male fertility. These issues are demonstrated by clinical observations revealing that β-arrestin-, but not cAMP-dependent signaling, is

maintained in oligozoospermic men bearing the inactivating Ala189Val *FSHR* mutation [99]. Notably, after binding its ligand, FSHR undergoes internalization into intracellular compartments via pathways not involving β-arrestins and with regulatory functions of cAMP signaling [100].

Additionally, recent investigations found increasing relevance of micro-RNAs (miRNAs) in regulating FSH action. Studies in rats with suppressed FSH and testosterone activity described the miRNA network at spermiation as susceptible to hormonal control [101]. Two of the transcripts identified in this model were attributable to the phosphatase and tensin homolog (*PTEN*) enzyme mRNA, suggesting that they would be a target of FSH and/or androgen action. Indeed, Sertoli cell treatment with FSH *in vitro* enhanced PTEN protein levels massively within minutes, restoring terminal cell differentiation and proliferation [102]. Moreover, FSH treatment induced the decline of miR-92a-3p levels, which could be linked to increased *FSHR* expression [103]. These data suggest that the miRNA network might have a crucial role in the modulation of the FSH signaling in Sertoli cells, although knowledge about the miRNA-mediated regulation of Sertoli cell functioning remains limited.

Currently, both urinary and recombinant FSH preparations are available for clinical use. Urinary FSH preparations are marketed in lyophilized vials (typically containing 75 IU), whereas recombinant FSH preparations are available in both lyophilized vials (typically containing 75 IU) and pen devices (with doses varying from 300 IU to 900 IU, for fractionated use) [42]. Both preparations are administered subcutaneously; the lyophilized products must be reconstituted using sterile water before injection, whereas the pen devices are manufactured ready-to-use. After each injection, the peak serum FSH levels are reached within 10–12 h; the FSH levels then decline until the next injection. Recently, a long-acting recombinant FSH preparation was developed for subcutaneous use by combining recombinant human FSH with the hCG *C*-terminal peptide (CTP) [42]; the preparation has a plasma half-life of 65 h. Menotropins (e.g., hMG) extracted from the urine of postmenopausal women also provide lyophilized FSH for daily subcutaneous or intramuscular administrations; these preparations contain FSH and LH activity (mainly derived from the addition of hCG) in a 1:1 ratio, i.e., 75 of IU FSH and 75 IU of LH activity [42].

Recombinant FSH has a better safety and quality profile than its urinary counterparts [42,104]. In general, recombinant FSH preparations are purer than urine-derived FSH, and the incorporation of vial filling by mass virtually eliminated batch-to-batch variations and enabled accurate dosing. While FSH preparations have been extensively used for ovarian stimulation in women undergoing fertility treatments [38,39,42,43], data concerning their use in the context of male infertility treatment are less robust. The label indication for FSH use in males is typically restricted to hypogonadotropic hypogonadism (HH), which must be combined with hCG. In HH males, the doses vary from 75 IU to 225 IU, administered twice or thrice a week [7,26,27]. Similar doses have been prescribed for stimulating spermatogenesis in idiopathic oligozoospermic and hypergonadotropic hypogonadal or eugonadal NOA patients [3,27–29,32,56,105], as an off-label prescription in most countries.

Effect of LH (and hCG) on leydig cells

Given the steroidogenic fingerprint of Leydig cells, which express LHCGRs to which both LH and hCG can bind, it is understandable that hCG found clinical utility in replacing LH functions [66]. Both glycoproteins share a common α and a specific β subunit, assembled to form a noncovalently linked heterodimer acting on specific leucine-rich repeats (LRRs) and rhodopsin-like G protein-coupled receptors (GPCRs). However, hCG is easily purified in high concentrations from the urine of pregnant women. In contrast, human LH of pituitary origin is difficult to obtain and lacks full biological activity because it embeds a proteolytic site leading to internally cleaved hormones [66]. Recombinant technology allowed the production of human recombinant LH, but it has only recently become available for clinical use in ovarian stimulation, and its use in male infertility has been anecdotal [42,66]. Additionally, hCG has a longer half-life than LH, making it more patient-friendly than LH, as less frequent injections would be required [42]. The differences in the half-lives of the two molecules relate mainly to oligosaccharide structures, especially O-linked [42,66]. Notably, hCG has a long carboxy-terminal segment with 24 amino acids containing four O-linked oligosaccharide sites, whereas LH has just one [42]. After intravenous administration, hCG has a terminal half-life of 23–31 h compared to 10–12 h of recombinant LH [106,107].

It is well known that hCG binds LHCGRs and has a higher steroidogenic potential than LH [66]. *In vitro* data from several primary and engineered cell models demonstrated that hCG acts

preferentially as an inducer of cAMP [66,67,108] and intracellular Ca^{2+} increase [109] rather than ERK1/2 or AKT phosphorylation, markedly upregulating the transcription of genes coding steroidogenic enzymes. For example, in a mouse Leydig tumor cell line, the activation of gonadal steroid synthesis is more pronounced using hCG than LH [110]. Another *in vitro* study confirmed the higher cAMP production after the treatment of mouse Leydig cells with hCG *vs.* LH, although no differences were found in testosterone production [68]. Such experiments are characterized by cell lines expressing non-human receptors and need a prudent interpretation. Nevertheless, these data support the use of hCG for testosterone biosynthesis and spermatogenesis.

Given the relative low availability of human Leydig cells for *in vitro* studies, assessing the differential action of LH and hCG has been provided by *in vivo* clinical studies. A trial conducted on 19 healthy men undergoing pituitary suppression with GnRH antagonist indicated that Leydig cell stimulation might be exerted by relatively low exogenous LH doses (787.5 IU/week), which induced similar testosterone levels to those obtained by administration of 5000 IU/week of hCG [111]. This finding may lead to the speculation that LH might be used at a lower dosage than hCG to induce androgen production *in vivo*, even if it is non-pulsatile, indicating a differential action of the two molecules in human males.

The results of the study mentioned above may be explained by the fact that LH exerts a preferential kinase-dependent activation of mitogenic signals [67,108], which could improve the metabolic state of the cell, positively impacting steroidogenesis (Fig. 1). This effect would be detectable in cells expressing the human receptor, which may discriminate between LH and hCG binding [112]. By contrast, *in vitro* studies using mouse receptor-expressing cells are unable to provide the complete pattern of LH- and hCG-specific intracellular pathways [68].

Three additional studies are noteworthy as they pioneered the comparisons between hCG and LH in the human male setting. Two decades ago, a case of an 18-year-old boy with a deletion of the exon 10-coded portion of the *LHCGR* gene was described [113]. The patient had Leydig cell hypoplasia, delayed pubertal development, small testes, high serum LH, and low testosterone levels. Interestingly, he was unresponsive to endogenous LH, but exogenous hCG administration restored androgen biosynthesis and spermatogenesis (Fig. 2). *In vitro* analysis confirmed the lack of LH-induced cAMP increase, while hCG activated the signal transduction pathway upon mutant receptor binding [114]. Although the clinical scenario described above is very rare, it highlights the capability of the LHCGR to discriminate between the two natural ligands (Fig. 2), possibly reflecting a different regulation of gametogenesis and placentation in humans. Most importantly, this case report suggested that hCG may have different actions than LH in male gonads.

Subsequently, in 2008, a randomized, single-blind study including 20 healthy eugonadal men aged 18–30 evaluated hCG's and LH's effect on Leydig cell function [115]. A subgroup received either vehicle alone or urinary hCG intramuscularly in doses varying from 50 to 5000 IU, while another group received recombinant hCG intramuscularly in a dose of 250 mcg (6500 IU) or recombinant LH intravenously in doses varying from 75 to 225 IU. A serial assessment of serum testosterone and estradiol was carried out to determine the response to stimulation. There was a dose-dependent increase in testosterone and estradiol levels with urinary hCG administration, with a peak observed at 48 h and 72 h after the injection of 500 IU and 5000 IU, respectively. The peaks in testosterone and estradiol obtained with urinary hCG (5000 IU) and recombinant hCG (6500 IU) did not differ. Serum LH levels increased dose-dependently after injection of recombinant LH; the peak value was obtained 30 min after injections, progressively dropping to reach basal values 6 h later. Similarly, a dose-dependent increase in testosterone (but not estradiol) was observed after recombinant LH administration, with a peak observed 6 h after administering 225 IU. The lack of a stimulatory effect on estradiol levels by recombinant LH is attributed to its lesser steroidogenic potency than hCG, probably explained by its short plasma half-life and the reversibility of its binding to the LHCGR. In this study, the highest serum testosterone levels attained within 5 h after the injection of either 150 or 225 IU recombinant LH correlated with the 48-h peak increase in testosterone in response to the injection of 50 IU recombinant hCG [115]. The results of this study confirm the ability of both recombinant hCG and recombinant LH to promote adequate androgen production in normal men.

Along these lines, a study comparing the effects of a daily, low (75 IU) dose of recombinant LH versus 75 IU hCG in a hypogonadotropic hypogonadal hypophysectomized man found no differences between the two hormones in terms of restoring the eugonadal status after 12–14 days of treatment

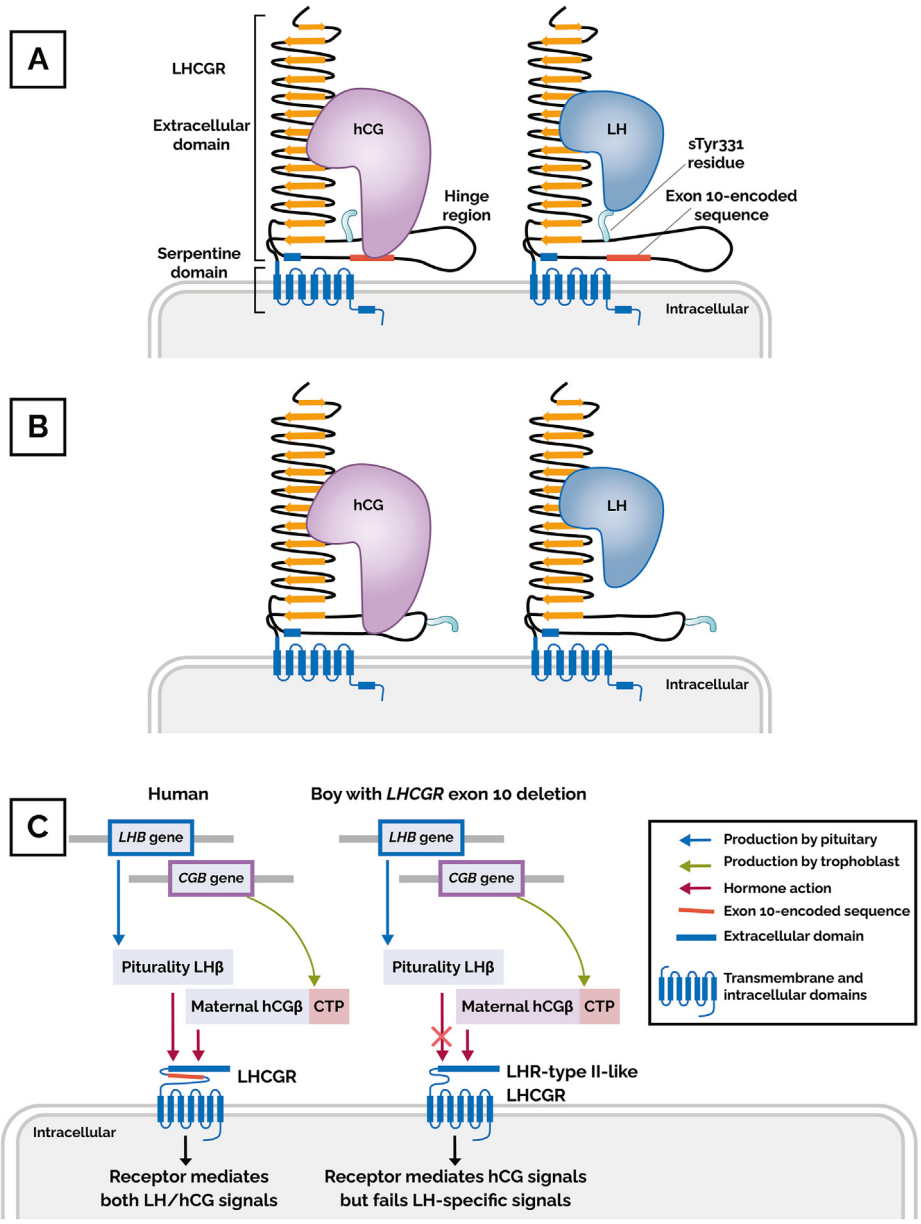

Fig. 2. Discrimination of LH- and hCG-mediated signaling stratified by the LHCGR hinge region. Both hormones bind the receptor's extracellular domain but interact differently with the "U-shaped" portion of the hinge region. (A) While hCG contacts the exon 10-encoded portion, LH spatial conformation leads to the interaction of the hormone with the sTyr331 residue. (B) These ligand–receptor interactions result in LHCGR conformational changes associated with hormone-specific intracellular signaling. Exon 10 deletion results in the shift of the sTyr residue impairing the interaction with LH, whereas the contact point of the "U-shaped" structure of the hinge region with hCG is maintained. Thus, exon 10 deletion results in a truncated LHCGR unable to mediate proper LH signaling, although it retains both LH and hCG binding capability. (C) Human LH and hCG isoforms are encoded by the specific genes belonging to a genetic cluster. Both human LH and hCG act through the same receptor displaying the amino acid sequence encoded by the exon 10, which is fundamental for activating hormone-specific intracellular signaling. The deletion of the LHCGR exon 10-encoded region leads to a truncated receptor capable of binding both LH and hCG, resulting in impaired LH signaling and male infertility. Reprint with permission from Oxford University Press, Copyright 2018, Casarini et al. Endocrine Reviews, volume 39:549–592.

[116]. These findings may be explained by the known mitotic effects of testosterone demonstrated in seminal vesicles of animal models [117], and other growth factors, such as estrogens, produced *in vivo*, which could have covered the gonadotropin-specific signal. In any case, more data are needed to elucidate the gonadotropin-specific action in human Leydig cells.

Historically, only hCG has been used in the context of male infertility, mainly for males with hypogonadism [7,26,118]. Urinary hCG preparations are currently marketed in lyophilized vials with 5000 or 10,000 IU for intramuscular use. In contrast, recombinant hCG is available in prefilled syringes or pen devices containing 250 mcg of pure hCG—equivalent to approximately 6750 IU of urinary hCG [42]. Like recombinant FSH preparations, recombinant hCG is purer than urine-derived hCG and has a better quality and safety profile than its urinary counterparts [42,104]. Nevertheless, data concerning recombinant hCG in the context of male infertility treatment are minimal, and its use remains off-label. By contrast, urinary hCG has a label indication to treat males with hypogonadism, including those with oligozoospermia. Given the shortage of urinary hCG availability in many countries, recombinant hCG must receive approval from regulatory agencies for use in male hypogonadism.

Clinical application

The rationale for using hCG therapy (as a surrogate for LH) off-label for treating male infertility conditions associated with hypogonadism relies on the fact that low ITT concentrations cause spermatogenesis disruption. In rodents, reductions of over 75% of the ITT concentration are incompatible with sperm maturation [119–121]. Furthermore, gonadotropin action is determined by its secretory pulses' frequency, amplitude, and duration [32,122–124]. In NOA males with elevated circulating levels of endogenous FSH and LH, the relative amplitudes of FSH and LH are low, leading to a paradoxically weak stimulation of Leydig and Sertoli cells [28,32,79,122–124]. Human studies have shown that hormonal therapy with hCG increases ITT and circulating testosterone in men with nonobstructive azoospermia [122,123]. hCG treatment also promotes spermatogonial DNA synthesis, as assessed by the expression of proliferating cell nuclear antigen (PCNA) [122]. Expression of the PCNA genes is associated with cell proliferation and, thus, with DNA synthesis during genome replication in the S phase of the cell cycle [122].

Nevertheless, it remains unclear what the optimal testosterone levels are for stimulating spermatogenesis and improving sperm retrieval rates (SRR) in NOA males. The assessment of ITT levels is challenging because measurements require testicular aspiration, which is an invasive procedure [76]. Measurement of circulating testosterone levels has been used as a proxy of ITT assessment because of the high correlation ($r = 0.82$) in the concentrations of both hormones in normal men [125]. It should be noted that ITT concentrations are markedly higher (~100–200*x*) than circulating testosterone concentrations [76,125], making direct extrapolations between intratesticular and circulating testosterone levels potentially inaccurate. For example, the correlations between ITT and testosterone levels are poor in men receiving testosterone replacement therapy because of its negative feedback on the hypothalamic–pituitary–gonadal axis [126]. Serum 17-hydroxyprogesterone—an intermediate steroid produced by the testicles and adrenal glands—has been proposed as an alternative biomarker for evaluating ITT levels [127].

Furthermore, human and animal data suggest pathological desensitization of the FSHR caused by high circulating FSH levels [128–131]. It has been postulated that hormone therapy using GnRH or hCG could benefit these patients by suppressing the endogenous gonadotrophin levels and thereby overcoming Sertoli cell receptor desensitization caused by chronically raised circulating FSH levels [132,133].

Lastly, as previously discussed, FSH promotes cell proliferation when acting in synergy with ITT [40]. Transgenic murine studies have suggested that spermatogenesis can be stimulated by high FSH concentrations using exogenous FSH administration, even in the presence of low ITT [40,134]. Moreover, it has been suggested that a subset of men with idiopathic oligozoospermia and low-to-normal circulating FSH levels are FSH deficient as a consequence of reduced FSH activity, which depends on the amount of circulating FSH, its glycosylation, as well as the genetically determined expression levels and function of the FSHR [56,60,134,135]. Therefore, hormonal therapy with FSH might help improve sperm parameters in some patients, as shown in carriers of the FSHR p. N680S homozygous N [56]. Although limited, these data have provided a rationale for the off-label administration of exogenous FSH to males with idiopathic oligozoospermia.

Real-world data

Idiopathic oligozoospermia

Men presenting with idiopathic oligozoospermia have no evident history of fertility problems, and both physical examination and endocrine laboratory testing are unremarkable. However, semen analysis, as routinely performed, reveals low sperm concentration [23,136]. According to the latest WHO manual for examining human semen, published in 2021, oligozoospermia is defined as a sperm concentration below the lower reference limit of 16 million/mL [1].

Several studies have explored the use of hormonal treatment with FSH for these patients [133,137–156]. The characteristics of the most relevant trials published are summarized in Table 2. The table comprises 21 studies using different designs and including 1803 patients. Among them, 1302 patients received gonadotropin therapy (FSH alone, hMG alone, hCG alone, or both FSH and hCG combined), whereas 501 did not. Of the 21 studies, 15 concerned controlled trials (13 RCTs), whereas six were non-controlled observational trials and case series. Specifically, the comparative trials included 1358 patients, of whom 857 received gonadotropin therapy (most often FSH alone, 13 trials) (Table 2). Among trials that provided semen analysis data, 11 reported improvements in semen parameters after gonadotropin therapy, particularly sperm concentration, whereas no improvement was noted in three studies.

To quantify the impact of gonadotropin therapy on pregnancy rates in the present review, we performed a statistical analysis of the data shown in Table 2. Pregnancy data were available in 13 trials, totaling 1070 patients. Overall, pregnancy rates (natural or assisted) were significantly higher in couples with male partners who received gonadotropin therapy (30.7%; 205/667) than those who did not (15.4%; 62/403; $p < 0.0001$). An odds ratio of 2.440 (95% confidence interval [CI] 1.778–3.349; $p < 0.0001$) for pregnancy success favored the gonadotropin therapy group. Our analysis revealed that an average of 7 patients with idiopathic oligozoospermia (95% CI 4.9–9.6) needed to be treated with gonadotropins to achieve an additional pregnancy.

The most common FSH included recombinant preparations, administered subcutaneously, twice or thrice a week for 3–6 months, in doses varying from 75 to 300 IU (Table 2). Notably, the studies listed in Table 2 are heterogeneous concerning the number of participants, gonadotropin therapy regimens, treatment duration, and follow-up. Most trials included few participants, reported pre- and post-semen outcomes based on single semen analysis, and had a short follow-up. Therefore, caution is required to adequately assess the beneficial effect of gonadotropin therapy on this patient population.

Our findings are consistent with the literature. A 2015 meta-analysis of 15 controlled studies, including 1275 couples whose male partners had idiopathic infertility, indicated that FSH therapy improved the chances of obtaining pregnancy naturally (odds ratio [OR] 4.5; 95% CI 2.17–9.33) or by ART (OR 1.60; 95% CI 1.08–2.37) [157]. In this study, sperm concentration also improved, with a mean difference of 2.66 million/mL (95% CI 0.47–4.84; 11 studies), favoring the group receiving FSH therapy. Additionally, a 2019 meta-analysis compiling the data of seven RCTs and including 444 participants showed that the mean difference in sperm concentration was about three million higher in men receiving recombinant FSH therapy than in controls [3.17 million/mL; 95% confidence interval [CI] 2.44–3.91 million/mL; $I^2 = 94\%$, $p < 0.00001$) [158]. In this study, the pregnancy rates were also higher for patients receiving recombinant FSH therapy (OR 3.30, 95% CI 1.39–7.82; 343 participants; 5 studies; $I2 = 0\%$; $p = 0.007$). Lastly, a 2020 meta-analysis of five RCTs, including 666 normogonadotropic men with idiopathic oligozoospermia, showed that FSH therapy using either urinary or recombinant preparations improved sperm parameters in a dose-dependent manner [159]. At low weekly doses (175–262.5 IU per week), FSH therapy improved only sperm motility, whereas intermediate doses (350–525 IU per week) ameliorated sperm concentration and morphology. Lastly, high doses (700–1050 IU per week) had a favorable effect on sperm concentration, total sperm count, and progressive motility; sperm morphology showed a trend toward an increase [159].

Despite indicating a beneficial effect, the existing trials included few participants and used different treatment protocols and follow-up periods, precluding the development of evidence-based clinical guidelines. Furthermore, it has been shown that polymorphisms in the FSH receptor might affect the response to FSH therapy. In one report, Selice and colleagues observed that the use of FSH therapy only conferred a statistically significant improvement in sperm parameters of men with idiopathic

Table 2
Characteristics of studies assessing the clinical utility of gonadotropin therapy for males with idiopathic oligozoospermia.

Author (y)	Study design	Country	Population; N	Mean age (SD or range)	Intervention regimen	Mean Tx period	Control group	Semen parameters outcomes	Pregnancy outcomes	Adverse events
Schill et al. (1982)	RC	Germany	NG (FSH levels <3.5 ng/ml; TT > 3.0 ng/ml) with idiopathic O (<20 million/ml); N = 48 Group A (N = 9): sperm concentration 1−5 million/mL; Group B (N = 12): 5.1 −10 million/ml; Group C (N = 18): 10.1 −15 million/ml; Group D (N = 9): 15.1 −20 million/ml	34.4 (5.0)	hMG 75 IU 3*x*/week + uhCG 2500 IU 2*x*/week	3 mo.	No	Increased sperm concentration, TSC, and progressive motility; morphology unchanged	Data of 33 patients available; natural pregnancies within 1 year after initiation of treatment; Overall PR: 10/33 (30.3%); Group A: 4/7 (57.1%); Group B: 1/7 (14.3%); Group C: 5/12 (41.7%); Group D: 0/7 (0%)	NR
Knuth et al. (1987)	RCT	Germany	NG (hormonal profile not specified) with idiopathic O (<10 million/ml); N = 37 IG: N = 17; CG:N = 20	IG: 31.1 (3.6); CG: 33.2 (6.5)	hMG 150 IU 3*x*/week + uhCG 2500 IU 2*x*/week	13 weeks	Yes	No difference in sperm concentration and motility between groups	IG: 2/17 (11.7%); CG: 0/20 (0%)	Breast tenderness and gynecomastia (1 case)
Foresta et al. (1998)	RCT	Italy	NG (FSH and TT threshold not specified) with idiopathic OA (<10 million/ml); N = 90 IG: N = 60; CG: N = 30	28.0 (5.0)	HP-uFSH 75 IU every 2 days	3 mo.	Yes	Increased sperm concentration in treatment group vs control group	NR	NR
Kamischke et al. (1998)	RCT	Germany	NG (FSH<7 IU/L; TT > 12 nmol/L) with OA (thresholds not specified); N = 65 IG: N = 34; CG: N = 31	32.9 (0.6)	rFSH 150 IU daily	3 mo.	Yes	No difference in sperm concentration and motility between groups	61 patients analyzed; IG: 2/31 (6.4%), both natural pregnancies; CG: 2/30 (6.7%),	None

(*continued on next page*)

Table 2 (*continued*)

Author (y)	Study design	Country	Population; N	Mean age (SD or range)	Intervention regimen	Mean Tx period	Control group	Semen parameters outcomes	Pregnancy outcomes	Adverse events
									by IUI (1 case) and ICSI (1 case)	
Ben-Rafael et al. (2000)	RCT	Israel	NG (FSH<12 IU/L; TT > 10 nmol/L) with OA (<20 million/ml; progressive motility <20%) and at least one previous failed IVF cycle (n = 40) IG: N = 20; CG: N = 20	Group A 36.3 (4.5); Group B 35.7 (5.2); CG: 35.4 (5.7)	Group A HP-uFSH 75 IU daily; Group B HP-uFSH 150 IU daily	at least 60 days	Yes	Increased in normal appearing intact acrosomes and axonemes, as assessed by electron microscopy, in treatment group (vs. CG); routine SA data not reported	PR by IVF: Group A 1/20 (5%); Group B: 2/20 (10%); CG: 0/20 (0%)	NR
Foresta et al. (2002)	RCT	Italy	NG (FSH<7 IU/L) with OA (<10 million/ml) and testicular histology showing HYPO; N = 45 IG: N = 30; CG: N = 15	Group A: 32.6 (4.5); Group B: 34.1 (3.8); CG: 32.2 (4.3)	Group A (N = 15): rFSH 50 IU every 2 days; Group B (N = 15): rFSH 100 IU every 2 days	3 mo.	Yes	Increased sperm concentration in IG (vs. CG), more pronounced in patients using 100 IU dose; motility and morphology unchanged.	NR	None
Fernandez-Arjona et al. (2003)	CS	Spain	NG (mean FSH levels: 3.8 IU/L; mean TT: 17.2 nmol/L) with idiopathic O (<20 million/ml); N = 29	33.0 (4.0)	HP-uFSH 75 IU daily	3 mo.	No	Increased sperm concentration, motility, viability, and morphology	NR	NR
Caroppo et al. (2003)	PC	Italy	NG (hormone profile not specified) with idiopathic O (<10 million/ml); N = 33 IG: N = 23; CG: N = 10	IG:34.1 (4.2); CG: 38.0 (5.6)	rFSH 150 IU 3x/ week	3 mo.	Yes	Increased TSC, motility, morphology, and vitality ($p < 0.01$)	PR: IG: 7/23 (30.4%) (1 natural and 6 by ICSI); CG: 0/10 (0%) LBR: IG: 6/7 (85.7%) (6 singletons)	None
Baccetti et al. (2004)	RCT	Italy	NG (FSH <12 IU/L) with idiopathic O (<20 miilion/ml); N = 44 IG: N = 24; CG: N = 20	28–45	HP-uFSH 150 IU daily	3 mo.	Yes	No differences in semen parameters between groups, but improved sperm ultrastructural	PR IG: 8/24 (33.3%) (1 abortion); CG: 4/20 (20%) (1 abortion); $p < 0.05$	NR

								characteristics in IG (vs. CG)		
Foresta et al. (2005)	RCT	Italy	NG (FSH<7 IU/L; TT > 3 ng/ml) with idiopathic O (<10 million/ml); N = 112 IG: N = 62; CG: N = 50	IG: 34.2 (4.8); CG: 34 (4.6)	rFSH 100 IU every 2 days	3 mo.	Yes	Increased TSC in responders vs. non-responders and CG; No difference in motility and morphology	IG: Natural pregnancy: 5/30 (16.7%) in responders; 1/32 (3.1%) in non-responders; p < 0.05 ART 6/25 (24%) in responders; 6/31 (19.3%) in non-responders; CG: Natural pregnancy: 2/50 (4%); ART: 10/48 (20.8%)	None
Paradisi et al. (2006)	RCT	Italy	NG (FSH<7.5 IU/L) with idiopathic O (<15 million/ml); N = 30 IG: N = 15; CG: N = 15	NR	rFSH 300 IU every 2 days	4 mo.	Yes	Increased concentration, TSC and motility between groups (p < 0.05), but morphology unchanged	CPR IG: 4/15 (26.7%); CG: 0/15 (0%) LBR: IG: 4/15 (26.7%)	None
Foresta et al. (2009)	RCT	Italy	HG (FSH threshold not specified) with idiopathic (N = 34) and non-idiopathic (N = 90) severe O (<3 million/ml) and testicular histopathology showing HYPO; N = 87 IG: N = 57; CG: N = 30	34.2 (4.5)	Leuprolide acetate 3.75 mg 1 dose, and after 30 days, leuprolide acetate 3.75 mg 1x/month + rFSH 150 IU every 2 days + hCG 2000 IU (type not specified) 2x/week	3 mo.	Yes	Increased sperm concentration and morphology in treatment group vs. control group, but motility unchanged	Natural pregnancy: IG: 4/57 (7%); CG: 0/30 (0%) ART: IG: IVF 6/21 (28.6%), ICSI 8/32 (25.0%) CG: only ICSI 6/30 (20.0%) Total PR:	10 patients experienced side effects related to androgen deprivation (asthenia, hot flushes, headache) that disappeared after hCG administration

(*continued on next page*)

Table 2 (*continued*)

Author (y)	Study design	Country	Population; N	Mean age (SD or range)	Intervention regimen	Mean Tx period	Control group	Semen parameters outcomes	Pregnancy outcomes	Adverse events
									IG: 18/57 (31.6%); CG: 6/30 (20.0%)	
Selice et al. (2011)	RCT	Italy	NG (FSH levels <8 IU/L, and TT > 10 nmol/L) with O (<20 million/ml) and HYPO; N = 105 IG: N = 70; CG: N = 35	NR	rFSH 150 IU 3*x*/week	3 mo.	Yes	Increased sperm concentration, TSC, motility, morphology, and total motile sperm in IG vs. CG	Natural pregnancy: IG: 10/70 (14.3%); CG: 2/35 (5.7%); NS	NR
Condorelli et al. (2014)	CS	Italy	NG (FSH<12 IU/L; TT > 2.8 ng/ml) with idiopathic O (<15 million/ml); N = 54	24.0 (6.0)	Group A (N = 20: rFSH alfa 150 IU 3*x*/week; Group B (N = 20): rFSH beta 150 IU 3*x*/week; Group C (N = 14): HP-uFSH 150 IU 3*x*/week	4 mo.	No	Improvement in semen parameters in all groups	NR	NR
Garolla et al. (2014)	PC	Italy	NG (FSH<8 IU/L; TT > 10 nmol/L) with idiopathic O (<10 million/ml); N = 174 Group A (N = 92): HYPO; Group B (N = 61): Maturative disturbance); Group C (N = 21): Normal spermatogenesis	Group A: 34.1 (5.9); Group B: 35.2 (6.0); Group C 32.3 (5.4)	HP-uFSH 150 IU 3*x*/week (Group A: N = 92)	3 mo.	Yes	Increased sperm concentration, total count, and motility; Morphology, sperm aneuploidy, and sperm DNA fragmentation rates unchanged	Natural pregnancy: IG: 25/92 (27.2%); CG: 4/82 (4.9%); p = 0.001 ART: IG: 16/49 (32.6); CG: 14/58 (24.1%) Total PR: IG: 41/92 (44.6%); CG: 18/82 (22.0%); p = 0.002)	None
Paradisi et al. (2014)	RCT	Italy	NG (FSH<7.5 IU/L) with idiopathic O (<15 million/ml); N = 60 IG: N = 45; CG: N = 15	NR	rFSH 300 IU every 2 days	4 mo.	Yes	Increased sperm conccentration and TSC in IG vs. CG	Natural pregnancy (8 mo follow-up): IG: 12/45	None

								(26.7%); CG: 0/15 (0%); p < 0.025); LBR: IG: 8/45 (17.8%)	
Farrag et al. (2015)	RCT	Italy	NG (FSH range: 2–12 IU/L; TT range: 10–30 nmol/L) with idiopathic O (<10 million/ml); N = 82 IG: N = 36 CG: N = 46	IG: 36.9 (5.1); CG: 38.4 (5.2)	rFSH 150 IU 3x/week	3 mo.	Yes	Increased sperm count, motility, and morphology in IG vs. CG	ICSI only (PR): IG: 42%; CG: 20%; p < 0.05	NR
Ding et al. (2015)	RCT	China	NG (FSH threshold not specified) with idiopathic O (<10 million/ml); N = 354 IG: N = 272; CG: N 82	IG: 32.5 (3.1); CG: 35.5 (4.1)	Different doses of rFSH: placebo, 50 IU, 100 IU, 200 IU, 300 IU rFSH every 2 days	3–5 mo.	Yes	Increased sperm concentration and TSC in IGs receiving 200 IU and 300 IU (vs. other groups); motility and morphology unchanged	After 3 months Natural pregnancy (3 mo follow-up): placebo: 2/30 (6.7%); 50 IU group 3/36 (8.3%); 100 UI group 3/38 (7.9%); 200 IU group 4/41 (9.7%); 300 IU group 6/40 (15%); p < 0.05 ART: placebo 7/28 (25%); 50 IU group 9/33 (27.2%); 100 IU group 11/35 (31.4%); 200 IU group 13/35 (37.1%); p < 0.05 300 IU group 14/34 (41.1%); p < 0.05	NR
La Vignera et al. (2020)	CS	Italy	NG or hypogonadal (TT < 350 ng/dl) with OA (<15 million/ml); N = 210	30.7	Various regimens including (i) Group A (N = 40; TV > 12 ml &	3–6 mo.	No	Improvement in parameters and decreased sperm	Natural pregnancy: Group A: 15/60 (25%);	NR

(*continued on next page*)

Table 2 (*continued*)

Author (y)	Study design	Country	Population; N	Mean age (SD or range)	Intervention regimen	Mean Tx period	Control group	Semen parameters outcomes	Pregnancy outcomes	Adverse events
					TT > 350 ng/dl): FSH 150 IU (type not specified) 3x/ week for at least 3 mo; (ii) Group B (N = 60; TV > 12 ml/ TT < 350 ng/dl): hCG 2000 IU (type not specfied) 2x/ week for 3 mo, if no sperm parameters inprovement, then FSH added for 3 mo; if TT remained low, then hCG continued for additional 3 mo; if TT normalized, hCG suspended; (iii) Group C (N = 65; TV < 12 ml/ TT < 50 ng/dl): FSH 150 IU (type not specified) 3x/ week + hCG 2000 IU (type not specified) 2x/week; (iv) Group D (N = 45; TV < 12 ml/ TT > 350 ng/dl): FSH 150 IU (type not specified) 3x/ week for 3 mo, if TV increased, but no improvement in sperm			DNA fragmentation rates	Group B: 12/40 (30%); Group C: 20/65 (31%); Group D: 15/45 (33%)	

					paramenters, hCG 2000 IU added (type not specified) 1*x*/week for 3 mo; if TV and sperm paramenters unchanged, FSH continued for another 3 mo.					
Condorelli et al. (2021)	CS	Italy	NG (FSH<8 mIU/ml; TT > 350 ng/dl) with idiopathic O (<15 million/ml); N = 48	Group A: 33.8 (6.1); Group B: 34.8 (5.2)	Group A (N = 24): HP-uFSH 75 IU every day; Group B (N = 24): HP-uFSH 150 IU 3*x*/week	3 mo.	No	Increased sperm concentration in group A vs. group B; Increased TSC, motility, morphology and vitality in both groups	NR	NR
Andrabi et al. (2022)	CS	India	Hypogonadic patients (TT < 400 ng/dl) with idiopathic severe O (<5 million/ml); N = 56	Responder group: 32.0 (4.3); Non-responder group: 30.4 (3.3)	rhCG 1*x*/week	6 mo.	No	Improvement in sperm concentration and TSC; motility unchanged	NR	NR

PC: prospective cohort study; RC: retrospective cohort study; CS: case series; RCT: randomized controlled trial; NG: normogonadotropic; HG: hypergonadotropic; IG: intervention group; CG: control group; O: oligozoospermia; OA: oligoasthenozoospermia; TSC: total sperm count; SA: semen analysis; HYPO: hypospermatogenesis; IU: international units; FSH: follicle-stimulating hormone; TT: total testosterone; TV: testicular volume; Mo.: months; hCG: human chorionic gonadotropin; rhCG: recombinant human chorionic gonadotropin; uhCG: urinary human chorionic gonadotropin; HP: highly purified; uFSH: urinary follicle-stimulating hormone; rFSH: recombinant follicle-stimulating hormone; hMG: human menopausal gonadotropin; T/E ratio: testosterone to estradiol ratio; AI: aromatase inhibitor; CC: clomiphene citrate; ART: assisted reproductive technology; IVF: *in vitro* fertilization; ICSI: intracytoplasmic sperm injection; PR: pregnancy rate; LBR: live birth rate; NR: not reported; NS: not significant.

oligozoospermia who had common allelic variants, particularly Ala307-Ser680/Ala307-Ser680 homozygosis or Thr307-Asn680/Ala307-Ser680 heterozygosis [148]. These findings suggest that response to FSH therapy might depend on the genetic background of affected men, but this hypothesis warrants validation in large clinical trials. Fig. 3 depicts a proposed algorithm for treating men with idiopathic oligozoospermia using exogenous FSH therapy.

Collectively, modest evidence indicates that exogenous FSH therapy might increase sperm quantity in men with idiopathic oligozoospermia, with an apparent positive effect on pregnancy rates. The current evidence is based on studies including few participants and using different treatment protocols and follow-up periods. Further high-quality research is needed to clarify the potential clinical role of FSH therapy in overcoming oligozoospermia and improving pregnancy rates. The optimal treatment regimen and duration and the role of pharmacogenomics in identifying the best candidates for treatment have also to be determined.

Nonobstructive azoospermia

NOA is associated with a spectrum of severe and untreatable conditions causing intrinsic testicular defects that affect sperm production [3]. The etiology includes genetic and congenital abnormalities, post-infectious testicular damage, exposure to gonadotoxins (such as radiotherapy/chemotherapy), and testicular trauma. In many cases, however, the etiology cannot be determined, and NOA is termed idiopathic [3,4].

Besides azoospermia (lack of sperm in the ejaculate), NOA males typically have small testicles and serum FSH levels greater than 7.6 mIU/mL, indicating spermatogenic failure [160]. Biochemical hypogonadism, characterized by low testosterone circulating levels [78], is noted in approximately half of the patients with NOA and generally reflects concurrent Leydig cell insufficiency [3,79,80]. However, men with NOA can also be eugonadal because serum FSH levels correlate with the number of spermatogonia [77,80]. If spermatogonia are absent or markedly reduced, the FSH level increases. By contrast, in cases of maturation arrest at the spermatocyte or spermatid stage, the number of spermatogonia is normal; therefore, testicular volume and FSH levels will be within the normal limits [77].

A diagnosis of NOA does not necessarily mean sterility because focal sperm production is found in the testicles of 30%–60% of the affected patients [3,77,161]. Such deficient production precludes sperm from appearing in the ejaculate. Nevertheless, sperm can be retrieved from the testis and used for intracytoplasmic sperm injection [161–163]. Testicular sperm can induce normal fertilization and embryo development and result in healthy offspring [163–165].

Sperm retrieval combined with ICSI is the only option for men with NOA to generate their biological offspring [163,166]. However, given the uncertainty of sperm acquisition and the suboptimal sperm retrieval success rates in NOA males [161,162,167], it would be ideal for optimizing spermatogenesis and hence the chances of successful sperm recovery. Although it is generally believed that empirical medical treatment is ineffective in men with spermatogenic failure because gonadotropins' plasma levels are already high, there might be a potential role for pharmacotherapy in men with NOA, as discussed in the previous sections.

Indeed, exogenous gonadotropins have been used off-label to modulate the male reproductive hormones and optimize ITT levels in men with NOA and hypogonadism. The goals are to induce the recovery of sperm in the ejaculate or improve surgical sperm retrieval rates. hCG has been the primary drug utilized, given its positive effect on ITT production and spermatogonial DNA synthesis [122]. Although a clear threshold of serum testosterone levels facilitating optimal spermatogenesis has yet to be established, a positive relationship exists between normal serum testosterone levels (vs. low testosterone levels) and the likelihood of successful sperm retrieval [168–170].

The characteristics of the relevant trials assessing the effectiveness of gonadotropin therapy in men with NOA are summarized in Table 3 [77,80,123,169,171–194]. The table comprises 28 studies using different designs and including 3875 patients. Among them, 1226 patients received gonadotropin therapy (alone or combined with aromatase inhibitors, selective estrogen receptor modulators, or other agents), whereas 2549 did not. The latter group comprised 1564 patients with no treatment and 1085 treated with various drugs (e.g., aromatase inhibitors and selective estrogen receptor modulators), except gonadotropins. Of the 28 studies, 13 concerned case reports or small case series using gonadotropin therapy,

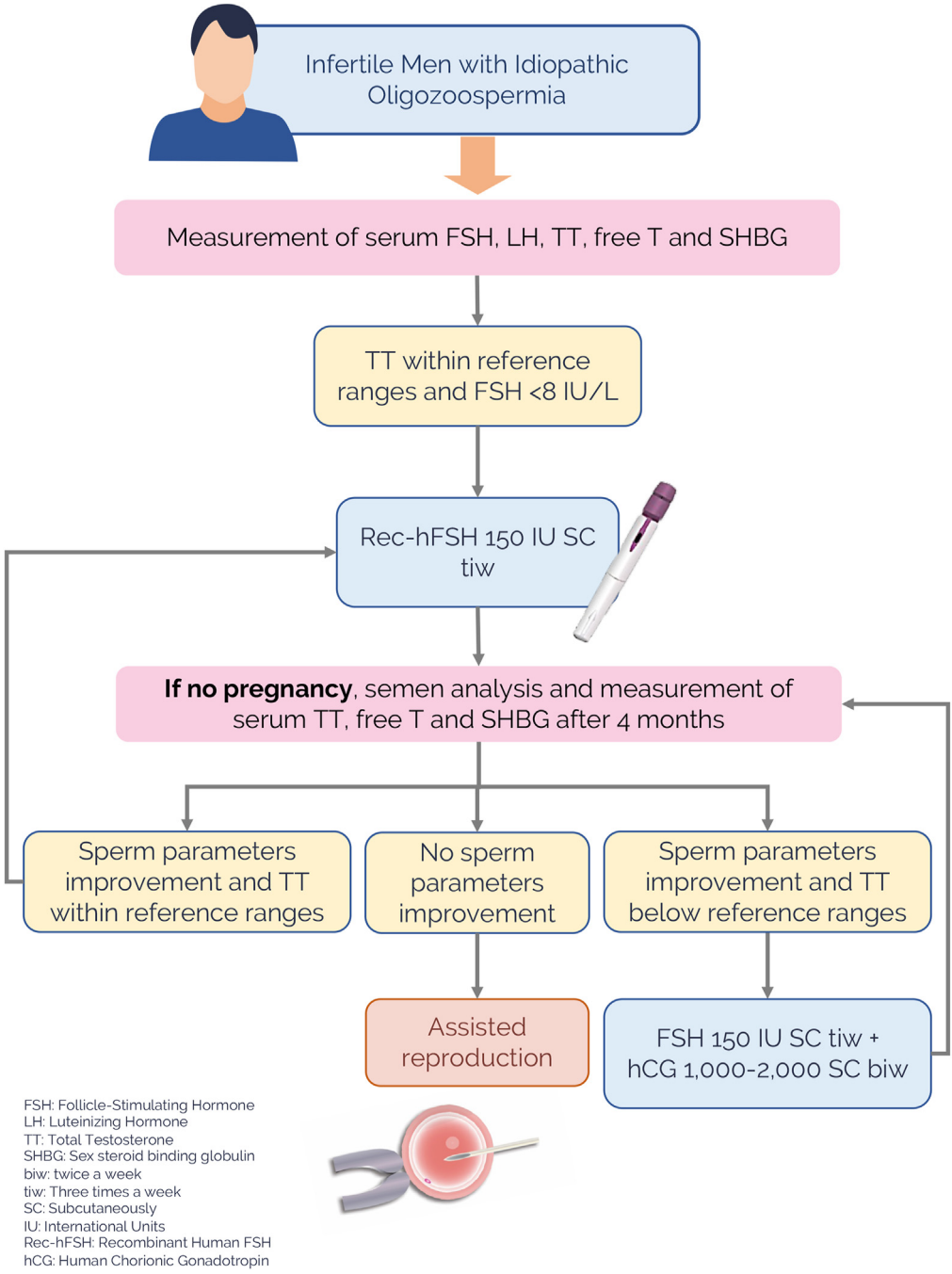

Fig. 3. Illustration depicting a proposed algorithm for FSH therapy in men with idiopathic oligozoospermia. Patients with baseline circulating FSH levels <8 IU/L are eligible for the treatment. The hormonal treatment relies on recombinant FSH, using a fixed dose of 150 IU 3*X* times a week for at least four months. The follow-up includes pregnancy achievement, semen analysis, and hormone measurements (serum FSH, LH, total testosterone, free testosterone, and SHBG). If pregnancy is not achieved, but improvement in sperm concentration is noticed, treatment can continue for an additional four months or longer. Patients with biochemical hypogonadism (e.g., TT levels <350 ng/dL) may be prescribed low-dose hCG (e.g., 1000–2000 IU twice a week) during FSH therapy.

whereas 15 were prospective or retrospective observational trials comparing hormonal therapy *versus* no treatment in eugonadal or hypergonadotropic hypogonadal infertile men with NOA.

To quantify the impact of gonadotropin therapy on SR success in the present review, we performed a statistical analysis of the data shown in Table 3. Specifically, the comparative trials included 3689 patients, of whom 1403 received any form of gonadotropin therapy (alone or combined with the agents mentioned above), whereas 1564 had not. Overall, SR success by testicular sperm extraction (TESE) using conventional or microsurgical methods was 39.4% in treated patients (559/1403) and 33.8% in untreated subjects (529/1564; $p = 0.0016$). An odds ratio of 1.295 (95% confidence interval [CI] 1.115–1.505; $p = 0.0007$) for a positive surgical sperm acquisition favored the gonadotropin therapy group. Our analysis revealed that an average of 17 patients (95% CI 10.5–39.3) needed to be treated with gonadotropins to achieve an additional positive sperm retrieval outcome.

Furthermore, according to our analysis, 9 of the 15 comparative trials included in Table 3 showed that gonadotropin therapy was beneficial, increasing sperm retrieval rates by 6% (95% CI 2.55%–9.49%). In some cases, treatment has been associated with the return of sperm to the ejaculate. In the complete analysis, including the 28 studies, 6.2% of the patients (87/1403) who received gonadotropin therapy exhibited few viable sperm in the ejaculate, which were used for ICSI in most cases; four couples achieved natural pregnancies (Table 3).

The most common gonadotropin regimens used to treat NOA males include urinary hCG, used alone or in combination with FSH, administered subcutaneously twice or thrice a week for three months or longer, in varying doses titrated to keep the endogenous FSH and testosterone levels at optimal levels (Table 3). The existing evidence indicates that the best candidates for gonadotropin therapy are NOA males with biochemical hypogonadism (i.e., serum levels of total testosterone <300–350 ng/dL) and those with histopathology showing maturation arrest (late stages) or hypospermatogenesis. Indeed, it seems that patients in whom the development of spermatozoa is disrupted during the early or later spermatogenic stages (i.e., germ cell maturation arrest) respond better to therapy than counterparts with minimal or lack of germ cells inside the testicle, yet the reasons why some of these individuals respond to treatment while others do not remain unknown.

Notably, the studies listed in Table 3 are highly heterogeneous concerning design, population, number of participants, gonadotropin therapy regimens, treatment duration, and sperm retrieval methods. Moreover, many lack pregnancy data, and no RCT has been published on the matter concerned. For these reasons, caution should be exercised to interpret our data compilation.

A 2022 meta-analysis compiled the data from 10 controlled studies using various types of hormonal stimulation, including 985 participants [195]. The authors showed that sperm retrieval rates were higher in subjects pre-treated with hormonal therapy (OR 1.96, 95% CI 1.08–3.56, $p = 0.03$; low-quality evidence). Subgroup analyses were carried out by the type of NOA patient subjected to hormonal therapy, classified as normogonadotropic and hypergonadotropic subjects. In their study, the baseline FSH level that distinguished these populations was 12 mIU/mL. A significant improvement in sperm retrieval rates was noted for normogonadotropic men (5 studies, OR 2.13, 95% CI 1.10–4.14, $p = 0.02$) but not in hypergonadotropic subjects (4 studies, OR 1.73, 95% CI 0.44–6.77) [189]. However, the authors excluded one study in the subanalysis of hypergonadotropic subjects, which enrolled patients ($n = 20$) with Klinefelter syndrome [181] who typically have hypergonadotropic hypogonadism. The study of Majzoub and colleagues showed a higher sperm retrieval rate by micro-TESE in patients subjected to hormonal modulation than those who did not (37.5% vs. 0%) [181]. Therefore, excluding this patient cohort from the meta-analysis of hypergonadotropic NOA males might have biased the results.

Notably, six of the ten trials in the meta-analysis mentioned above have explored gonadotropins as sole agents for hormonal modulation. hCG monotherapy was used in two studies [178,186] and FSH monotherapy in two trials [166,181], whereas in two trials, therapy was initiated with hCG and, subsequently, FSH was added in patients with a marked pituitary down-regulation [177,180]. A single study reported recombinant hCG monotherapy as the drug of choice, with positive results [186], but its data are only available from conference proceedings. Various regimens, including SERMs, aromatase inhibitors, and gonadotropins, were utilized in the remaining trials. Given the few studies, the authors could not perform a subanalysis by drug classes.

Table 3
Characteristics of studies reporting the use of gonadotropin therapy for males with nonobstructive azoospermia.

Author (year)	Study design	Country	NOA population; N	Mean age (SD or range)	Intervention regimen	Mean Tx period	Control group	SR method	Sperm return to ejaculate N (%)	Sperm retrieval success, N (%)	Pregnancy data	Adverse events
Aydos et al. (2003)	PC	Turkey	NG (FSH levels<8IU/L; N = 108 (IG: N = 63; CG: N = 45)	29 (21–29)	HP-uFSH 75 IU 3x/week	3 mo.	Yes	cTESE	0/108 (0%)	IG: 40/63 (64%); CG: 15/45 (33%); OR: 3.48, 95% CI: 1.56 −7.78	NR	None
Selman et al. (2004)	CR	Italy	NG; N = 1; (hormonal profile not specified) with an AZFc microdeletion and MA	32	rFSH 75IU 3x/week for 2 mo; followed by rFSH 150 IU 3x/ week for 1 mo, and lastly, rFSH 150 IU 3x/week + uhCG 2000 IU 2x/week for 3 mo.	5 mo.	No	NA	1/1 (100.0%) Crypto zoospermia	NA	Twin delivery from ICSI using ejaculated sperm)	NR
Schiff et al. (2005)	CS	USA	HGH (mean FSH: 33.2 IU/L; mean TT: 190.2 ng/dL) and HG eugonadal, mostly non-mosaic KS; N = 42 (N = 54 SR attempts) IG: N = 36 (HGH patients); CG: N = 6 (eugonadal patients)	32.8 (24–52)	Various regimens: (i) testolactone 50–100 mg 2x/ day + uhCG 1500 IU 2x/week (hCG dose titrated based on TT levels with maximum dose of 2500 IU 3x/week; N = 13); (ii) testolactone 50–100 mg 2x/day (N = 19); (iii) Anastrazol 1 mg/day (N = 5); (iv) anastrazol 1 mg/ day + hCG (regimen not specified) (N = 1); (v) CC 25 mg/day (N = 3); (vi) rFSH (regiment not specified) (N = 1)	4 mo.	Yes	mTESE	NR	IG: 25/42 (69.4%), (i) anastrazole: 5/5 (100%); (ii) anastrazole + hCG: 1/ 1 (100%); (iii) CC: 3/3 (100%); (iv) rFSH: 1/1 (100%); (v) testolactone: 14/19 (74%); (vi) testolactone + hCG: 7/13 (54%); (vii) unknown treatment: 2/6 (33%) CG: 4/6 (66.6%) p value NR	PR: 18/29 (62%) (21 babies born); pregnancy data NR by type of treatment	NR
Selman et al. (2006)	CS	Italy	NG (hormonal profile not specified) with MA; N = 49	32–41	rFSH 75 IU every other day for 2 mo, followed by rFSH 150 IU every other day for 1 mo, and lastly, rFSH 150 IU every other day + uhCG 2000 IU 2x/week for 3 mo.	6 mo.	No	cTESE	0/49 (0%)	11/49 (22.4%)	CPR: 3/11 (27.2%); LBR: 3/11 (27.2%)	NR
Cao et al. (2007)	CR	China	NG hypogonadal; N = 1; (FSH: 2.5 IU/L; TT: 8.1 nmol/L) with MA	36	hCG 3000 IU daily (type not specified)	NR	No	TESE	NR	1/1 (100.0%)	Twin delivery from ICSI using testicular sperm	NR
Efesoy et al. (2009)	CS	Turkey	NG (mean FSH: 5.7 mIU/mL; TT: NR) with MA; N = 11	31.1 (4.5)	rFSH 100–150 IU 2–3x/ week	7.4 mo (±4.5)	No	TESE	2/11 (18.2%)	2/11 (18.1%)	NR	NR
Ramasamy et al. (2009)	RC	USA	Non-mosaic KS (N = 68, including NG eugonadal (CG, TT > 300 ng/dL; N = 12) and HGH (IG, mean FSH: 34.4 IU/L; mean TT: 172.3 ng/dL; N = 56); N = 91 SR attempts (68 in IG and 23 in CG)	33.0 (6.0)	Various regimens: (i) testolactone 50–100 mg 2x/ day + uhCG 1500 IU 2x/week (N = 12); (ii) testolactone 50–100 mg 2x/day (N = 28); (iii) anastrazol 1 mg/day (N = 9); (iv) anastrazol 1 mg/ day + uhCG (regiment not specified) (N = 1); (v) CC 25 mg/day (N = 3);	+SR: 194 days (±161); -SR: 246 days (±173)	Yes	mTESE	NR	IG: 43/68 (62.2%), (i) anastrazol: 5/9 (55.5%); (ii) anastrazol + hCG: 1/1 (100%); (iii) CC: 4/4 (100%); (iv) testolactone: 23/35 (65%); (v) testolactone + hCG: 7/ 15 (47%); (vi) hCG alone: 3/4 (75%) CG: 19/23 (82.6%); p = 0.06	CPR: 33/62 (53%); LBR: 28/62 (45%), pregnancy data NR by type of treatment	NR

(*continued on next page*)

Table 3 (*continued*)

Author (year)	Study design	Country	NOA population; N	Mean age (SD or range)	Intervention regimen	Mean Tx period	Control group	SR method	Sperm return to ejaculate N (%)	Sperm retrieval success, N (%)	Pregnancy data	Adverse events
					uhCG (regimen not specified) (N = 4)							
Shiraishi et al. (2012)	RC	Japan	Mixed population (N = 48) of NG and HGH (mean FSH: 28 IU/L; mean TT: 372 ng/dl) with failed TESE IG: N = 28; CG: N = 20	IG: 34.0 (5.7) CG: 33.0 (4.9)	uhCG 5000 IU 3x/week for 3 mo (N = 28) and continued for another 1−2 mo if follow-up FSH levels >3 IU/L (N = 13); or rFSH added (150 IU 3x/week for 2 mo) if follow-up FSH levels dropped to <3 IU/L (N = 15)	3−6 mo (range)	Yes	mTESE	NR	IG: 6/28 (21.4%); CG: 0/20 (0%); p < 0.05	NR	Acne: 3/28 (10.7%); Gynecomastia 2/28 (7.1%)
Reifsnyder et al. (2012)	RC	USA	Mixed population of eugonadal (N = 388) and hypogonadal men (TT < 300 ng/dL; N = 348); IG: N = 307 (hypogonadal); CG: N = 41 (hypogonadal) and N = 388 (eugonadal)	IG: 34.0 (4.0) CG: 37.0 (3.0)	Various regimens: (i) anastrazol 1 mg/d (N = 180); (ii) anastrazol 1 mg/d + uhCG 1500−2000 IU 2−3x/week (N = 29); (iii) CC (N = 66; regiment not specified); (iv) testolactone 50−100 mg 2x/day (N = 14); (v) testolactone 50−100 mg 2x/day + uhCG 1500−2000 IU 2−3x/week (N = 12); (v) uhCG 1500−2000 IU 2−3x/week (N = 9); (vi) Other combinations/unknown (N = 38)	2−3 mo (range)	Yes	mTESE	NR	IG: 157/307 (51.1%); CG (hypogonadal): 25/41 (61%) CG (eugonadal): 217/388 (56%); p = 0.31	IG: CPR = 79/157 (50%); LBR = 60/157 (38%); CG (eugonadal): CPR = 14/25 (56%); LBR = 12/25 (48%); p > 0.05 (pregnancy data NR by type of treatment); CG (eugonadal): CPR = 105/217 (48%); LBR:94/217 (43%)	NR
Hussein et al. (2013)	RC	Turkey	Mixed population (N = 612) of NG eugonadal and NG hypogonadal men with idiopathic NOA (mean FSH levels: 6.4 mIU/mL; TT levels <300 ng/dL in 140 patients); IG: N = 496; CG: N = 116	26.7 (4.9)	CC 50 mg every other day for 2 weeks, followed by various regimens based on initial response to CC and follow-up FSH and TT levels, including (i) group 1: CC only (varying doses ranging from 50 mg every 3 days to 75 mg every other day) for 6.4 ± .4 mo (N = 372), if follow-up FSH levels increased >50% from baseline and TT target (500−800 ng/dl) achieved; ii. group 2: CC 50 mg every other day +5000 IU uhCG 2x/week for 4.1 ± 2.4 mo (hCG dose adjusted to achieve TT target) (N = 62), if suboptimal increase/no increase in follow-up LH and TT levels; iii. group 3: stop CC, and 5000 IU uhCG 1x/week +75 IU hMG 1x/week added for 4.2 ± 1.1 mo (hCG dose adjusted for TT target; N = 46), if no obvious	5.4 mo (range: 3−9)	Yes	mTESE	Group 1: 41/372 (11%); Group 2: 7/62 (11.3%); Group 3: 4/46 (8.7%); Group 4: 2/16 (12.6%) CG: 0/116 (0%)	IG: Group1 = 191/331 (57.7%), Group 2 = 31/55 (56.4%), Group 3 = 22/42 (52.4%), Group 4 = 8/14 (57.1%) CG: 39/116 (33.6%) p < 0.05 (CG vs. IGs)	NR	None

					increase in FSH, LH and TT levels despite CC adjustments; iv. group 4: stop CC, and uhCG 5000 IU 1x/ week + hMG 75 IU1x/week added for 4.2 ± 1.1 mo (hCG dose adjusted for TT target; N = 16), If TT paradoxically decreased after increased CC dosage						
Shiraishi et al. (2014)	CS	Japan	Mixed population (N = 6) of post-CT (mean FSH: 23.6 mIU/mL; mean TT: 349.3 ng/dL) with failed mTESE and had a testicular histology showing immature germ cells	34.6 (23–42)	uhCG 5000 IU 3x/week for 3 mo, followed by same regimen for another 1–2 mo if follow-up FSH levels remained >2 mIU/mL (N = 2), or rFSH added (150 IU 3x/week for 2 mo; N = 4)	4–5 mo (range)	No	mTESE	NR	2/6 (33.3%)	NR	Gynecomastia: 1/6 (16.6%)
Kobori et al. (2015)	CS	Japan	NG and HG (N = 26) with MA and failed mTESE (FSH range: 4.2–14.4 mIU/mL) eugonadal (TT levels range: 344–661 ng/dL)	34.6 (29–38)	rFSH 75 IU 2x/week for 3 mo, followed by 150 IU rFSH 150 IU 2x/week for 3 mo.	5 mo.	No	NA	5/26 (19.2%)	NA	NR	Gynecomastia: 1/26 (3.8%)
Majzoub et al. (2016)	RC	Qatar	HGH men with non-mosaic KS; N = 43; (FSH>19 IU/L and TT < 10.4 nmol/L); mTESE group (N = 20), including: (i) IG (N = 16) and (ii) CG (N = 4); cTESE group (N = 23; no pre-treatment)	32.9 (6.3)	Anastrazole 1 mg/d for 6 mo (N = 10) or CC 25 mg/ day + uhCG 5000 IU 1x/week (N = 6)	NR (hCG)	Yes	mTESE (N = 20) cTESE (N = 23)	NR	mTESE: 6/20 (30%); IG: 6/16 (37.5%); Anastrazole group: 5/10 (50%), CC + hCG group: 1/6 (12.5%); CG: mTESE: 0/4 (0%), p = 0.06 (mTESE IG vs. CG overall); cTESE: 0/23 (0%)	PR: 3/6 (50%) OPR/LBR: 3/6 (50%); all obtained in treated patients	NR
Shiraishi et al. (2016)	CS	Japan	HG eugonadal (mean FSH: 22.1 IU/L; mean TT: 453 ng/dL) with idiopathic NOA and failed mTESE (N = 21)	32.2 (3.1)	u-hCG 5000 IU 3x/week for 1 mo, followed by u-hCG 5000 IU 3x/week + rFSH 150 IU 3x/week for 3 mo.	4 mo.	No	Repeat mTESE	NR	2/21 (9.5%)	1/21 (4.8%)	Acne: 3/21 (14.3%)
Gul et al. (2016)	RC	Turkey	NG (FSH between 1 and 12 mIU/mL) with idiopathic NOA; N = 83 IG: N = 34 CG: N = 49	IG: 34.0 (5.7) CG: 33.1 (3.8)	u-hCG 2500 IU 2x/week	10–14 weeks	Yes	cTESE or mTESE	NR	IG: 17/34 (50%); CG: 28/49 (57.1%), p = 0.338	LBR: IG = 10/16 (62.5%) CG = 17/27 (63%), (p = 0.613)	None
Barbotin et al. (2017)	CR	France	NG; N = 1 (FSH: 5.8 IU/L) with MA and failed TESE	36.0	CC 50 mg/d for 10 mo, followed by and then replaced with rFSH 150 IU 3x/week for 9 mo.	9 mo.	No	NA	CC: 2 of 4 samples with abnormal sperm (not frozen) rFSH: 1 of 3 samples with 50 motile sperm (frozen)	NA	Twin delivery from ICSI using ejaculated sperm	None
Hu et al. (2018)	RC	China	HG eugonadal; N = 35 (FSH>5.5 IU/L; TT > 9.4 nmol/L) with idiopathic NOA and failed TESE, all with Hypo;	IG: 25.8 (3.4) CG: 26.6 (3.3)	Goserelin 3.6 mg single dose for 1 mo, followed by goserelin 3.6 single dose + u 2000 IU 1x/week for 1 mo, and lastly, goserelin 3.6 mg 1x/mo. + uhCG 2000 IU 1x/	6 mo.	Yes	cTESE	NR	IG: 2/25 (8%); CG: 0/10 (0%)	NR	Goserelin alone: erectile dysfunction, libido loss, asthenia) 10/25

(continued on next page)

Table 3 (*continued*)

Author (year)	Study design	Country	NOA population; N	Mean age (SD or range)	Intervention regimen	Mean Tx period	Control group	SR method	Sperm return to ejaculate N (%)	Sperm retrieval success, N (%)	Pregnancy data	Adverse events
			IG: N = 25; CG: N = 10		week + hMG 150 IU 2x/w for 4 mo.							(40%), resolved with hCG
Cocci et al. (2018)	PC	Italy	NG; N = 50 (FSH levels <12 mIU/mL) with idiopathic NOA; IG: N = 25; CG: N = 25	IG: 34.0 (4.0) CG: 35.5 (4.3)	HP-uFSH 150 IU 3x/week	3 mo.	Yes	TESE	IG: 5/25 (20%); CG:0/25 (0%)	IG: 10/25 (40%); CG: 7/25 (28%), p < 0.05	PR per intention to treat: IG: 7/25 (28%); CG: 3/25 (15%), p < 0.05	NR
Amer et al. (2019)	RC	Egypt	HG; N = 1395 (FSH >8 mIU/mL); IG: N = 426; CG: N = 969	+SR: 37.2 (9.7) -SR: 36.4 (7.6)	Various regimens (doses not specified), including hCG (type not specified) + hMG (N = 131; anti-oestrogen (N = 66); testosterone (N = 79); HP-uFSH or rFSH (n = 22); hCG + hMG + anti-oestrogen (N = 41); hCG + hMG + testosterone (N = 34); anti-oestrogen + testosterone (N = 37) + AI (n = 6); hCG + hMG + FSH + anti-oestrogen + testosterone (N = 10)	NR	Yes	mTESE	NR	IG: 118/426 (27.7%); CG: 332/969 (34.3%); p = 0.21 Stratified by hormonal therapy regimen: (i) hCG + HMG: 33/131 (25.2%); (ii) anti-estrogen: 16/66 (24.2%); (iii) T: 21/79 (26.6%); (iv) FSH: 9/22 (40.9%); (v) hCG + HMG + anti-estrogen: 10/41 (24.4%); (vi) hCG + HMG + T: 6/34 (17.6%); anti-estrogen + T: 15/37 (40.5%); AI: 2/6 (33.3%) hCG + HMG + FSH + anti-estrogen + T: 6/10 (60%); p = 0.198	NR	NR
Laursen et al. (2019)	CR	Denmark	NG eugonadal; N = 1 (FSH 11.6 IU/L; TT 18.2 nmol/L); cryptorchidism	28	rhCG 1560 IU 2x/week for 1 mo, followed by (i) dose adjustment as needed to keep TT between 23 and 29 nmol/L or (ii) rFSH added (150–225 IU) 2x/week if follow-up FSH levels <1.5 IU/L	40 weeks	No	No	Few ejaculate sperm (five cell-sleepers stored with motile sperm)	NA	Singleton delivery from ICSI using ejaculated sperm	NR
Amer et al. (2020)	PC	Egypt	HG; N = 40 (mean FSH: 25.4 IU/L) with failed mTESE IG: N = 20; CG: N = 20	IG: 35.9 (5.4) CG: 36.2 (4.3)	Tenanthate 250 mg 1/week for 1 mo, followed by Tenanthate 250 mg 1/week + hCG 5000 IU (type not specified) 1/week + HP-uFSH 150 IU 3x/week for 3 mo.	4 mo.	Yes	mTESE	NR	IG: 2/20 (10%); CG: 0/20 (0%); p = 0.07	NR	NR
Sen et al. (2020)	PC	Singapore	NG hypogonadal N = 24; (FSH range: 1.5–12.4 IU/L; TT < 11 nmol/L); IG: N = 12; CG: N = 12	IG: 36.6 (2.0) CG: 41.0 (2.4)	r-hCG 250 mcg 1x/week	6 mo.	Yes	mTESE	IG: 3/12 25%); CG: 0/12 (0%)	IG: 6/9 (66.6%); CG: 4/12 (33.3%), p < 0.05	NR	NR
Tharakan et al. (2020)	CR	UK	HG eugonadal (FSH: 24.1 IU/L; TT:	42 33	Tamoxifen 20 mg/day for 2 weeks followed by hCG (type not specified) 2500 IU 2x/	9 mo.	No	mTESE	Immotile sperm	1/1 (100%)	Singleton delivery from ICSI using testicular sperm	NR

			10.1 nmol/L) with idiopathic NOA		week + hMG 75 IU 2x/weeks for 9 mo.							
Guo et al. (2020)	RC	China	HG; N = 184 (mean FSH levels: 29.1) with non-mosaic KS; IG: N = 134; CG: n = 50	30.2 (4.8)	2000 IU hCG every 2 days	3 mo.	Yes	mTESE	NR	IG: 58/134 (43.3%); CG: 22/50 (44%)	CPR: IG: 22/49 (44.9%) CG: 9/20 (45%), p = 0.99 LBR: IG: 15/49 (30.6%); CG: 7/20 (35%), p = 0.69	NR
Andrade et al. (2021)	CR	Brazil	NG with MA; Case 1: FSH: 6.1 mIU/mL; TT: 266 ng/dL; Case 2: FSH: 4.4 mIU/mL; TT: 360 ng/dL	Case 1: 36 Case 2: 35	Case 1: rhCG 125 mcg 2x/week for 2 months, followed by rFSH 150 IU 2x/week + anastrazole 1 mg/day for 4 mo. Case 2: rhCG 125 mcg 2x/week + rFSH150 IU 2x/week for 5 mo.	4–5 mo.	No	mTESE	Case 1: no sperm after 6 months of Tx; Case 2: rare abnormal motile sperm after 5 mo of Tx	2/2 (100%)	Case 1: Singleton delivery from ICSI using testicular sperm; Case 2: No pregnancy	NR
Peng et al. (2022)	RC	China	HG; N = 569 (mean FSH levels: 18.1 IU/L); IG: N = 395; CG: n = 174	IG: 30.0 (28–33) CG: 32.0 (28.0–35.0)	hCG (type not specified) 2000 IU every 2 days for 1 mo, followed by adjustmemnts based on follow-up FSH levels: (i) if FSH >11.1 IU/L, treatment continued with 2000 IU hCG every 2 days for 1 mo; (ii) if FSH between 0.7 and 11.1 IU/L), hCG 2000 IU + HP-uFSH every 2 days for 2 mo.	2–3 mo.	Yes	mTESE	IG: 27/395 (6.8%); CG: 0/174 (0%)	IG: 115/368 (31.2%); CG: 34/174 (19.5%), p = 0.006	LBR: IG = 54/107 (50.5%); CG = 14/31 (45.2%), p = 0.752	NR
Laursen et al. (2022)	CS	Denmark	Mixed population of NG and HG with failed TESA; N = 8	36.3 (28.0–45.0)	rhCG 1620 IU 2x/week for 1 mo, followed by (i) same regimen with (n = 1; T/E ratio <10) or without addition of anastrazaol 1 mg/day (N = 1), or (ii) rFSH added (150–225 IU) 2x/week if follow-up FSH levels <1.5 IU/L; N = 6)	10 mo (range: 8–15)	No	TESA	2/8 (25%)	2/8 (25%)	PR: 4/8 (50%); OPR/LBR: 3/8 (37.5%)	None
Andrabi et al. (2022)	CS	India	HG hypogonadal (TT < 400 ng/dL); N = 58	31.4 (3.7)	rhCG 6500 IU 1x/week	at least 3 mo.	No	NA	25/58 (43.1%)	NR	LBR (natural pregnancy): 4/25 (16%)	Weight gain (1 case)

PC: prospective cohort study; RC: retrospective cohort study; CS: case series; CR: case report; NG: normogonadotropic; HG: hypergonadotropic; HGH: hypergonadotropic hypogonadal; IG: intervention group; CG: control group; MA: maturation arrest; Hypo: hypospermatogenesis; SCO: Sertoli cell only; KS: Klinefelter Syndrome; post-CT: post-chemotherapy; IU: international units; FSH: follicle-stimulating hormone; LH: luteinizing hormone; TT: total testosterone; T: testosterone; AZF: azoospermia factor; TESE: testicular sperm extraction; cTESE: conventional testicular sperm extraction; mTESE: microdissection testicular sperm extraction; TESA: testicular sperm aspiration; SR: sperm retrieval; +SR: positive sperm retrieval; −SR: negative sperm retrieval; Tx: treatment; Mo.: months; hCG: human chorionic gonadotropin; rhCG: recombinant human chorionic gonadotropin; uhCG: urinary human chorionic gonadotropin; HP: highly purified; uFSH: urinary follicle-stimulating hormone; rFSH: recombinant follicle-stimulating hormone; hMG: human menopausal gonadotropin; T/E ratio: testosterone to estradiol ratio; AI: aromatase inhibitor; CC: clomiphene citrate; PR: pregnancy rate; LBR: live birth rate; NR: not reported; NA: not applicable.

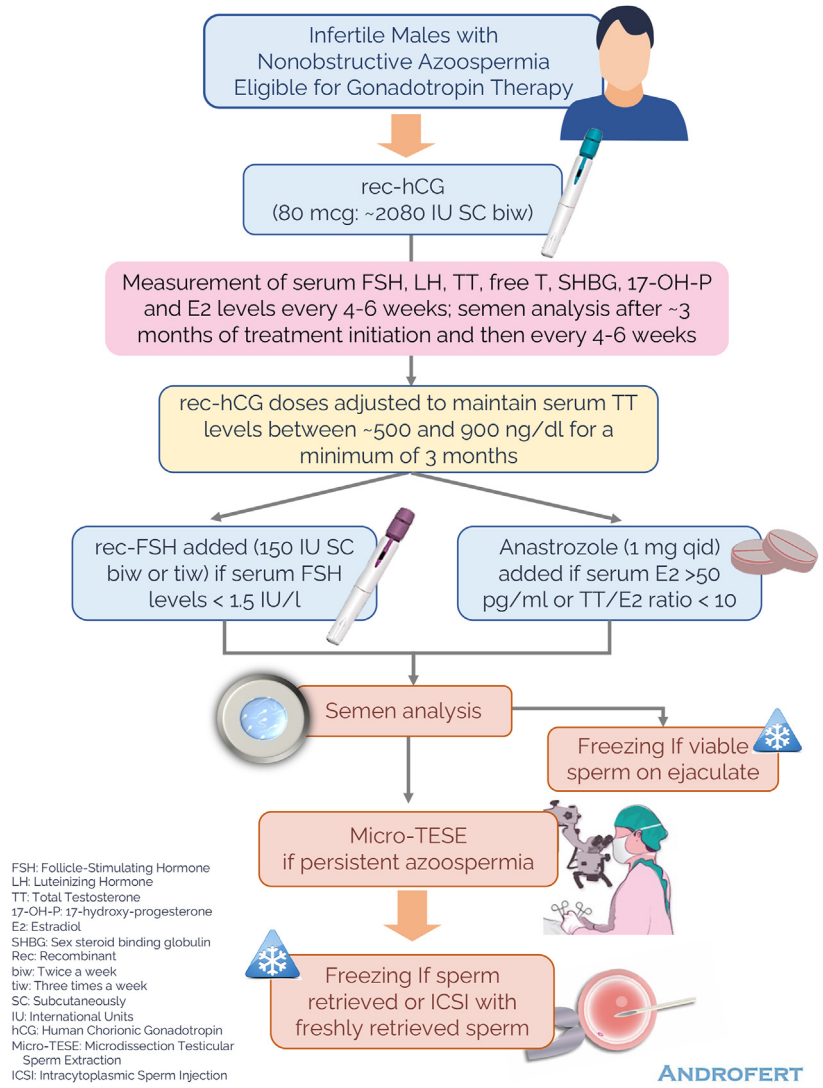

Fig. 4. Illustration depicting the gonadotropin therapy algorithm used at the ANDROFERT Center for infertile males with non-obstructive azoospermia. The hormonal treatment relies on the off-label use of hCG alone or in association with FSH. After signed informed consent, patients are commenced on recombinant human chorionic gonadotropin (rec-hCG; choriogonadotropin alfa, Ovidrel 250 μg/0.5 ml prefilled pen ready for injection, Merck, Brasil), with the dose of 80 μg (~2080 IU) applied subcutaneously, twice weekly. The rec-hCG dose is lowered to a minimum of 40 μg or increased to 250 mcg (6500 IU) per injection to maintain the total testosterone level between 500 and 900 ng/dl. If the serum FSH level drop below 1.5 IU/l during rec-hCG stimulation, supplementation with rec-FSH (rec-FSH; follitropin alfa, Gonal-f 300 IU/0.5 ml, prefilled multidose pen ready for injection, Merck, Brasil) is commenced. A fixed dose of 150 IU 2−3X times a week is given concomitantly with rec-hCG therapy for at least three months. An aromatase inhibitor (AI; anastrozole; 1 mg, Eurofarma, Brasil, or Arimidex; 1 mg, AstraZeneca, Brasil) is added off-label, in a dose of 1 mg daily, anytime during the treatment course if the estradiol levels exceeded 50 pg/mL or total testosterone (ng/dl) to estradiol (pg/mL) ratio (T/E ratio) turned <10. The aromatase inhibitor is administered orally in a fixed dose to keep estradiol levels below 50 pg/mL and a T/E ratio >10. The follow-up includes hormone measurements (serum FSH, LH, estradiol, total testosterone, free testosterone, SHBG, and 17-hydroxy-progesterone levels) and liver enzymes (patients taking AIs) every three to four weeks. Semen analysis is carried out three months after the treatment commencement and then every four weeks in patients who continued therapy for over three months. If viable sperm are found in any semen analysis during treatment, sperm cryopreservation is carried out. Otherwise, patients are subjected to microdissection testicular sperm extraction (micro-TESE) for at least a 3-month treatment. Reprinted with permission, ANDROFERT© 2022. All rights reserved.

The treatment protocol used at the ANDROFERT Clinic relies primarily on recombinant hCG used off-label to boost ITT production (Fig. 4). Our experience shows that most patients' circulating testosterone levels increase after hCG treatment. The increased testosterone levels reset some patients' elevated baseline FSH levels to normal levels. This is beneficial as the FSH reset might increase FSH receptors' expression and improve Sertoli cell function [123,123,132]. Notably, some patients exhibit a remarkable decline in circulating FSH levels during hCG treatment; we add recombinant FSH to the hCG regimen when the FSH levels drop below 1.5 IU/L. Patients are followed up with a monthly hormonal assessment, and an aromatase inhibitor is added during the treatment when the testosterone-to-estradiol ratio turns less than 10 [3,77,188,193].

Collectively, limited evidence, overwhelmingly based on observational studies and case series, suggests that gonadotropin therapy for males with NOA might increase sperm retrieval success rates. In a few cases, it was associated with the return of minimal sperm numbers to the ejaculate. Further high-quality studies are warranted to confirm whether gonadotropin therapy improves sperm retrieval success rates in this population. Additionally, more data are needed to identify the NOA patients who might benefit the most from gonadotropin therapy and the optimal treatment regimen and duration.

Summary

RWD on gonadotropin stimulation for eugonadal and hypogonadal infertile males with idiopathic oligozoospermia or NOA indicate an overall positive therapeutic effect. FSH therapy in patients with idiopathic oligozoospermia, mainly using recombinant preparations, may increase sperm quantity and quality because of the FSH proliferative action on Sertoli cells, synergically complemented by the action of androgens and other growth factors. However, the effect is not universal, and patients' specific genotypes might impact therapeutic effectiveness. Leydig cells express LHCGRs to which both LH and hCG can bind; thus, hCG preparations, mainly urine-derived, have been the drugs of choice to boost ITT production in eugonadal and hypergonadotropic hypogonadal men with NOA. Low ITT concentration is typical in men with NOA and intrinsic testicular pathology; it causes spermatogenesis disruption that hCG can revert. The increase in testosterone production by hCG might suppress the elevated endogenous gonadotrophin levels, overcoming the Sertoli cell receptor desensitization caused by chronically raised circulating FSH levels commonly seen in NOA males. Although the optimal testosterone level for stimulating spermatogenesis is unknown, RWD on hCG-based therapy in NOA males—overwhelmingly based on small case series and observational studies—has shown increased sperm retrieval rates (by 10–15%) than with no treatment, and in some cases, the return of small sperm numbers to the ejaculate. The most suitable NOA patients for gonadotropin therapy seem to be hypogonadal men with testicular histopathology showing maturation arrest (late stages) or hypospermatogenesis. Still, the evidence concerning the role of exogenous gonadotropins in male infertility treatment is limited and needs validation by large-scale, well-designed studies. Further research is required to identify the best candidates for treatment and optimal gonadotropin regimens.

Practice points

- Empiric hormonal therapy for male infertility patients is widely practiced and relies on the knowledge that FSH- and LH-driven testosterone primarily regulate spermatogenesis.
- Exogenous FSH is the gonadotropin treatment of choice for men with idiopathic oligozoospermia seeking fertility.
- Exogenous FSH therapy might increase sperm quantity in men with idiopathic oligozoospermia, with an apparent positive effect on natural and medically assisted reproduction pregnancy rates.

- Gonadotropin therapy for males with nonobstructive azoospermia might boost intratesticular testosterone production, spermatogenesis, and spermiogenesis, potentially improving sperm retrieval success or the presence of sperm in the ejaculate.
- Evidence from cohort studies and case series suggests that gonadotropin therapy with exogenous hCG and/or FSH could lead to a 10–15% higher sperm retrieval rate than with no treatment in NOA males.
- Based on limited data, the most suitable NOA patients for gonadotropin therapy seem to be hypogonadal men (i.e., serum levels of total testosterone <300 ng/dL) with baseline FSH levels $\leq$12 UI/L, and those with histopathology showing maturation arrest (late stages) or hypospermatogenesis.

Research agenda

- High-quality research, including real-world evidence studies and prospective clinical trials, to confirm the potential clinical benefit of FSH therapy to overcome infertility in men with idiopathic oligozoospermia.
- Real-world evidence studies and prospective clinical trials exploring the effectiveness and efficacy of gonadotropin therapy for males with nonobstructive azoospermia seeking fertility.
- The optimal treatment regimen and duration of gonadotropin therapy for males with idiopathic infertility or nonobstructive azoospermia.
- The role of pharmacogenomics is to identify the best candidates for treatment.
- Prospective clinical trials exploring gonadotropin therapy's safety for males with idiopathic infertility and nonobstructive azoospermia seeking fertility.
- Investigation of serum testosterone level thresholds facilitating optimal spermatogenesis.
- Long-term effect of gonadotropin therapy on testicular function in men with nonobstructive azoospermia subjected or not to sperm retrieval.
- Comparative effect of therapy with exogenous gonadotropins on sperm retrieval rates and the likelihood of sperm return to the ejaculate in hypergonadotropic, hypogonadal, and eugonadal males.
- The health of the resulting offspring after gonadotropin therapy for males with idiopathic oligozoospermia or nonobstructive azoospermia.

Funding

None.

Declaration of competing interest

SCE and LC declare receipt of unrestricted research grants from Merck and lecture fees from Merck and Med. E.A and Event Planet. Other authors have no conflicts of interest to declare.

Acknowledgments

The authors are grateful to Chloé Xilinas and Roberto Cavalieri from Med. E.A. for their help with the artwork.

References

[1] Esteves SC. Evolution of the World Health Organization semen analysis manual: where are we? Nat Rev Urol 2022;19:439–46. https://doi.org/10.1038/s41585-022-00593-2.
[2] Hamada AJ, Esteves SC, Agarwal A. A comprehensive review of genetics and genetic testing in azoospermia. Clinics 2013;68(Suppl 1):39–60.
[3] Esteves SC. Clinical management of infertile men with nonobstructive azoospermia. Asian J Androl 2015;17:459–70.

[4] Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. Clinics 2011;66:691–700.
[5] Achermann APP, Esteves SC. Diagnosis and management of infertility due to ejaculatory duct obstruction: summary evidence. Int Braz J Urol 2021;47:868–81.
[6] Cho CL, Esteves SC, Agarwal A. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian J Androl 2016;18:186–93.
[7] Fraietta R, Zylberstejn DS, Esteves SC. Hypogonadotropic hypogonadism revisited. Clinics 2013;68(Suppl 1):81–8.
[8] Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen Quality: a new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. Eur Urol 2016;70:635–45.
[9] Gunes S, Esteves SC. Role of genetics and epigenetics in male infertility. Andrologia 2021;53:e13586.
[10] Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol 2015;13:37. https://doi.org/10.1186/s12958-015-0032-1.
[11] Esteves SC. Who cares about oligozoospermia when we have ICSI. Reprod Biomed Online 2022;44:769–75. https://doi.org/10.1016/j.rbmo.2021.11.026.
[12] Humaidan P, Haahr T, Povlsen BB, Kofod L, Laursen RJ, Alsbjerg B, et al. The combined effect of lifestyle intervention and antioxidant therapy on sperm DNA fragmentation and seminal oxidative stress in IVF patients: a pilot study. Int Braz J Urol 2022;48:131–56. https://doi.org/10.1590/S1677-5538.IBJU.2021.0604.
[13] Lira Neto FT, Roque M, Esteves SC. Effect of varicocelectomy on sperm deoxyribonucleic acid fragmentation rates in infertile men with clinical varicocele: a systematic review and meta-analysis. Fertil Steril 2021;116:696–712. https://doi.org/10.1016/j.fertnstert.2021.04.003.
[14] Esteves SC, Santi D, Simoni M. An update on clinical and surgical interventions to reduce sperm DNA fragmentation in infertile men. Andrology 2020;8:53–81. https://doi.org/10.1111/andr.12724.
[15] Esteves SC. Novel concepts in male factor infertility: clinical and laboratory perspectives. J Assist Reprod Genet 2016;33:1319–35. https://doi.org/10.1007/s10815-016-0763-8.
[16] Datta J, Palmer MJ, Tanton C, Gibson LJ, Jones KG, Macdowall W, et al. Prevalence of infertility and help seeking among 15 000 women and men. Hum Reprod 2016;31:2108–18. https://doi.org/10.1093/humrep/dew123.
[17] Cocuzza MS, Tiseo BC, Srougi V, Wood GJA, Cardoso JPGF, Esteves SC, et al. Diagnostic accuracy of physical examination compared with color Doppler ultrasound in the determination of varicocele diagnosis and grading: impact of urologists' experience. Andrology 2020;8:1160–6. https://doi.org/10.1111/andr.12797.
[18] Esteves SC, Zini A, Coward RM, Evenson DP, Gosálvez J, Lewis SEM, et al. Sperm DNA fragmentation testing: summary evidence and clinical practice recommendations. Andrologia 2021;53:e13874. https://doi.org/10.1111/and.13874.
[19] Esteves SC, Zini A, Coward RM. Best urological practices on testing and management of infertile men with abnormal sperm DNA fragmentation levels: the SFRAG guidelines. Int Braz J Urol 2021;47:1250–8. https://doi.org/10.1590/S1677-5538.IBJU.2020.1004.
[20] Esteves SC, López-Fernández C, Martínez MG, Silva EA, Gosálvez J. Reliability of the sperm chromatin dispersion assay to evaluate sperm deoxyribonucleic acid damage in men with infertility. Fertil Steril 2022;117:64–73. https://doi.org/10.1016/j.fertnstert.2021.08.045.
[21] Esteves SC. Are specialized sperm function tests clinically useful in planning assisted reproductive technology? Int Braz J Urol 2020;46:116–23. https://doi.org/10.1590/S1677-5538.IBJU.2020.01.03.
[22] Majzoub A, Esteves SC, Gosálvez J, Agarwal A. Specialized sperm function tests in varicocele and the future of andrology laboratory. Asian J Androl 2016;18:205–12. https://doi.org/10.4103/1008-682X.172642.
[23] Minhas S, Bettocchi C, Boeri L, Capogrosso P, Carvalho J, Cilesiz NC, et al. EAU working group on male sexual and reproductive health. European association of urology guidelines on male sexual and reproductive health: 2021 update on male infertility. Eur Urol 2021;S0302–2838(21):1982–5.
[24] Ambulkar SS, Darves-Bornoz AL, Fantus RJ, Wren J, Bennett NE, Halpern JA, et al. Prevalence of hyperprolactinemia and clinically apparent prolactinomas in men undergoing fertility evaluation. Urology 2022;159:114–9.
[25] Esteves SC, Spaine DM, Filho JH. The testis: function and hormonal control. In: Agarwal A, Rizk RMB, Aziz N, Sabanegh Jr E, editors. Medical & surgical management of male infertility. first ed. Jaypee Brothers Medical Publishers (P) Ltd.; 2014. p. 8–13. doi.org/10.5005/jp/books/11840_2.
[26] Yang L, Zhang SX, Dong Q, Xiong ZB, Li X. Application of hormonal treatment in hypogonadotropic hypogonadism: more than ten years experience. Int Urol Nephrol 2012;44:393–9. https://doi.org/10.1007/s11255-011-0065-0.
[27] Behre HM. Clinical Use of FSH in male infertility. Front Endocrinol 2019;24(10):322. https://doi.org/10.3389/fendo.2019.00322.
[28] Santi D, Crépieux P, Reiter E, Spaggiari G, Brigante G, Casarini L, et al. Follicle-stimulating hormone (FSH) action on spermatogenesis: a focus on physiological and therapeutic roles. J Clin Med 2020;9:1014. https://doi.org/10.3390/jcm9041014.
[29] Al Khayal AM, Balaraj FK, Alferayan TA, Al Sait MA, Abumelha SM, Alrabeeah KA. Empirical therapy for male factor infertility: survey of the current practice. Urol Ann 2021;13:346–50. https://doi.org/10.4103/UA.UA_22_20.
[30] Thaker H, Ko EY, Sabanegh ES, Brannigan RE, Alukal JP, Samplaski MK. Empirical medical therapy for idiopathic male infertility. F S Rep 2020;1:15–20. https://doi.org/10.1016/j.xfre.2020.04.003.
[31] Santi D, De Vincentis S, Alfano P, Balercia G, Calogero AE, Cargnelutti F, et al. Use of follicle-stimulating hormone for the male partner of idiopathic infertile couples in Italy: results from a multicentre, observational, clinical practice survey. Andrology 2020;8(3):637–44. https://doi.org/10.1111/andr.12748.
[32] Shiraishi K, Matsuyama H. Gonadotoropin actions on spermatogenesis and hormonal therapies for spermatogenic disorders [Review]. Endocr J 2017;64:123–31.
[33] Schlegel PN, Sigman M, Collura B, De Jonge CJ, Eisenberg ML, Lamb DJ, et al. Diagnosis and treatment of infertility in men: AUA/ASRM guideline part II. Fertil Steril 2021;115:62–9. https://doi.org/10.1016/j.fertnstert.2020.11.016.
[34] Benson K, Hartz AJ. A comparison of observational studies and randomized, controlled trials. Am J Ophthalmol 2000;130:688. https://doi.org/10.1016/s0002-9394(00)00754-6.

[35] Dreyer NA, Bryant A, Velentgas P. The GRACE Checklist: a validated assessment tool for high quality observational studies of comparative effectiveness. J Manag Care Spec Pharm 2016;22:1107–13. https://doi.org/10.18553/jmcp.2016.22.10.1107.
[36] Wang R, Chen ZJ, Vuong LN, Legro RS, Mol BW, Wilkinson J. Large randomized controlled trials in infertility. Fertil Steril 2020;113:1093–9. https://doi.org/10.1016/j.fertnstert.2020.04.037.
[37] Santi D, De Vincentis S, Alfano P, Balercia G, Calogero AE, Cargnelutti F, et al. Use of follicle-stimulating hormone for the male partner of idiopathic infertile couples in Italy: results from a multicentre, observational, clinical practice survey. Andrology 2020;8:637–44. https://doi.org/10.1111/andr.12748.
[38] Esteves SC, Yarali H, Vuong LN, Carvalho JF, Özbek İY, Polat M, et al. Cumulative delivery rate per aspiration IVF/ICSI cycle in POSEIDON patients: a real-world evidence study of 9073 patients. Hum Reprod 2021;36:2157–69. https://doi.org/10.1093/humrep/deab152.
[39] Esteves SC, Yarali H, Vuong LN, Carvalho JF, Özbek İY, Polat M, et al. Low prognosis by the POSEIDON criteria in women undergoing assisted reproductive technology: a multicenter and multinational prevalence study of over 13,000 patients. Front Endocrinol 2021;12:630550. https://doi.org/10.3389/fendo.2021.630550.
[40] Oduwole OO, Peltoketo H, Huhtaniemi IT. Role of follicle-stimulating hormone in spermatogenesis. Front Endocrinol 2018;9:763. https://doi.org/10.3389/fendo.2018.00763.
[41] França LR, Hess RA, Dufour JM, Hofmann MC, Griswold MD. The Sertoli cell: one hundred fifty years of beauty and plasticity. Andrology 2016;4:189–212. https://doi.org/10.1111/andr.12165.
[42] Leão Rde B, Esteves SC. Gonadotropin therapy in assisted reproduction: an evolutionary perspective from biologics to biotech. Clinics 2014;69:279–93. https://doi.org/10.6061/clinics/2014(04)10.
[43] Fiddes JC, Goodman HM. The gene encoding the common alpha subunit of the four human glycoprotein hormones. J Mol Appl Genet 1981;1:3–18.
[44] Shome B, Parlow AF. Human follicle stimulating hormone: first proposal for the amino acid sequence of the hormone-specific, beta subunit (hFSHb). J Clin Endocrinol Metab 1974;39:203–5. https://doi.org/10.1210/jcem-39-1-203.
[45] Watkins PC, Eddy R, Beck AK, Vellucci V, Leverone B, Tanzi RE, et al. DNA sequence and regional assignment of the human follicle-stimulating hormone beta-subunit gene to the short arm of human chromosome 11. DNA 1987;6:205–12. https://doi.org/10.1089/dna.1987.6.205.
[46] Benson CA, Kurz TL, Thackray VG. A human FSHB promoter SNP associated with low FSH levels in men impairs LHX3 binding and basal FSHB transcription. Endocrinology 2013;154:3016–21. https://doi.org/10.1210/en.2013-1294.
[47] Grigorova M, Punab M, Ausmees K, Laan M. FSHB promoter polymorphism within evolutionary conserved element is associated with serum FSH level in men. Hum Reprod 2008;23:2160–6. https://doi.org/10.1093/humrep/den216.
[48] Schubert M, Pérez Lanuza L, Wöste M, Dugas M, Carmona FD, Palomino-Morales RJ, et al. A GWAS in idiopathic/unexplained infertile men detects a genomic region determining follicle-stimulating hormone levels. J Clin Endocrinol Metab 2022;107:2350–61. https://doi.org/10.1210/clinem/dgac165.
[49] Plant TM, Marshall GR. The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. Endocr Rev 2001;22:764–86. https://doi.org/10.1210/edrv.22.6.0446.
[50] Bergmann M, Behre HM, Nieschlag E. Serum FSH and testicular morphology in male infertility. Clin Endocrinol 1994;40:133–6. https://doi.org/10.1111/j.1365-2265.1994.tb02455.x.
[51] Wide L, Eriksson K. Low-glycosylated forms of both FSH and LH play major roles in the natural ovarian stimulation. Ups J Med Sci 2018;123:100–8. https://doi.org/10.1080/03009734.2018.1467983.
[52] Jiang C, Hou X, Wang C, May JV, Butnev VY, Bousfield GR, et al. Hypoglycosylated hFSH has greater bioactivity than fully glycosylated recombinant hFSH in human granulosa cells. J Clin Endocrinol Metab 2015;100:E852–60. https://doi.org/10.1210/jc.2015-1317.
[53] Agwuegbo UT, Colley E, Albert AP, Butnev VY, Bousfield GR, Jonas KC. Differential FSH glycosylation modulates FSHR oligomerization and subsequent cAMP signaling. Front Endocrinol 2021;12:765727. https://doi.org/10.3389/fendo.2021.765727.
[54] Simoni M, Gromoll J, Nieschlag E. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. Endocr Rev 1997;18:739–73. https://doi.org/10.1210/edrv.18.6.0320.
[55] Perez Mayorga M, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab 2000;85:3365–9. https://doi.org/10.1210/jcem.85.9.6789.
[56] Simoni M, Santi D, Negri L, Hoffmann I, Muratori M, Baldi E, et al. 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 2016;31:1960–9. https://doi.org/10.1093/humrep/dew167.
[57] Ahda Y, Gromoll J, Wunsch A, Asatiani K, Zitzmann M, Nieschlag E, et al. Follicle-stimulating hormone receptor gene haplotype distribution in normozoospermic and azoospermic men. J Androl 2005;26:494–9. https://doi.org/10.2164/jandrol.04186.
[58] Wunsch A, Ahda Y, Banaz-Yaşar F, Sonntag B, Nieschlag E, Simoni M, et al. Single-nucleotide polymorphisms in the promoter region influence the expression of the human follicle-stimulating hormone receptor. Fertil Steril 2005;84:446–53.
[59] Pengo M, Ferlin A, Arredi B, Ganz F, Selice R, Garolla A, et al. FSH receptor gene polymorphisms in fertile and infertile Italian men. Reprod Biomed Online 2006;13:795–800. https://doi.org/10.1016/s1472-6483(10)61026-7.
[60] Tüttelmann F, Laan M, Grigorova M, Punab M, Sõber S, Gromoll J. Combined effects of the variants FSHB -211G>T and FSHR 2039A>G on male reproductive parameters. J Clin Endocrinol Metab 2012;97:3639–47. https://doi.org/10.1210/jc.2012-1761.
[61] Bousfield GR, Butnev VY, Gotschall RR, Baker VL, Moore WT. Structural features of mammalian gonadotropins. Mol Cell Endocrinol 1996;125:3–19. https://doi.org/10.1016/s0303-7207(96)03945-7.
[62] Bo M, Boime I. Identification of the transcriptionally active genes of the chorionic gonadotropin beta gene cluster in vivo. J Biol Chem 1992;267:3179–84.

[63] Nagirnaja L, Rull K, Uusküla L, Hallast P, Grigorova M, Laan M. Genomics and genetics of gonadotropin beta-subunit genes: unique FSHB and duplicated LHB/CGB loci. Mol Cell Endocrinol 2010;329:4–16. https://doi.org/10.1016/j.mce.2010.04.024.
[64] Levilliers J, Alsat E, Laudat P, Cedard L. Hormone-stimulated cAMP production in human placenta perfused in vitro. FEBS Lett 1974;47:146–8. https://doi.org/10.1016/0014-5793(74)80446-1.
[65] Waeber G, Meyer TE, LeSieur M, Hermann HL, Gérard N, Habener JF. Developmental stage-specific expression of cyclic adenosine 3',5'-monophosphate response element-binding protein CREB during spermatogenesis involves alternative exon splicing. Mol Endocrinol 1991;5:1418–30. https://doi.org/10.1210/mend-5-10-1418. Erratum in: Mol Endocrinol 1993;vol. 7:1501.
[66] Casarini L, Santi D, Brigante G, Simoni M. Two hormones for one receptor: evolution, biochemistry, actions, and pathophysiology of LH and hCG. Endocr Rev 2018;39:549–92. https://doi.org/10.1210/er.2018-00065.
[67] Casarini L, Lispi M, Longobardi S, Milosa F, La Marca A, Tagliasacchi D, et al. LH and hCG action on the same receptor results in quantitatively and qualitatively different intracellular signalling. PLoS One 2012;7:e46682. https://doi.org/10.1371/journal.pone.0046682.
[68] Riccetti L, De Pascali F, Gilioli L, Potì F, Giva LB, Marino M, et al. Human LH and hCG stimulate differently the early signalling pathways but result in equal testosterone synthesis in mouse Leydig cells in vitro. Reprod Biol Endocrinol 2017;15:2. https://doi.org/10.1186/s12958-016-0224-3.
[69] Martinat N, Crépieux P, Reiter E, Guillou F. Extracellular signal-regulated kinases (ERK) 1, 2 are required for luteinizing hormone (LH)-induced steroidogenesis in primary Leydig cells and control steroidogenic acute regulatory (StAR) expression. Reprod Nutr Dev 2005;45:101–8. https://doi.org/10.1051/rnd:2005007.
[70] Min L, Galet C, Ascoli M. The association of arrestin-3 with the human lutropin/choriogonadotropin receptor depends mostly on receptor activation rather than on receptor phosphorylation. J Biol Chem 2002;277:702–10. https://doi.org/10.1074/jbc.M106082200.
[71] Lyga S, Volpe S, Werthmann RC, Götz K, Sungkaworn T, Lohse MJ, et al. Persistent cAMP signaling by internalized LH receptors in ovarian follicles. Endocrinology 2016;157:1613–21. https://doi.org/10.1210/en.2015-1945.
[72] Casarini L, Crépieux P. Molecular mechanisms of action of FSH. Front Endocrinol 2019;10:305. https://doi.org/10.3389/fendo.2019.00305.
[73] Coffey DS. Androgen action and the sex accessory tissues. In: Knobil E, Neill, editors. The physiology of reproduction. New York: J Raven Press; 1988. p. 1081–119.
[74] Esteves SC, Miyaoka R. Sperm physiology and assessment of spermatogenesis kinetics in vivo. In: Watson RR, editor. Handbook of fertility. Academic Press; 2015. p. 383–96. https://doi.org/10.1016/B978-0-12-800872-0.00034-2.
[75] Coviello AD, Bremner WJ, Matsumoto AM, Herbst KL, Amory JK, Anawalt BD, et al. Intratesticular testosterone concentrations comparable with serum levels are not sufficient to maintain normal sperm production in men receiving a hormonal contraceptive regimen. J Androl 2004;25:931–8. 10.1002/j.1939-4640.2004.tb03164.x.
[76] Jarow JP, Chen H, Rosner TW, Trentacoste S, Zirkin BR. Assessment of the androgen environment within the human testis: minimally invasive method to obtain intratesticular fluid. J Androl 2001;22:640–5.
[77] Andrade DL, Viana MC, Esteves SC. Differential diagnosis of azoospermia in men with infertility. J Clin Med 2021;10:3144. https://doi.org/10.3390/jcm10143144.
[78] Dean JD, McMahon CG, Guay AT, Morgentaler A, Althof SE, Becher EF, et al. The International Society for Sexual Medicine's process of care for the assessment and management of testosterone deficiency in adult men. J Sex Med 2015;12:1660–86. https://doi.org/10.1111/jsm.12952.
[79] Sussman EM, Chudnovsky A, Niederberger CS. Hormonal evaluation of the infertile male: has it evolved? Urol Clin 2008;35:147–55. https://doi.org/10.1016/j.ucl.2008.01.010. vii.
[80] Reifsnyder JE, Ramasamy R, Husseini J, Schlegel PN. Role of optimizing testosterone before microdissection testicular sperm extraction in men with nonobstructive azoospermia. J Urol 2012;188:532–6. https://doi.org/10.1016/j.juro.2012.04.002.
[81] Lei ZM, Mishra S, Zou W, Xu B, Foltz M, Li X, et al. Targeted disruption of luteinizing hormone/human chorionic gonadotropin receptor gene. Mol Endocrinol 2001;15:184–200. https://doi.org/10.1210/mend.15.1.0586.
[82] Latronico AC, Arnhold IJ. Gonadotropin resistance. Endocr Dev 2013;24:25–32. https://doi.org/10.1159/000342496.
[83] Ma X, Dong Y, Matzuk MM, Kumar TR. Targeted disruption of luteinizing hormone beta-subunit leads to hypogonadism, defects in gonadal steroidogenesis, and infertility. Proc Natl Acad Sci U S A 2004;101:17294–9. https://doi.org/10.1073/pnas.0404743101.
[84] Kumar TR. Functional analysis of LHbeta knockout mice. Mol Cell Endocrinol 2007;269:81–4. https://doi.org/10.1016/j.mce.2006.10.020.
[85] Abel MH, Wootton AN, Wilkins V, Huhtaniemi I, Knight PG, Charlton HM. The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction. Endocrinology 2000;141:1795–803. https://doi.org/10.1210/endo.141.5.7456.
[86] Huhtaniemi I. Mechanisms in Endocrinology: hormonal regulation of spermatogenesis: mutant mice challenging old paradigms. Eur J Endocrinol 2018;179:R143–50. https://doi.org/10.1530/EJE-18-0396.
[87] Oduwole OO, Peltoketo H, Poliandri A, Vengadabady L, Chrusciel M, Doroszko M, et al. Constitutively active follicle-stimulating hormone receptor enables androgen-independent spermatogenesis. J Clin Invest 2018;128:1787–92. https://doi.org/10.1172/JCI96794.
[88] Hill CM, Anway MD, Zirkin BR, Brown TR. Intratesticular androgen levels, androgen receptor localization, and androgen receptor expression in adult rat Sertoli cells. Biol Reprod 2004;71:1348–58. https://doi.org/10.1095/biolreprod.104.029249.
[89] Larose H, Kent T, Ma Q, Shami AN, Harerimana N, Li JZ, et al. Regulation of meiotic progression by Sertoli-cell androgen signaling. Mol Biol Cell 2020;31:2841–62. https://doi.org/10.1091/mbc.E20-05-0334.
[90] Wang G, Weng CC, Shao SH, Zhou W, de Gendt K, Braun RE, et al. Androgen receptor in Sertoli cells is not required for testosterone-induced suppression of spermatogenesis, but contributes to Sertoli cell organization in Utp14b jsd mice. J Androl 2009;30:338–48. https://doi.org/10.2164/jandrol.108.006890.

[91] Dorrington JH, Roller NF, Fritz IB. Effects of follicle-stimulating hormone on cultures of Sertoli cell preparations. Mol Cell Endocrinol 1975;3:57–70. https://doi.org/10.1016/0303-7207(75)90031-3.
[92] Swinnen JV, Tsikalas KE, Conti M. Properties and hormonal regulation of two structurally related cAMP phosphodiesterases from the rat Sertoli cell. J Biol Chem 1991;266:18370–7.
[93] Crépieux P, Marion S, Martinat N, Fafeur V, Vern YL, Kerboeuf D, et al. The ERK-dependent signalling is stage-specifically modulated by FSH, during primary Sertoli cell maturation. Oncogene 2001;20:4696–709. https://doi.org/10.1038/sj.onc.1204632.
[94] Ruthig VA, Lamb DJ. Updates in Sertoli cell-mediated signaling during spermatogenesis and advances in restoring Sertoli cell function. Front Endocrinol 2022;13:897196. https://doi.org/10.3389/fendo.2022.897196.
[95] Musnier A, Heitzler D, Boulo T, Tesseraud S, Durand G, Lécureuil C, et al. Developmental regulation of p70 S6 kinase by a G protein-coupled receptor dynamically modelized in primary cells. Cell Mol Life Sci 2009;66:3487–503. https://doi.org/10.1007/s00018-009-0134-z.
[96] Bhattacharya I, Gautam M, Sarkar H, Shukla M, Majumdar SS. Advantages of pulsatile hormone treatment for assessing hormone-induced gene expression by cultured rat Sertoli cells. Cell Tissue Res 2017;368:389–96. https://doi.org/10.1007/s00441-016-2410-1.
[97] Kara E, Crépieux P, Gauthier C, Martinat N, Piketty V, Guillou F, et al. A phosphorylation cluster of five serine and threonine residues in the C-terminus of the follicle-stimulating hormone receptor is important for desensitization but not for beta-arrestin-mediated ERK activation. Mol Endocrinol 2006;20:3014–26. https://doi.org/10.1210/me.2006-0098.
[98] Tréfier A, Musnier A, Landomiel F, Bourquard T, Boulo T, Ayoub MA, et al. G protein-dependent signaling triggers a β-arrestin-scaffolded p70S6K/rpS6 module that controls 5'TOP mRNA translation. Faseb J 2018;32:1154–69. https://doi.org/10.1096/fj.201700763R.
[99] Tranchant T, Durand G, Gauthier C, Crépieux P, Ulloa-Aguirre A, Royère D, et al. Preferential β-arrestin signalling at low receptor density revealed by functional characterization of the human FSH receptor A189 V mutation. Mol Cell Endocrinol 2011;331:109–18. https://doi.org/10.1016/j.mce.2010.08.016.
[100] Sposini S, De Pascali F, Richardson R, Sayers NS, Perrais D, Yu HN, et al. Pharmacological programming of endosomal signaling activated by small molecule ligands of the follicle Stimulating Hormone Receptor. Front Pharmacol 2020;11:593492. https://doi.org/10.3389/fphar.2020.593492.
[101] Nicholls PK, Harrison CA, Walton KL, McLachlan RI, O'Donnell L, Stanton PG. Hormonal regulation of Sertoli cell microRNAs at spermiation. Endocrinology 2011;152:1670–83. https://doi.org/10.1210/en.2010-1341.
[102] Dupont J, Musnier A, Decourtye J, Boulo T, Lécureuil C, Guillou H, et al. FSH-stimulated PTEN activity accounts for the lack of FSH mitogenic effect in prepubertal rat Sertoli cells. Mol Cell Endocrinol 2010;315:271–6. https://doi.org/10.1016/j.mce.2009.09.016.
[103] Gupta A, Vats A, Ghosal A, Mandal K, Sarkar R, Bhattacharya I, et al. Follicle-stimulating hormone-mediated decline in miR-92a-3p expression in pubertal mice Sertoli cells is crucial for germ cell differentiation and fertility. Cell Mol Life Sci 2022;79:136. https://doi.org/10.1007/s00018-022-04174-9.
[104] Esteves SC. Efficacy, efficiency and effectiveness of gonadotropin therapy for infertility treatment. Med Dent J 2015;2. https://doi.org/10.5935/MedicalExpress.2015.03.02.
[105] Casarini L, Crépieux P, Reiter E, Lazzaretti C, Paradiso E, Rochira V, et al. FSH for the treatment of male infertility. Int J Mol Sci 2020;21:2270. https://doi.org/10.3390/ijms21072270.
[106] Esteves SC, Alviggi C. The role of LH in controlled ovarian stimulation. In: Ghumman S, editor. Principles and practice of controlled ovarian stimulation in ART. New Delhi: Springer; 2015. https://doi.org/10.1007/978-81-322-1686-5_16.
[107] Le Cotonnec JY, Porchet HC, Beltrami V, Munafo A. Clinical pharmacology of recombinant human luteinizing hormone: Part I. Pharmacokinetics after intravenous administration to healthy female volunteers and comparison with urinary human luteinizing hormone. Fertil Steril 1998;69:189–94.
[108] Casarini L, Riccetti L, De Pascali F, Gilioli L, Marino M, Vecchi E, et al. Estrogen modulates specific life and death signals induced by LH and hCG in human primary granulosa cells in vitro. Int J Mol Sci 2017;18:926. https://doi.org/10.3390/ijms18050926.
[109] Jonas KC, Fanelli F, Huhtaniemi IT, Hanyaloglu AC. Single molecule analysis of functionally asymmetric G protein-coupled receptor (GPCR) oligomers reveals diverse spatial and structural assemblies. J Biol Chem 2015;290:3875–92. https://doi.org/10.1074/jbc.M114.622498.
[110] Riccetti L, Yvinec R, Klett D, Gallay N, Combarnous Y, Reiter E, et al. Human luteinizing hormone and chorionic gonadotropin display biased agonism at the LH and LH/CG receptors. Sci Rep 2017;7:940. https://doi.org/10.1038/s41598-017-01078-8.
[111] Veldhuis JD, Liu PY, Takahashi PY, Weist SM, Wigham JR. Analysis of the impact of intravenous LH pulses versus continuous LH infusion on testosterone secretion during GnRH-receptor blockade. Am J Physiol Regul Integr Comp Physiol 2012;303:R994–1002. https://doi.org/10.1152/ajpregu.00314.2012.
[112] Lazzaretti C, Secco V, Paradiso E, Sperduti S, Rutz C, Kreuchwig A, et al. Identification of key receptor residues discriminating human chorionic gonadotropin (hCG)- and luteinizing hormone (LH)-specific signaling. Int J Mol Sci 2020;22:151. https://doi.org/10.3390/ijms22010151.
[113] Gromoll J, Eiholzer U, Nieschlag E, Simoni M. Male hypogonadism caused by homozygous deletion of exon 10 of the luteinizing hormone (LH) receptor: differential action of human chorionic gonadotropin and LH. J Clin Endocrinol Metab 2000;85:2281–6. https://doi.org/10.1210/jcem.85.6.6636.
[114] Müller T, Gromoll J, Simoni M. Absence of exon 10 of the human luteinizing hormone (LH) receptor impairs LH, but not human chorionic gonadotropin action. J Clin Endocrinol Metab 2003;88:2242–9. https://doi.org/10.1210/jc.2002-021946.
[115] Cailleux-Bounacer A, Reznik Y, Cauliez B, Menard JF, Duparc C, Kuhn JM. Evaluation of endocrine testing of Leydig cell function using extractive and recombinant human chorionic gonadotropin and different doses of recombinant human LH in normal men. Eur J Endocrinol 2008;159:171–8. https://doi.org/10.1530/EJE-07-0876.
[116] Santi D, Spaggiari G, Casarini L, Fanelli F, Mezzullo M, Pagotto U, et al. Central hypogonadism due to a giant, "silent" FSH-secreting, atypical pituitary adenoma: effects of adenoma dissection and short-term Leydig cell stimulation by luteinizing

hormone (LH) and human chorionic gonadotropin (hCG). Aging Male 2017;20:96–101. https://doi.org/10.1080/13685538.2016.1276161.
[117] Lysoń K, Pawlikowski M. Suppression of the proliferative response of the seminal vesicles to testosterone by inhibitors of prostaglandin synthesis. Testosterone, indomethacin, and proliferation in seminal vesicles. J Androl 1983;4:167–70. https://doi.org/10.1002/j.1939-4640.1983.tb00745.x.
[118] Kobori Y, Suzuki K, Iwahata T, Shin T, Sato R, Nishio K, et al. Hormonal therapy (hCG and rhFSH) for infertile men with adult-onset idiopathic hypogonadotropic hypogonadism. Syst Biol Reprod Med 2015;61:110–2. https://doi.org/10.3109/19396368.2014.994789.
[119] Ahmad N, Haltmeyer GC, Eik-Nes KB. Maintenance of spermatogenesis in rats with intratesticular implants containing testosterone or dihydrotestosterone (DHT). Biol Reprod 1973;8:411–9. https://doi.org/10.1093/biolreprod/8.4.411.
[120] Cunningham GR, Huckins C. Persistence of complete spermatogenesis in the presence of low intratesticular concentrations of testosterone. Endocrinology 1979;105:177–86. https://doi.org/10.1210/endo-105-1-177.
[121] Zirkin BR, Santulli R, Awoniyi CA, Ewing LL. Maintenance of advanced spermatogenic cells in the adult rat testis: quantitative relationship to testosterone concentration within the testis. Endocrinology 1989;124:3043–9. https://doi.org/10.1210/endo-124-6-3043.
[122] Shinjo E, Shiraishi K, Matsuyama H. The effect of human chorionic gonadotropin-based hormonal therapy on intratesticular testosterone levels and spermatogonial DNA synthesis in men with non-obstructive azoospermia. Andrology 2013;1:929–35. https://doi.org/10.1111/j.2047-2927.2013.00141.x.
[123] Shiraishi K, Ohmi C, Shimabukuro T, Matsuyama H. Human chorionic gonadotrophin treatment prior to microdissection testicular sperm extraction in non-obstructive azoospermia. Hum Reprod 2012;27:331–9. https://doi.org/10.1093/humrep/der404.
[124] Levalle OA, Zylbersztein C, Aszpis S, Mariani V, Ponzio R, Aranda C, et al. Serum luteinizing hormone pulsatility and intratesticular testosterone and oestradiol concentrations in idiopathic infertile men with high and normal follicle stimulating hormone serum concentrations. Hum Reprod 1994;9:781–7. https://doi.org/10.1093/oxfordjournals.humrep.a138596.
[125] Roth MY, Lin K, Amory JK, Matsumoto AM, Anawalt BD, Snyder CN, et al. Serum LH correlates highly with intratesticular steroid levels in normal men. J Androl 2010;31:138–45.
[126] Patel AS, Leong JY, Ramos L, Ramasamy R. Testosterone is a contraceptive and should not be used in men who desire fertility. World J Mens Health 2019;37:45–54. https://doi.org/10.5534/wjmh.180036.
[127] Lima TFN, Patel P, Blachman-Braun R, Madhusoodanan V, Ramasamy R. Serum 17-hydroxyprogesterone is a potential biomarker for evaluating intratesticular testosterone. J Urol 2020;204:551–6.
[128] Gnanaprakasam MS, Chen CJ, Sutherland JG, Bhalla VK. Receptor depletion and replenishment processes: in vivo regulation of gonadotropin receptors by luteinizing hormone, follicle stimulating hormone and ethanol in rat testis. Biol Reprod 1979;20:991–1000. https://doi.org/10.1095/biolreprod20.5.991.
[129] Namiki M, Okuyama A, Sonoda T, Miyake A, Aono T, Matsumoto K. Down-regulation of testicular follicle-stimulating hormone receptors by human menopausal gonadotropin in infertile men. Fertil Steril 1985 Nov;44(5):710–2. https://doi.org/10.1016/s0015-0282(16)48994-6.
[130] Namiki M, Nakamura M, Okuyama A, Sonoda T, Itatani H, Sugao H, et al. Reduction of human and rat testicular follicle stimulating hormone receptors by human menopausal gonadotrophin in vivo and in vitro. Clin Endocrinol;26:675-684. doi: 10.1111/j.1365-2265.1987.tb00825.x.
[131] Themmen AP, Blok LJ, Post M, Baarends WM, Hoogerbrugge JW, Parmentier M, et al. Follitropin receptor down-regulation involves a cAMP-dependent post-transcriptional decrease of receptor mRNA expression. Mol Cell Endocrinol 1991;78:R7–13. https://doi.org/10.1016/0303-7207(91)90130-k.
[132] Foresta C, Bettella A, Spolaore D, Merico M, Rossato M, Ferlin A. Suppression of the high endogenous levels of plasma FSH in infertile men are associated with improved Sertoli cell function as reflected by elevated levels of plasma inhibin B. Hum Reprod 2004;19:1431–7. https://doi.org/10.1093/humrep/deh255.
[133] Foresta C, Selice R, Moretti A, Pati MA, Carraro M, Engl B, et al. Gonadotropin administration after gonadotropin-releasing-hormone agonist: a therapeutic option in severe testiculopathies. Fertil Steril 2009;92:1326–32. https://doi.org/10.1016/j.fertnstert.2008.07.1766.
[134] Simoni M, Brigante G, Rochira V, Santi D, Casarini L. Prospects for FSH treatment of male infertility. J Clin Endocrinol Metab 2020;105:dgaa243. https://doi.org/10.1210/clinem/dgaa243.
[135] Casarini L, Simoni M. Recent advances in understanding gonadotropin signaling. Fac Rev 2021;10:41. https://doi.org/10.12703/r/10-41.
[136] Esteves SC, Schatmann G, Agarwal A. Definitions and relevance of unexplained infertility in reproductive medicine. In: Schattman G, Esteves SC, Agarwal A, editors. Unexplained infertility. New York, NY: Springer; 2015. https://doi.org/10.1007/978-1-4939-2140-9_1.
[137] Schill WB, Jüngst D, Unterburger P, Braun S. Combined hMG/hCG treatment in subfertile men with idiopathic normogonadotrophic oligozoospermia. Int J Androl 1982;5:467–77. https://doi.org/10.1111/j.1365-2605.1982.tb00278.x.
[138] Knuth UA, Hönigl W, Bals-Pratsch M, Schleicher G, Nieschlag E. Treatment of severe oligospermia with human chorionic gonadotropin/human menopausal gonadotropin: a placebo-controlled, double blind trial. J Clin Endocrinol Metab 1987;65:1081–7. https://doi.org/10.1210/jcem-65-6-1081.
[139] Foresta C, Bettella A, Ferlin A, Garolla A, Rossato M. Evidence for a stimulatory role of follicle-stimulating hormone on the spermatogonial population in adult males. Fertil Steril 1998;69:636–42. https://doi.org/10.1016/s0015-0282(98)00008-9.
[140] Kamischke A, Behre HM, Bergmann M, Simoni M, Schäfer T, Nieschlag E. Recombinant human follicle stimulating hormone for treatment of male idiopathic infertility: a randomized, double-blind, placebo-controlled, clinical trial. Hum Reprod 1998;13:596–603. https://doi.org/10.1093/humrep/13.3.596.
[141] Ben-Rafael Z, Farhi J, Feldberg D, Bartoov B, Kovo M, Eltes F, et al. Follicle-stimulating hormone treatment for men with idiopathic oligoteratoasthenozoospermia before in vitro fertilization: the impact on sperm microstructure and fertilization potential. Fertil Steril 2000;73:24–30. https://doi.org/10.1016/s0015-0282(99)00461-6.

[142] Foresta C, Bettella A, Merico M, Garolla A, Ferlin A, Rossato M. Use of recombinant human follicle-stimulating hormone in the treatment of male factor infertility. Fertil Steril 2002;77:238–44. https://doi.org/10.1016/s0015-0282(01)02966-1.
[143] Fernández-Arjona M, Díaz J, Cortes I, González J, Rodriguez JM, Alvarez E. Relationship between gonadotrophin secretion, inhibin B and spermatogenesis in oligozoospermic men treated with highly purified urinary follicle-stimulating hormone (uFSH-HP): a preliminary report. Eur J Obstet Gynecol Reprod Biol 2003;107:47–51. https://doi.org/10.1016/s0301-2115(02)00313-5.
[144] Caroppo E, Niederberger C, Vizziello GM, D'Amato G. Recombinant human follicle-stimulating hormone as a pretreatment for idiopathic oligoasthenoteratozoospermic patients undergoing intracytoplasmic sperm injection. Fertil Steril 2003;80:1398–403. https://doi.org/10.1016/s0015-0282(03)02202-7.
[145] Baccetti B, Piomboni P, Bruni E, Capitani S, Gambera L, Moretti E, et al. Effect of follicle-stimulating hormone on sperm quality and pregnancy rate. Asian J Androl 2004;6:133–7.
[146] Foresta C, Bettella A, Garolla A, Ambrosini G, Ferlin A. Treatment of male idiopathic infertility with recombinant human follicle-stimulating hormone: a prospective, controlled, randomized clinical study. Fertil Steril 2005;84:654–61. https://doi.org/10.1016/j.fertnstert.2005.03.055.
[147] Paradisi R, Busacchi P, Seracchioli R, Porcu E, Venturoli S. Effects of high doses of recombinant human follicle-stimulating hormone in the treatment of male factor infertility: results of a pilot study. Fertil Steril 2006;86:728–31. https://doi.org/10.1016/j.fertnstert.2006.02.087.
[148] Selice R, Garolla A, Pengo M, Caretta N, Ferlin A, Foresta C. The response to FSH treatment in oligozoospermic men depends on FSH receptor gene polymorphisms. Int J Androl 2011;34:306–12. https://doi.org/10.1111/j.1365-2605.2010.01086.x.
[149] Condorelli RA, Calogero AE, Vicari E, Mongioi L, Burgio G, Cannarella R, et al. Reduced seminal concentration of CD45pos cells after follicle-stimulating hormone treatment in selected patients with idiopathic oligoasthenoteratozoospermia. Internet J Endocrinol 2014;2014:372060. https://doi.org/10.1155/2014/372060.
[150] Garolla A, Selice R, Engl B, Bertoldo A, Menegazzo M, Finos L, et al. Spermatid count as a predictor of response to FSH therapy. Reprod Biomed Online 2014;29:102–12. https://doi.org/10.1016/j.rbmo.2014.02.014.
[151] Paradisi R, Natali F, Fabbri R, Battaglia C, Seracchioli R, Venturoli S. Evidence for a stimulatory role of high doses of recombinant human follicle-stimulating hormone in the treatment of male-factor infertility. Andrologia 2014;46:1067–72. https://doi.org/10.1111/and.12194.
[152] Farrag A, Sagnella F, Pappalardo S, Costantini A, Lisi F, Carfagna P, et al. The use of r-hFSH in treatment of idiopathic male factor infertility before ICSI. Eur Rev Med Pharmacol Sci 2015;19:2162–7.
[153] Ding YM, Zhang XJ, Li JP, Chen SS, Zhang RT, Tan WL, et al. Treatment of idiopathic oligozoospermia with recombinant human follicle-stimulating hormone: a prospective, randomized, double-blind, placebo-controlled clinical study in Chinese population. Clin Endocrinol 2015;83:866–71. https://doi.org/10.1111/cen.12770.
[154] La Vignera S, Condorelli RA, Duca Y, Mongioi LM, Cannarella R, Giacone F, et al. FSH therapy for idiopathic male infertility: four schemes are better than one. Aging Male 2020;23:750–5. https://doi.org/10.1080/13685538.2019.1590696.
[155] Condorelli RA, Cannarella R, Crafa A, Barbagallo F, Mongioì LM, Aversa A, et al. Retrospective monocentric clinical study on male infertility: comparison between two different therapeutic schemes using follicle-stimulating hormone. J Clin Med 2021;10:2665. https://doi.org/10.3390/jcm10122665.
[156] Andrabi SW, Makker GC, Makker R, Mishra G, Singh R. Human chorionic gonadotropin therapy in hypogonadic severe-oligozoospermic men and its effect on semen parameters. Clin Exp Reprod Med 2022;49:57–61. https://doi.org/10.5653/cerm.2021.04742.
[157] Santi D, Granata AR, Simoni M. FSH treatment of male idiopathic infertility improves pregnancy rate: a meta-analysis. Endocr Connect 2015;4:R46–58. https://doi.org/10.1530/EC-15-0050.
[158] Omar MI, Pal RP, Kelly BD, Bruins HM, Yuan Y, Diemer T, et al. Benefits of empiric nutritional and medical therapy for semen parameters and pregnancy and live birth rates in couples with idiopathic infertility: a systematic review and meta-analysis. Eur Urol 2019;75:615–25. https://doi.org/10.1016/j.eururo.2018.12.022.
[159] Cannarella R, La Vignera S, Condorelli RA, Mongioì LM, Calogero AE. FSH dosage effect on conventional sperm parameters: a meta-analysis of randomized controlled studies. Asian J Androl 2020;22:309–16. https://doi.org/10.4103/aja.aja_42_19.
[160] Schoor RA, Elhanbly S, Niederberger CS, Ross LS. The role of testicular biopsy in the modern management of male infertility. J Urol 2002;167:197–200.
[161] Esteves SC. Microdissection TESE versus conventional TESE for men with nonobstructive azoospermia undergoing sperm retrieval. Int Braz J Urol 2022;48:569–78. https://doi.org/10.1590/S1677-5538.IBJU.2022.99.14.
[162] Achermann APP, Pereira TA, Esteves SC. Microdissection testicular sperm extraction (micro-TESE) in men with infertility due to nonobstructive azoospermia: summary of current literature. Int Urol Nephrol 2021;53:2193–210. https://doi.org/10.1007/s11255-021-02979-4.
[163] Esteves SC, Roque M, Bedoschi G, Haahr T, Humaidan P. Intracytoplasmic sperm injection for male infertility and consequences for offspring. Nat Rev Urol 2018 Sep;15(9):535–62. https://doi.org/10.1038/s41585-018-0051-8.
[164] Esteves SC, Prudencio C, Seol B, Verza S, Knoedler C, Agarwal A. Comparison of sperm retrieval and reproductive outcome in azoospermic men with testicular failure and obstructive azoospermia treated for infertility. Asian J Androl 2014 16:602–6. https://doi.org/10.4103/1008-682X.126015.
[165] Esteves SC, Agarwal A. Reproductive outcomes, including neonatal data, following sperm injection in men with obstructive and nonobstructive azoospermia: case series and systematic review. Clinics 2013;68(Suppl 1):141–50. https://doi.org/10.6061/clinics/2013(sup01)16. Suppl 1.
[166] Miyaoka R, Orosz JE, Achermann AP, Esteves SC. Methods of surgical sperm extraction and implications for assisted reproductive technology success. Panminerva Med 2019 Jun;61(2):164–77. https://doi.org/10.23736/S0031-0808.18.03508-5.
[167] Arshad MA, Majzoub A, Esteves SC. Predictors of surgical sperm retrieval in non-obstructive azoospermia: summary of current literature. Int Urol Nephrol 2020;52:2015–38. https://doi.org/10.1007/s11255-020-02529-4.

[168] Mehmood S, Aldaweesh S, Junejo NN, Altaweel WM, Kattan SA, Alhathal N. Microdissection testicular sperm extraction: overall results and impact of preoperative testosterone level on sperm retrieval rate in patients with nonobstructive azoospermia. Urol Ann 2019;11:287–93. https://doi.org/10.4103/UA.UA_36_18.
[169] Guo F, Fang A, Fan Y, Fu X, Lan Y, Liu M, et al. Role of treatment with human chorionic gonadotropin and clinical parameters on testicular sperm recovery with microdissection testicular sperm extraction and intracytoplasmic sperm injection outcomes in 184 Klinefelter syndrome patients. Fertil Steril 2020;114:997–1005. https://doi.org/10.1016/j.fertnstert.2020.05.043.
[170] Caroppo E, Colpi GM. Hormonal treatment of men with nonobstructive azoospermia: what does the evidence suggest? J Clin Med 2021;10:387. https://doi.org/10.3390/jcm10030387.
[171] Aydos K, Unlü C, Demirel LC, Evirgen O, Tolunay O. The effect of pure FSH administration in non-obstructive azoospermic men on testicular sperm retrieval. Eur J Obstet Gynecol Reprod Biol 2003;108:54–8. https://doi.org/10.1016/s0301-2115(02)00412-8.
[172] Selman HA, Cipollone G, Stuppia L, De Santo M, Sterzik K, El-Danasouri I. Gonadotropin treatment of an azoospermic patient with a Y-chromosome microdeletion. Fertil Steril 2004;82:218–9. https://doi.org/10.1016/j.fertnstert.2003.11.055.
[173] Schiff JD, Palermo GD, Veeck LL, Goldstein M, Rosenwaks Z, Schlegel PN. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. J Clin Endocrinol Metab 2005;90:6263–7. https://doi.org/10.1210/jc.2004-2322.
[174] Selman H, De Santo M, Sterzik K, Cipollone G, Aragona C, El-Danasouri I. Rescue of spermatogenesis arrest in azoospermic men after long-term gonadotropin treatment. Fertil Steril 2006;86:466–8. https://doi.org/10.1016/j.fertnstert.2005.12.055.
[175] Cao YX, Zhang ZG. A successful pregnancy outcome using testicular sperm from an infertile male pretreated with HCG. Arch Androl 2007;53:1–3. https://doi.org/10.1080/01485010600889076.
[176] Efesoy O, Cayan S, Akbay E. The efficacy of recombinant human follicle-stimulating hormone in the treatment of various types of male-factor infertility at a single university hospital. J Androl 2009;30:679–84. https://doi.org/10.2164/jandrol.108.007278.
[177] Ramasamy R, Ricci JA, Palermo GD, Gosden LV, Rosenwaks Z, Schlegel PN. Successful fertility treatment for Klinefelter's syndrome. J Urol 2009;182:1108–13. https://doi.org/10.1016/j.juro.2009.05.019.
[178] Hussein A, Ozgok Y, Ross L, Rao P, Niederberger C. Optimization of spermatogenesis-regulating hormones in patients with non-obstructive azoospermia and its impact on sperm retrieval: a multicentre study. BJU Int 2013;111(3 Pt B):E110–4. https://doi.org/10.1111/j.1464-410X.2012.11485.x.
[179] Shiraishi K, Matsuyama H. Microdissection testicular sperm extraction and salvage hormonal treatment in patients with postchemotherapy azoospermia. Urology 2014;83:100–6. https://doi.org/10.1016/j.urology.2013.08.043.
[180] Kobori Y, Suzuki K, Iwahata T, Shin T, Sato R, Nishio K, et al. Induction of spermatogenesis by rhFSH for azoospermia due to spermatogenic dysfunction with maturation arrest: five case series. Syst Biol Reprod Med 2015;61(3):168–70. https://doi.org/10.3109/19396368.2015.1024897.
[181] Majzoub A, Arafa M, Al Said S, Agarwal A, Seif A, Al Naimi A, et al. Outcome of testicular sperm extraction in nonmosaic Klinefelter syndrome patients: what is the best approach? Andrologia 2016;48:171–6. https://doi.org/10.1111/and.12428.
[182] Shiraishi K, Ishikawa T, Watanabe N, Iwamoto T, Matsuyama H. Salvage hormonal therapy after failed microdissection testicular sperm extraction: a multi-institutional prospective study. Int J Urol 2016;23:496–500. https://doi.org/10.1111/iju.13076.
[183] Gul U, Turunç T. The effect of human chorionic gonadotropin treatment before testicular sperm extraction in non-obstructive azoospermia. J Clin Anal Med 2016;7(1):55–9. https://doi.org/10.4328/JCAM.3332.
[184] Barbotin AL, Ballot C, Sigala J, Leroy M, Rigot JM, Dewailly, et al. Pregnancy after intracytoplasmic sperm injection following extended sperm preparation and hormone therapy in an azoospermic man with maturation arrest and microlithiasis: a case report and literature review. Andrologia 2017;49. https://doi.org/10.1111/and.12665.
[185] Hu X, Ding Z, Hong Z, Zou Z, Feng Y, Zhu R, et al. Spermatogenesis improved by suppressing the high level of endogenous gonadotropins in idiopathic non-obstructive azoospermia: a case control pilot study. Reprod Biol Endocrinol 2018;16:91. https://doi.org/10.1186/s12958-018-0401-7.
[186] Cocci A, Cito G, Russo GI, Falcone M, Capece M, Timpano M, et al. Effectiveness of highly purified urofollitropin treatment in patients with idiopathic azoospermia before testicular sperm extraction. Urologia 2018;85:19–21. https://doi.org/10.5301/uj.5000253.
[187] Amer MK, Ahmed AR, Abdel Hamid AA, GamalEl Din SF. Can spermatozoa be retrieved in non-obstructive azoospermic patients with high FSH level?: a retrospective cohort study. Andrologia 2019;51:e13176. https://doi.org/10.1111/and.13176.
[188] Laursen RJ, Elbaek HO, Povlsen BB, Lykkegaard J, Jensen KBS, Esteves SC, et al. Hormonal stimulation of spermatogenesis: a new way to treat the infertile male with non-obstructive azoospermia? Int Urol Nephrol 2019;51:453–6. https://doi.org/10.1007/s11255-019-02091-8.
[189] Amer MK, Ahmed HEH, GamalEl Din SF, Fawzy Megawer A, Ahmed AR. Evaluation of neoadjuvant gonadotropin administration with downregulation by testosterone prior to second time microsurgical testicular sperm extraction: a prospective case-control study. Urologia 2020;87:185–90. https://doi.org/10.1177/0391560320913401.
[190] Tharakan T, Salonia A, Corona G, Dhillo W, Minhas S, Jayasena C. The role of hormone stimulation in men with non-obstructive azoospermia undergoing surgical sperm retrieval. J Clin Endocrinol Metab 2020;105. https://doi.org/10.1210/clinem/dgaa556. dgaa556.
[191] Sen S, Chaw TE, Ling YS, Xuemei Z, Rajesh H. Effect of recombinant HCG pretreatment on surgical sperm retrieval rates in patients – an audit of our practice. Fertil Steril 2020;113:E21–2.
[192] Peng T, Liao C, Ye X, Chen Z, Lan Y, Fu X, et al. Gonadotropins treatment prior to microdissection testicular sperm extraction in non-obstructive azoospermia: a single-center cohort study. Reprod Biol Endocrinol 2022;20:61. https://doi.org/10.1186/s12958-022-00934-1.

[193] Laursen RJ, Alsbjerg B, Elbaek HO, Povlsen BB, Jensen KBS, Lykkegaard J, et al. Recombinant gonadotropin therapy to improve spermatogenesis in nonobstructive azoospermic patients - a proof of concept study. Int Braz J Urol 2022;48: 471–81. https://doi.org/10.1590/S1677-5538.IBJU.2022.9913.
[194] Andrabi SW, Saini P, Joshi M, Mehta P, Makker GC, Mishra G, et al. HCG therapy in azoospermic men with lower or borderline testosterone levels and the prognostic value of Y-deletion analysis in its outcome. Andrologia 2022;54(1): e14251. https://doi.org/10.1111/and.14251.
[195] Tharakan T, Corona G, Foran D, Salonia A, Sofikitis N, Giwercman A, et al. Does hormonal therapy improve sperm retrieval rates in men with non-obstructive azoospermia: a systematic review and meta-analysis. Hum Reprod Update 2022: dmac016. https://doi.org/10.1093/humupd/dmac016.