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REVIEW

Investigational follicle-stimulating hormone receptor agonists for male infertility therapy

Rossella Cannarella [a,b], Cristina M.B. Petralia[a], Rosita A. Condorelli [a], Antonio Aversa [c], Aldo E. Calogero [a] and Sandro La Vignera [a]

[a]Department of Clinical and Experimental Medicine, University of Catania, Catania, Italy; [b]Glickman Urological & Kidney Institute, Cleveland Clinic Foundation, Cleveland, OH, USA; [c]Department of Experimental and Clinical Medicine, University Magna Graecia of Catanzaro, Catanzaro, Italy

ABSTRACT

Introduction: According to estimates by the World Health Organization, about 17.5% of the adult population – roughly 1 in 6 globally – experience infertility. The causes of male infertility remain poorly understood and have yet to be fully evaluated. Follicle-stimulating hormone (FSH) represents an available and useful therapeutic strategy for the treatment of idiopathic infertility.

Areas covered: We provide here an overview of the molecular mechanisms by which FSH stimulates Sertoli cells and the schemes, dosages, and formulations of FSH most prescribed so far and reported in the literature. We also evaluated the possible predictor factors of the response to FSH administration and the indications of the latest guidelines on the use of FSH for the treatment of male infertility.

Expert opinion: FSH therapy should be considered for infertile male patients with oligoasthenoteratozoospermia and normal serum FSH levels to quantitatively and qualitatively improve sperm parameters and pregnancy and birth rates. The grade of evidence is very low to low, due to the limited number of randomized controlled studies and patients available, the heterogeneity of the studies, and the limited effect size. To overcome these limitations, preclinical and clinical research is needed to evaluate the most effective dose and duration of FSH administration.

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1. Introduction

Infertility represents one of the biggest challenges of modern medicine. The Centers for Disease Control and Prevention of the United States emphasizes that infertility has an impact on public health, as it leads to psychological distress, social stigmatization, economic problems, and family discord.

Infertility is defined as a condition in which the couple is unable to conceive after at least 12 months of regular unprotected sexual intercourse [1]. Recent esteems from the World Health Organization indicate that approximately 17.5% of the adult population – roughly 1 in 6 worldwide – suffer from infertility [2]. About 50% of cases are due to female causes, whereas cases of male infertility amount to about 20–30% [3]. Forty percent of infertility cases are determined by a combination of both female and male factors [3]. At least 30 million men worldwide are estimated to be infertile, with the highest prevalence in Africa and Eastern Europe [4].

While commonly cited, these statistics may be biased by the specific population being analyzed and, therefore, are not representative of worldwide prevalence. This reflects discrepancies in data collection across countries and patriarchal societies may hinder accurate and systematic data collection. The paucity of available data prompted Sun and colleagues to undertake a study to estimate evaluate the infertility burden by sex in more than 195 countries, spanning the period from 1990 to 2017. The '2017 Global Burden of Disease (GBD)' study reported that the age-standardized infertility prevalence rate increased by 0.370% per year for females and 0.291% per year for males from 1990 to 2017. In addition, the age-standardized Disability-Adjusted Life-Years of infertility rose by 0.396% per year for females and 0.293% per year for males during the observation period. Male infertility rate increased by 0.291% annually [5].

Interestingly, recent research has suggested a decline in sperm counts globally. According to a meta-regression analysis, the average number of sperm ejaculate declined by 51.6% between 1973 and 2018, with a steeper slope when considering post-2000 data, when the annual percentage doubled, rising from 1.16% post-1972 to 2.64% post-2000. Interestingly, total sperm count data follow the same pattern [6]. While not without controversy [7], this topic has generated much interest and has been a reason for many to intensify research into male infertility. Several causes of male infertility have been identified. Among these we recall the scarce or absent stimulation of the testis by the hypothalamic-pituitary axis due to congenital or acquired [local (intrinsic to the axis) or systemic] diseases, the inability of the testis to produce spermatozoa for primary (congenital or acquired) alterations of spermatogenesis [leading to quantitative (alteration in the number) or quantitative (alteration in the quality) defects of spermatogenesis], the inability of seminal fluid to be ejaculated due to obstruction of the

CONTACT Rossella Cannarella rossella.cannarella@phd.unict.it Department of Clinical and Experimental Medicine, University of Catania, Via S. Sofia 78, Catania 95123, Italy

Article highlights

- FSH therapy should be considered for infertile male patients with oligozoospermia and normal serum FSH levels to quantitatively and qualitatively improve sperm parameters and pregnancy and birth rates.
- The grade of evidence is very low to low, due to the limited number of randomized controlled studies and patients available, the heterogeneity of the studies, and the limited effect size.
- Efforts have been made and are ongoing to find predictors of response to FSH administration. Apart from testicular histology, no other reliable predictors have been acknowledged by international societies' guidelines so far.
- Studies are urgently needed to standardize the weekly dosage, as well as the duration of the treatment, for male infertility.

seminal tract [8]. However, many other causes still remain poorly studied and therefore poorly understood. This is supported by a 9-year single-center prospective study aimed at understanding the causes of male infertility in patients attending a fertility center. In this study, Punab and colleagues highlighted that it was not possible to identify the primary etiology of infertility for 60% of the male patients examined [9]. Therefore, there is a need for further research, particularly for patients with oligozoospermia, which accounts for 83.6% of idiopathic infertility cases [9]. Similarly, according to Tüttelmann and colleagues, among 26,091 men in infertile couples who attended the Center for Reproductive Medicine and Andrology in Münster (Germany), in the last 30 years, up to 72% of cases remained undiagnosed after an appropriate diagnostic procedure [10]. While treatments to restore fertility are readily available for specific causes of male infertility (hypogonadotropic hypogonadism, obstructive azoospermia, etc.), in cases of idiopathic infertility or oligozoospermia of testicular etiopathogenesis, therapeutic approaches are still largely empirical or unsuccessful. Further research is therefore needed to understand the etiology of apparently idiopathic male infertility and thus be able to address it with appropriate therapeutic weapons.

The therapies currently available for the treatment of male infertility include surgical and medical approaches.

As far as surgical options, these include diagnostic procedures, such as multi-site fine needle aspiration (or 'testicular mapping') and open testicular biopsy, or therapeutic techniques, performed to correct existing diseases to improve in sperm production (varicocele repair, hydrocelectomy, reverse vasectomy – vasestomy, vasoepididymostomy), procedures aimed at removing the cause of the obstruction in cases of obstructive azoospermia (i.e. transurethral resection of ejaculatory ducts) and sperm retrieval by percutaneous epididymal sperm aspiration (PESA), epididymal microscopic sperm aspiration (MESA), testicular aspiration (TESA), extraction (TESE), or microTESE [11].

Regarding medical treatment, according to the American Urological Association (AUA)/American Society for Reproductive Medicine (ASRM) guidelines, physicians may benefit from the use human chorionic gonadotropin (hCG), selective estrogen receptor modulators (SERMs), aromatase inhibitors (AIs), or a combination thereof for infertile patients with low serum testosterone (T) levels [12]. Although some data suggest the usefulness of antioxidants and vitamins for the treatment of male infertility (Calogero et al., 2017) [13], patients should be informed of their limited clinical efficacy, as the evidence in not robust enough to provide a recommendation in favor of their use [12]. Finally, treatment with follicle-stimulating hormone (FSH) can be considered for idiopathic infertility [12]. Because of its LH-like effect, hCG is used to promote or restore the increase of intratesticular T concentration and to induce spermatogenesis. It can be prescribed alone or together with FSH in patients with hypogonadotropic hypogonadism. It can also be used in patients with late-onset hypogonadism and a desire for fatherhood [14]. Furthermore, hCG therapy has been shown to improve spermatogenesis in men undergoing T replacement therapy [15]. This occurs because, when injective T is administered, LH secretion is suppressed, which in turn results in a cessation of Leydig cell activity [14].

Drugs such as SERMs and AIs, while acting on different targets, share the same mechanism of action, which consists in blocking the negative feedback exerted by estrogens on the hypothalamic-pituitary-gonadal axis. This blockade causes an increase in the secretion of gonadotropin-releasing hormone (GnRH) and gonadotropins. By blocking the action of aromatase, AIs prevent the conversion of androgens to estrogens. Thus, they reduce the negative feedback of estrogens on the hypothalamic-pituitary-testicular axis, increasing intratesticular T levels and spermatogenesis [16,17]. Overall, the choice of therapy will depend on the underlying cause of infertility and should therefore be determined after a careful clinical evaluation of the patient. SERM administration is associated with a 3-fold improvement in the chance of pregnancy [odd ratio (OR) 3.42, 95% CI: 1.37–8.52] [18], which is similar to the efficacy of the therapy with FSH, as discussed in section 3.3. However, the latter drug is more expensive. Due to its efficacy and low cost, clomiphene is the most used drug in the empirical medical management of idiopathic male infertility, at least in the United States [19].

In addition to hypogonadotropic hypogonadism, FSH can also be used to achieve an increase in sperm concentration in cases of idiopathic infertility or oligozoospermia in patients with normal serum of gonadotropins. Several studies have shown that FSH therapy positively has a positive impact the sperm parameters such as concentration, motility, and morphology, and DNA fragmentation [18]. However, the role of FSH in the treatment of idiopathic infertility is still debated. Despite supporting evidence conducted primarily in Caucasian cohorts, its use is still not recognized in clinical practice worldwide. Therefore, the purpose of this review was to provide an evidence-based state of the art of the use of FSH for male infertility. We will first discuss the physiology and molecular signaling pathways of FSH; then we will cover the indications for treatment in detail.

2. Physiology and molecular signaling

Effects of FSH on Sertoli cells and spermatogenesis - FSH regulates gametogenesis by acting on target cells in the gonads. Its biosynthesis is regulated by the pulsatile release of GnRH, which

stimulates its secretion from gonadotropic cells located of the anterior pituitary gland into the systemic circulation to control the development, maturation, and function of gonads [20]. It is a 35.5 kDa dimeric glycoprotein, which shares the same α-subunit with LH, thyroid-stimulating hormone, and hCG, while the ß-subunit is specific for each hormone, and gives to FSH its specific biological activity, performed by binding to its receptor [21]. Furthermore, FSH promotes the growth and maturation of Sertoli cells and supports spermatogenesis [21].

Sertoli cells proliferate during the fetal and neonatal periods, ceasing proliferation during puberty when they begin their terminal differentiation into the adult form. During embryonic testicular development, fetal Sertoli cells aggregate and encase male precursor germ cells, called gonocytes, to form testicular cords that eventually become seminiferous tubules in the adult testis [22]. In neonatal life and childhood, Sertoli cells are the most represented cell types in the testis, accounting for most of pre-pubertal testicular volume. In this phase, while the levels of LH, FSH, and T are very low, the Sertoli cells secrete a high amount of AMH [23,24]. Therefore, this hormone has been suggested as a useful marker of testicular function in pre-pubertal age [25]. More specifically, the measurement of AMH and inhibin B can be useful for the early diagnosis of puberty disorders and the presence of possible primary testicular damage. Indeed, low levels of these hormones have been found in children with primary testicular disorders. By measuring their levels, it may also be possible to discriminate between conditions such as congenital hypogonadotropic hypogonadism, constitutional delay in growth and puberty, or when precocious puberty is clinically suspected [25,26].

Spermatogenesis involves multiple autocrine, paracrine, and hormonal stimuli, as well as nutrients that support germ cell development through the mechanisms of mitotic development, meiotic recombination, and sperm morphological maturation. Once spermatogenesis is initiated by FSH, it can be qualitatively maintained with T alone [27]. Gametogenesis results from fine-tuning between cell growth and survival and cross-linked steroidogenic signals for apoptosis. The interaction between the different components and the consequent negative feedback exerted by the T and the inhibin B produced by Sertoli cells is essential in the first place for the regulation of the feedback and the secretion of GnRH and gonadotropins for the maintenance of the correct homeostasis of the hypothalamus-pituitary-testicular axis [28].

FSHR expression and signaling - The FSH receptor (FSHR) is a transmembrane protein belonging to the G protein-coupled receptor family that activates the protein kinase A (PKA) pathway [29]. In males, it is typically expressed in Sertoli cells, which are found in the basolateral portion of the seminiferous tubules and regulate spermatogenesis. The gene responsible for encoding the receptor consists of 10 exons. Of these, exons 1–9 encode the intracellular domain, and the long exon 10 encode the transmembrane and intracellular domains, where numerous polymorphisms have been described and studied over the years [30].

Recently, the *FSHR* gene has also been identified in extragonadal sites, including bone cells [31], monocytes [32], hepatocytes [33], and several sites of the female reproductive tract [34]. Furthermore, *FSHR* is expressed by the blood vessel endothelium in most metastatic tumors [35]. These findings suggest that the extragonadal *FSHR* gene expression may have a role in various physiological processes. One of the reasons behind this hypothesis is that there are several alternatively spliced isoforms of FSHR, which may explain why FSH has such pleiotropic effects [36,37]. More recently, FSHR protein expression has been reported in human spermatozoa [38,39], with FSH being present in human seminal plasma at a concentration ranging between 4.4 and 35.4 mIU/mL [40,41]. These observations support the possibility of a direct effect of FSH on spermatozoa. Spermatogenesis encompasses a complex network of interactions between various cell types, including Sertoli, Leydig, peritubular, and germ cells. This process occurs in the epithelium of seminiferous tubules. To achieve successful spermatogenesis, the integrity of the blood-testis-barrier is therefore required [42].

FSH exerts its effects through a wide range of complex signaling pathways. Promotes Sertoli cell proliferation via cyclic adenosine monophosphate (cAMP)/PKA/extracellular regulated kinase (ERK) and phosphatidylinositol 3-kinases/protein kinase B/mammalian target of rapamycin complex 1 (PI3K/Akt/mTORC1) pathways [43] and differentiation and apoptosis via the cAMP/PKA pathway [28]. This evidence suggests that FSH has a dual action, retaining both pro-apoptotic and anti-apoptotic effects [29].

The role of FSH is not yet fully understood. Recent studies have shown that its effect is influenced by insulin-like growth factor 1 (IGF1) and its receptor (IGF1R). These molecules modulate FSH signaling. According to *in vitro* evidence in porcine prepubertal Sertoli cells, IGF1 appears to stimulate Sertoli cell proliferation. Furthermore, it downregulates *anti-Müllerian hormone* (*AMH*) gene expression and protein secretion, while enhancing inhibin B secretion [44]. Moreover, IGF1R has been shown to play a role during embryogenesis in mice, stimulating testicular development from the undifferentiated gonad [45]. There is evidence that FSH and insulin/IGFs share common molecular pathways. Indeed, the insulin/IGFs family, through its receptors, mainly activates the kinase/mitogen-activated protein kinase (MAPK) and the PI3K/AKT pathways. Similarly, although FSH primarily functions through the G protein-coupled receptor superfamily, it also activates the kinase/MAPK and the PI3K/AKT pathways [46]. In the presence of covalent inhibition of IGF1R, FSH loses the ability to phosphorylate downstream proteins [47] (Figure 1). This evidence supports the interconnections between the FSHR and the IGF1R pathways and that IGF1R is required for FSH to exert its molecular signaling. The similarities and connections between the FSH and insulin/IGF1 pathways could explain why insulin resistance affects FSH responsiveness in patients with oligozoospermia [48].

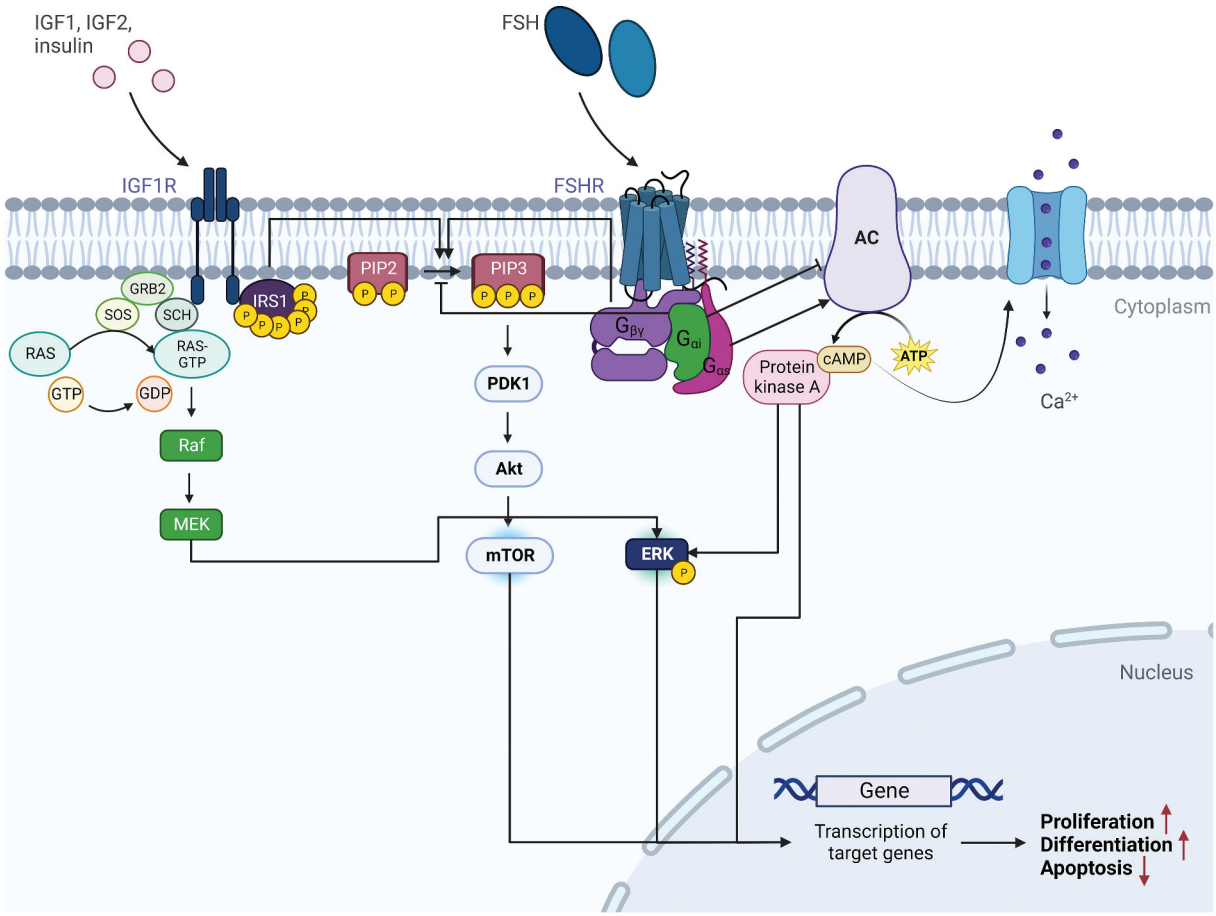

Figure 1. FSH, IGF1, IGF2, and insulin share common molecular pathways and target genes. IGF1, IGF2 and insulin bind to IGF1 receptors in the cell membrane of Sertoli cells. This activates the Raf/mek pathway leading to ERK phosphorylation and transcription on genes target, thereby enhancing proliferation, differentiation, and downregulation of apoptosis. This also occurs when phosphorylation of IRS1 takes place: phosphatidylinositol (4,5)-bisphosphate and phosphatidylinositol (3,4,5)-trisphosphate direct the signaling cascades of mTOR. By binding to its receptor, FSH also activates the ERK pathway. FSH acts through the G protein-coupled receptors superfamily by activating the kinase/MAPK and the PI3K/AKT pathways..

Abbreviations: AC, adenylate cyclase; akt, Serine/threonine protein kinase; ATP, adenosine triphosphate; Ca^{2+}: calcium, ionized; cAMP, cyclic adenosine monophosphate; ERK, extracellular signal-regulated kinases; FSH, follicle-stimulating hormone; FSHR, follicle-stimulating hormone receptor; GDP, Guanosine diphosphate; GRB2, growth factor receptor-bound protein 2; GTP, Guanosine triphosphate; Gαi – Gαs – Gβγ, G-protein subunits; IGF1: insulin-like growth factor 1; IGF1r: insulin-like growth factor receptor; IGF2, insulin-like growth factor 2; IRS1, insulin receptor substrate 1; MEK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PDK1, 3-phosphoinositide-dependent protein kinase 1; PIP2: phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; Raf, rapidly accelerated fibrosarcoma; RAS, rat sarcoma

3. Indications

3.1. Puberty induction

Gonadotropins are used for the induction of puberty, as indicated by the joint clinical practice guidelines of the European Society for Pediatric Endocrinology, the European Society of Endocrinology, and the European Academy of Andrology (EAA) [49]. The 2022 European Association of Urology (EAU) sexual and reproductive health guidelines mention the use of hCG (1000 IU x 2/week to 2000–5000 IU x 2–3/week), until levels of serum total T reach at least half of the upper reference value. FSH could be added later at the dose of 75–150 IU x 3/week until the testicular volume reaches 12–15 ml. Spermatozoa in the ejaculate appear after 3–8 months of treatment [50].

Despite these indications, to date, there is no unanimous agreement on the therapeutic scheme to be used. Some evidence suggests that FSH priming is better than initiating with hCG alone to achieve more complete Sertoli cell proliferation and maturation of and, therefore, improved sperm production in adulthood. In this regard, intratubular T levels, which increase after hCG administration, promote the maturation Sertoli cell and thus cause the loss of their proliferative capacity [51]. In line with this, a prospective randomized study evaluated the effects of FSH priming followed by GnRH administration compared to GnRH therapy alone on testicular histology, serum inhibin B levels, testicular volume, and sperm concentration. Thirteen patients with congenital hypogonadotropic hypogonadism and testicular volume <4 ml who had not received any previous treatment were enrolled in this clinical trial. Patients underwent treatment with recombinant FSH (rFSH) for 4 months, followed by GnRH for 24 months (Group 1) or GnRH alone for 24 months (Group 2). At the end of treatment, patients in Group 1 showed Sertoli cell proliferation, increased number of spermatogonia, and decreased Sertoli cell/germ cell ratio (from 0.74 to 0.35) on

testicular histology. Furthermore, these patients had a significant increase in serum inhibin B levels, testicular volume, and sperm concentration. In contrast, mature Sertoli cells and Leydig cells were found in boys treated with GnRH alone, probably due to increased intratubular T levels [52]. Therefore, FSH priming may represent a more effective strategy for puberty induction. By mimicking physiology, this approach could support Sertoli cell proliferation, allowing the testis to reach its maximum capacity in terms of stimulating germ cell proliferation and differentiation into adulthood.

3.2. Hypogonadotropic hypogonadism

In the case of the post-pubertal onset of hypogonadotropic hypogonadism (testicular volume ≥4 ml), the EAU guidelines on sexual and reproductive health suggest treatment with hCG (250 IU x 2/week to 2000 IU x 2/week). The dose can be adjusted according on the serum T levels that should be maintained at least in half of the normal range. Semen analysis should be requested every 3 months. FSH could be prescribed concurrently or subsequently, at a dose ranging from 75 to 150 IU x 3/week [50].

3.3. Idiopathic infertility

Who is FSH therapy indicated for? - FSH is not recommended for all infertile patients and finding the patients with the most appropriate characteristics is of pivotal importance to ensure a higher probability of response to therapy. In the past thirty years, FSH has been indicated as a possible therapy for the treatment of idiopathic oligozoospermia, therefore it should be prescribed only in those patients in whom, after a proper diagnostic work-up, no apparent can be found. Two main groups can be identified among patients with idiopathic oligozoospermia: those with high serum FSH levels and those with FSH within the normal range. One old study evaluated the effect of treatment in three different groups, the first with serum FSH levels of 5–15 mIU/mL, the second with levels of 16–25 mIU/mL, and the third with levels > 25 mIU/mL [53]. Although sperm concentration, motility and morphology did not change significantly in any of the groups compared to pre-therapy values, the fertilization rate improved significantly after FSH therapy in subgroups with secondary infertility of all three groups [53]. Most subsequent studies have used FSH treatment for patients with FSH serum levels < 8 mIU/mL [54–63], while fewer studies used the cutoff value of 12 mIU/mL [64–66]. This is based on the evidence that high serum FSH levels are indicative of a primary failure of spermatogenesis, especially at the premeiotic stage [67]. Evidence suggests that the presence of maturation arrest of spermatogenesis is associated with poor response to treatment [68–70]. This is why FSH therapy is recommended for patients with normal serum levels of this hormone [71].

Regarding patients with non-obstructive azoospermia (NOA), a retrospective study of 569 patients undergoing mTESE in combination with ICSI found a higher sperm retrieval rate in those treated with gonadotropins (both FSH and hCG), compared with untreated patients [72]. In another study of 50 patients with idiopathic NOA and FSH < 12 mIU/mL who underwent TESE and ICSI, FSH treatment was associated with improved sperm retrieval and pregnancy rates, compared with untreated controls [73]. Despite these data, the evidence is insufficient to suggest treatment with FSH in patients with NOA; further better-sized and designed studies are needed (Table 1).

What formulations and therapeutic regimes should be used? - The first available formulation, the so-called human purified FSH (hpFSH), has been in use since the 1960s and was extracted and purified from the urine of post-menopausal women. Another formulation, however, was subsequently obtained by *in vitro* recombinant technology (rhFSH) [76,77]. This formulation constitutes a safe and valid alternative to human urine preparations, which avoids the possible risks related to infections due to the human origin of urine preparations [78]. In any case [79], the evidence currently present in the literature does not report any differences between the two formulations in terms of efficacy or side effects [18].

Regarding the therapeutic regimen, the available guidelines do not indicate a specific dose of FSH. Although standardized protocols for ovarian stimulation have been developed, the schemes to be used for FSH stimulation of spermatogenesis in the infertile patient are still empirical. Most studies use a dose of 150 IU x 3/week for 3 months. However, a variety of doses has been reported in the literature thus far, and, overall, doses can be roughly divided into low (175–262.5 IU/week), intermediate (350–525 IU/week), and high (700–1050 IU/week)

Table 1. Effects of follicle-stimulating hormone (FSH) for the treatment of male infertility. This table describes the characteristics of the patients to whom FSH should be prescribed, including information on serum FSH levels and sperm parameters. The parameters on which the therapy has demonstrated its efficacy are also reported.

FSH levels	Population	Effects	References
High (FSH ≥12 mIU/mL)	FSH non indicated	FSH non indicated	
Within the normal range (FSH <12 mIU/mL)	Idiopathic oligozoospermia	Increase in sperm concentration	[18,74]
		Increase in total sperm count	[18,74]
		Increase in sperm progressive motility	[18]
		Increase in sperm normal morphology	[74]
		Decrease in sperm DNA fragmentation	[74]
		Increase in spontaneous pregnancy rate	[75]
		Increase in pregnancy post-ART	[75]
	Idiopathic non-obstructive azoospermia (NOA)	Increase in sperm retrieval rate	[72,73]
		Increase in pregnancy rate after TESE	[73]

Abbreviations. ART, assisted reproductive technique; FSH, follicle-stimulating hormone; TESE, testicular sperm extraction.
Notes. While [18,74,75] are meta-analyses [72,73], are single studies whose design is limited by multiple factors. Based on current evidence, FSH treatment is not recommended/suggested for patients with idiopathic NOA.

Table 2. Follicle-stimulating hormone (FSH) for the treatment of male infertility: summary of guideline recommendations. Scientific societies suggest the use of FSH for the treatment of patients with idiopathic oligozoospermia and normal serum gonadotropin levels, to improve conventional sperm parameters, sperm DNA fragmentation, and pregnancy rate, although with a low or very low level of evidence.

	SIAMS 2022 (Ferlin et al., 2022) [71]	AUA/ASRM 2021 (Schlegel et al., 2021) [12]	EAA 2018 (Colpi et al., 2018) [84]	SIAMS 2018 (Barbonetti et al., 2018) [83]
Type of document	Position statement	Guideline	Guideline	Position statement
FSH treatment	Can be suggested	Clinicians may consider it	Can be suggested	Can be suggested
Targeted population	Selected men with oligozoospermia or OAT, FSH < 8 IU/L and no signs of obstruction	Men with idiopathic infertility	Selected men from infertile couples (normogonadotropic men with idiopathic oligozoospermia or OAT)	Normogonadotropic male partners of couples with idiopathic male factor infertility
End-points	Sperm parameters (qualitative/quantitative) Pregnancy rate (spontaneous or after ART)	Sperm concentration Pregnancy rate Live birth rate	Sperm parameters (qualitative/quantitative)Pregnancy ratePregnancy rate Pregnancy rate	Sperm conventional parameters Sperm DNA fragmentation Pregnancy rate (spontaneous or after ART)
Level of evidence	Low (2ØØOO)	Conditional Recommendation; Evidence Level: Grade B	Very low (2ØOOO)	Very low (2ØOOO)
Remarks	The therapeutic scheme should be personalized. RCTs on this topic have not been performed and usually a fixed scheme is used	Measurable but limited fertility benefits Not FDA-approved for this use Questionable cost-to-benefit ratio	The evidence is limited by the unprecise selection criteria used in the available studies	Low number of RCTs (low sample size) Dropout rate correctly reported only in few RCTs Heterogeneous infertile population (unknown female factor)

Abbreviations: ASRM, American Society for Reproductive Medicine; AUA, American Urological Association; EAA, European Academy of Andrology; FDA, Food and Drug Administration; OAT, oligo-astheno-teratozoospermia; RCT, randomized controlled trial; SIAMS, Società Italiana di Andrologia e Medicina della Sessualità.

(Table 2). A meta-analysis from our group demonstrated the existence of a dose-dependent efficacy of FSH treatment, with the effect size achieved by the high dose exceeding that achieved by the low or intermediate dose [18]. More in detail, higher doses of FSH provided better results in terms of sperm concentration and total sperm count and on conventional sperm parameters, while progressive motility benefited from both low and high doses [18]. A dose of 300 IU every other day for ≥4 months was used in the RCT authored by Ding and coworkers, who reported no side effects in patients treated with high doses of FSH [57]. Additionally, patients with idiopathic oligozoospermia might benefit from longer duration protocols (>3 months), which have been associated with little or no side effects. Further studies are therefore needed to standardize the weekly dosage as well as the duration of treatment for male infertility.

On which parameters is the treatment effective? - Most scientific society guidelines suggest FSH for treating idiopathic infertile patients with normal serum FSH levels, to improve conventional sperm parameters, sperm DNA fragmentation, and pregnancy and live birth rates.

The effect of FSH therapy on conventional sperm parameters is debated. Many studies have shown improvements in sperm parameters [80]. Conversely, other authors have not reported clear signs of improvement on these parameters [53,58,81], although some of these studies indicate a normalization of sperm ultrastructural morphology and reduction of sperm DNA fragmentation or aneuploidy, and production of reactive oxygen species. Some evidence also points to the benefit of FSH on biofunctional sperm parameters, which correlate with higher embryo quality and a reduced rate of offspring abnormalities [56,59,82]. According to a previous meta-analysis, the mean difference in improvement in sperm concentration and total sperm count after FSH treatment is + 4.53 mil/ml (95% CI: 2.14–6.92) and + 10.74 mil/ejaculate (95% CI: 4.40–17.07), respectively [18]. Similarly, a more recent study found an improvement in sperm concentration of + 4.52 mil/ml (95% CI: 1.46–7.58), total sperm count of 17.75 mil/ejaculate (95% CI: 11.53–23.98), and a reduction in sperm DNA fragmentation of 12.62% (95% CI: −19.27–5.97) [74] (Table 1).

Furthermore, it would appear that treatment with FSH could improve spontaneous conception and, more generally, the success rate in assisted reproductive technique (ART) programs. Likewise, it would allow the use of less invasive ART techniques (intrauterine insemination rather than more invasive ones, like intracytoplasmic sperm injection and *in vitro* fertilization [54,69]. A meta-analysis published in 2015 found that treatment with FSH increased the chance of pregnancy with an OR of 2.09 (95% CI: 1.49–3.01). In particular, the OR was 4.50 (95% CI: 2.17–9.33) for spontaneous pregnancy and 1.60 (95% CI: 1.08–2.37) for ART [75] (Table 1).

However, the evidence is rated very-low or low, as the number of randomized controlled trials (RCTs) is limited as is the number of patients treated. Furthermore, the selection criteria are heterogeneous. Some RCTs do not provide information on female factors of infertility and not all RCTs have been conducted in patients with idiopathic oligozoospermia. Finally, the effect size is low and, although statistical significance emerged in RCTs and meta-analyses [18], the size of the improvement is not deemed clinically relevant [12,71,83,84] (Table 3).

Table 3. Doses of follicle-stimulating hormone used for the treatment of male infertility as reported in the literature [18]. The weekly dosages used can be classified into low (175 IU – 262.5 IU), intermediate (350 IU – 525 IU), and high (700 IU – 1050 IU). FSH is prescribed for up to 3 months in the vast majority of studies. More frequently it is administered with the scheme of 150 IU three times a week, every other day.

Therapeutic scheme	Weekly dosage (IU)	
hpFSH, 50 IU on alternate days for 3 months	175	Low
rhFSH, 50 IU on alternate days for 3 months		
hpFSH, 75 IU 3 times a week for 3 months	225	
hpFSH, 75 IU on alternate days for 3 months	262.5	
hpFSH, 100 IU on alternate days for 3 months	350	Intermediate
rhFSH, 100 IU on alternate days for 3 months		
hpFSH, 150 IU 3 times a week for 3 to 6 months	450	
rhFSH, 150 IU 3 times a week for 3 to 4 months		
hpFSH, 75 IU daily for 3 months	525	
hpFSH, 150 IU on alternate days for 3 months		
rhFSH, 150 IU on alternate days for 3 months		
hpFSH, 200 IU on alternate days for 3 months	700	High
hpFSH, 150 IU daily for 3 months	1050	
hpFSH, 300 IU on alternate days for 3 months		
rhFSH, 150 IU daily for 3 months		
rhFSH, 300 IU on alternate days for ≥4 months		

Abbreviations: hpFSH, human purified FSH; rhFSH, recombinant human FSH.

Are there factors that predict the response to FSH? - The response to FSH administration differs between studies [85]. Therefore, the identification of factors that can be reliably predict the efficacy of FSH is important in selecting patients for FSH prescription. Single nucleotide polymorphisms (SNPs) of *FSHB* and *FSHR* genes have long been studied as possibly involved in the modulation of the response to FSH. More in detail, *FSHB* −211 G/T affects the transcription of the FSH ß-subunit. The TT SNP reduces gene expression and is associated with lower serum FSH levels than in G carriers. Treatment with FSH showed greater efficacy on sperm count and quality in TT homozygotes than in G allele carriers [86]. Other studies have also analyzed the effect of FSH therapy based on the G/T polymorphism FSHR 2039 [87], which maps to exon 10 and causes a change of amino acid 680 from asparagine to serine in the transmembrane domain of the FSHR. Evidence has demonstrated this polymorphism is able to influence the efficiency of signal transduction. Consequently, *in vitro* data on granulosa cells indicate that the GG genotype (Ser660Ser) is associated with the synthesis of a more FSH-resistant FSHR than the AA genotype (Asp680Asp) [88]. According to Simoni and colleagues, FSH therapy can improve sperm DNA fragmentation, and this effect is influenced by the FSHR c. 2039 G/A genotype, with greater efficacy in GG carriers [89]. Despite this evidence, the role of these SNPs of the FSHß and FSHR genes in predicting the efficacy of FSH treatment is still not fully understood. The guidelines, indeed, indicate considering their evaluation only for experimental purposes [83].

Physiologically, inhibin B exerts negative feedback on GnRH-secreting neurons when serum FSH levels rise. Being involved in the regulation of FSH secretion, this hormone reflects the function of Sertoli cells which are, in turn, stimulated by FSH. Inhibin B is involved in type A spermatogonial proliferation and spermatogenesis. For this reason, it can be considered as an important serum marker of testicular function [90]. Regarding the response to FSH, low inhibin B levels have been shown to reflect the poor function of seminiferous tubules, suggesting a poor response to FSH. On the other hand, inhibin B within the normal range indicates preserved spermatogenesis and Sertoli cells integrity, supporting the hypothesis that FSH administration may be effective [85]. The various guidelines do not consider inhibin B among the factors capable of predicting the response to FSH [83].

Testicular histology can provide useful information to predict the efficacy of FSH therapy. Although its assessment is not recommended in patients with oligozoospermia, research has shown that hypospermatogenesis associated with spermatid maturation arrest results in a lack of response to FSH. According to the authors, patients with hypospermatogenesis will respond to the administration of FSH, while FSH is not able to stimulate spermatogenesis in patients in whom spermatids are not retrieved, as occurs in cases of arrested germ cell maturation [68–70].

Based on what has been said so far, although several lines of evidence have indicated the role of the aforementioned factors in predicting the responsiveness to FSH, there is no agreement on their usefulness. Therefore, further research is needed, possibly identifying and delineating the role of other markers. On this regard, serum levels of 17α-hydroxyprogesterone (17αOH-P) have recently been taken into consideration. The rationale is that being an upstream product of the steroidogenic cascade leading to T synthesis, it can represent a marker of intratubular T levels, important for supporting the differentiation phase of spermatogenesis (from spermatocytes to spermatozoa) [91]. In the presence of *CYP171A* gene polymorphisms that slightly alter the function of its enzyme product, steroidogenesis could slight reduce its efficiency, reducing intratubular T levels and causing a slight increase in serum 17αOH-P levels. In such cases, patients would respond poorly to FSH (Figure 2). In support of this hypothesis, Amory and colleagues found a positive correlation between serum 17αOH-P levels and intratubular T concentrations after the administration of exogenous hCG, confirming 17αOH-P as a marker of intratubular T levels [92]. Lima and colleagues conducted a prospective study in which 31 infertile patients were given clomiphene citrate and/or hCG for three months. They found that the only factor able to predict an improvement of semen quality were serum 17αOH-P levels. Low levels of 17αOH-P have been positively associated with improved semen quality. Conversely, patients with higher levels of 17αOH-P did not show the same upgrade [93]. Mouzannar and colleagues have published similar results [94]. This evidence suggests that serum 17αOH-P levels could be regarded as a marker of FSH response in patients with idiopathic oligozoospermia. However, further studies are needed.

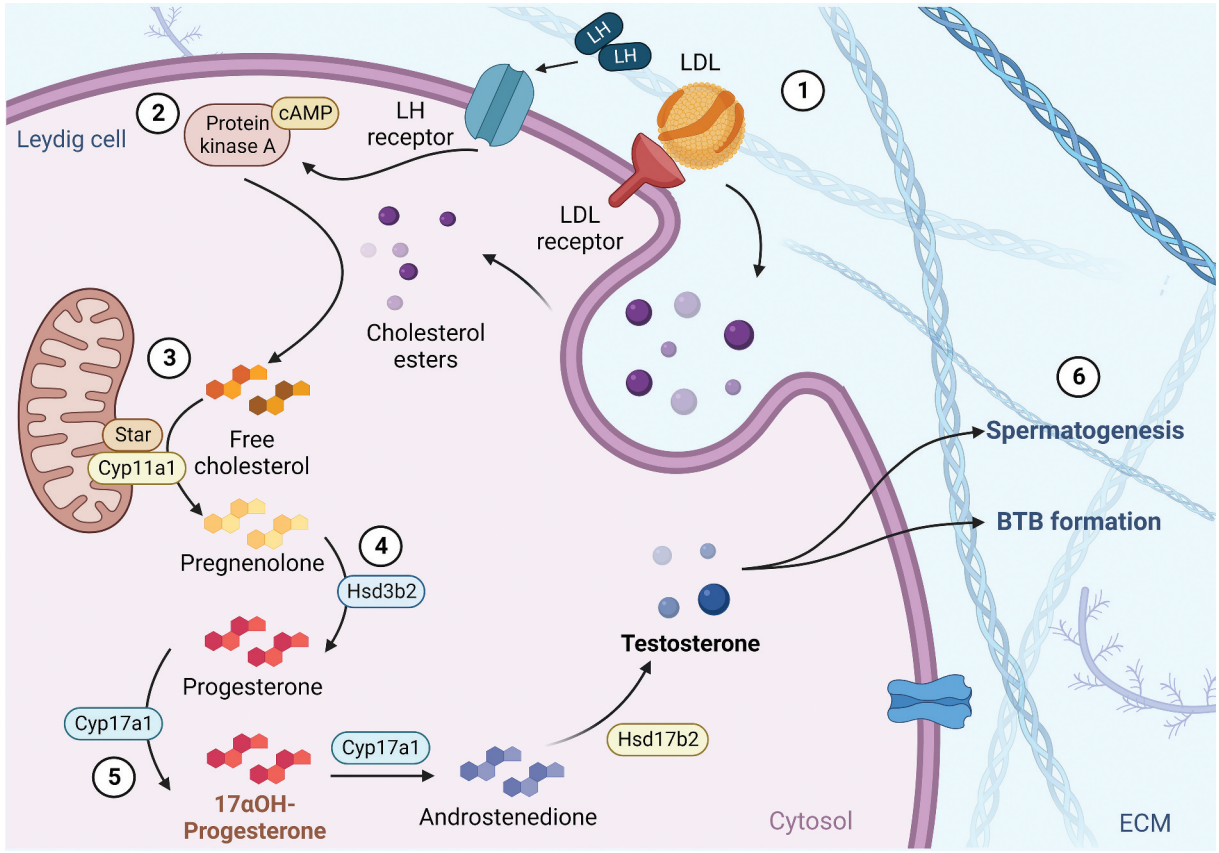

Figure 2. 17 α-hydroxy-progesterone in testicular steroidogenesis. 1) Leydig cells use LDL as a source of cholesterol esters. 2) in a LH-mediated manner and via the protein kinase A, these cells derive free cholesterol from cholesterol esters. 3) star 1 and Cyp11a1 enzymes convert free cholesterol into pregnenolone in the mitochondrial membrane. 4) pregnenolone is converted to progesterone by the Hsd3b2 enzyme. 5) in turn, Cyp17a1 converts progesterone into 17α-hydroxy-progesterone and the latter into androstenedione, which is finally converted into testosterone by the Hsd17b2 enzyme. 6) testosterone plays a relevant role in spermatogenesis by allowing the differentiation from spermatocytes into spermatozoa and in the formation of BTB, through the expression of tight junctions in Sertoli cells. In case of a slight reduced Cyp17a1 activity, testosterone synthesis decreases, consequently 17α-hydroxy-progesterone accumulates and spills over in the blood stream circulation.

Abbreviations. BTB, blood testicular barrier; Cyp11a1, 11-alpha-hydroxylase; Cyp17A1, 17α-hydroxylase; ECM, extracellular matrix; Hsd17b2, hydroxysteroid 17ß-dehydrogenase 2; Hsd3b2, hydroxy-Δ5-steroid dehydrogenase, 3ß and steroid Δ-isomerase 2; LDL, low-density lipoproteins; LH, luteinizing hormone; star, Steroidogenic acute regulatory protein.

Exciting new evidence points to FSHR expression in the post-acrosomal region, neck, midpiece, and tail of human spermatozoa [38,39]. These findings revolutionize the concept of the mechanism by which FSH would affects spermatogenesis. Indeed, FSHR has classically been considered to act only on Sertoli cells. Recent data suggest FSH expression in extragonadal tissues, such as osteoclasts, or adipocytes [31]. According to recent *in vitro* studies, however, this receptor is also expressed in spermatozoa, where it is able to influence sperm motility, AKT phosphorylation, and mitochondrial membrane potential [39]. FSH has been reported in human seminal plasma at a concentration ranging between 4.4 and 35.4 mIU/mL [40,41], therefore the possibility that it may interact with the FSHR at the sperm level should not be excluded. The implications of this finding for the treatment of idiopathic infertile patients (i.e. possible FSH pre-treatment of spermatozoa used for ART) still need to be clarified.

Finally, the relationship between FSH and the previously discussed IGF family signaling pathway represents the basis for hypothesizing a connection between response to FSH therapy and metabolic disorders. This hypothesis is clinically supported by the better response to FSH administration found in patients with insulin resistance and oligozoospermia treated with metformin, an insulin-sensitizing drug, compared to those treated with FSH alone [48].

4. Conclusion

Male infertility represents one of the greatest challenges of modern medicine. Studies have shown that its prevalence is steadily increasing. The reasons behind this are still not entirely clear and often we are faced with a diagnosis of idiopathic infertility. While on the one hand, for some well-defined causes, there are therapeutic protocols that can help restore fertility, this is not fully true in the case of idiopathic infertility. Further research is therefore needed to understand the etiology and treatment of these forms of infertility.

Therapies that can be considered for male infertility treatment include surgical and medical approaches. As far as medical approaches are concerned, more and more importance is being given to FSH-based therapy. It is available in two formulations: the so-called purified human FSH obtained and then purified from the urine of post-menopausal women, and other formulations obtained by *in vitro* recombinant technology. The

most used scheme in the treatment of infertile patients is the administration of FSH at a dose of 150 IU three times a week, even if there is evidence that more prolonged therapies or at different doses may have greater efficacy. Furthermore, several parameters have been called into play to predict the response to FSH administration and, among all, the guidelines cite testicular cytology and the presence of spermatids as predictors of treatment efficacy [83]. Furthermore, exciting new evidence is progressively expanding our understanding of the molecular mechanisms in which FSH is involved, with clinical implications.

In conclusion, FSH-based therapies can represent a valid support in a selected cohort of patients presenting well-defined characteristics, such as idiopathic oligozoospermia, normal serum levels of FSH and inhibin B, presence of hypospermatogenesis but not a testicular cytology picture of germ cell maturation arrest.

5. Expert opinion

FSH therapy has been studied for the treatment of male infertility since the 1990s. Italian researchers have published most of the evidence. The latest AUA/ASRM, EAA and SIAMS guidelines agree in suggesting FSH therapy to patients with idiopathic non-obstructive infertility and/or oligozoospermia and normal gonadotropin levels to improve quantitatively and qualitatively sperm parameters and pregnancy and live birth rates [12,71,84].

However, the evidence is rated as very-low or low, due to the limited number of published RCTs and patients, the heterogeneity of the studies in terms of the etiology of female factor infertility, and the presence of idiopathic oligozoospermia. Importantly, despite the study-proven treatment efficacy, the effect size is considered low and therefore clinically of little relevance by the AUA/ASRM guidelines [12]. This may be due to the small sample size of the RCTs with sperm count as the primary outcome: since this parameter physiologically has a large variation, the power calculation results in a large number of cases, which is not achieved in most of the studies. To overcome the aforementioned limits, a double-blind, placebo-controlled registered clinical trial is ongoing to compare the efficacy and safety of follitropin delta (12 µg/die for 6 months) versus placebo in 800 patients with idiopathic infertility on the pregnancy rate (NCT05403476). It is hoped that this study, expected to be completed in 2025, will clarify whether the effect size and the Number Needed to Treat are large enough to support the use of FSH for the treatment of idiopathic male infertility.

The lack of knowledge regarding which therapeutic schemes are more effective represent another current limit of the research performed so far. *In vitro* studies to analyze the saturation kinetics of FSHR in Sertoli cells are needed to evaluate the possible advantage of a high dose of FSH. If supported by *in vitro* data, *ad hoc* studies in humans should be designed to compare 'low' (175–262.5 IU/week), 'intermediate' (350–525 IU/week), and 'high' (700–1050 IU/week) FSH dosages [18]. A duration of therapy longer than 3 months (the one more commonly prescribed) should be investigated in subsequent studies. Indeed, evidence has indicated that higher doses over a longer duration are more effective [18,57].

Finding predictors of FSH responsiveness represent another key-aspect of the research on this topic. Testicular histology, *FSHR* and *FSH* polymorphisms, inhibin B have been investigated as possible predictors. An aspect little considered by the research concerns the possibility that serum 17αOH-P could also be a predictor of response to therapy. In fact, its levels could provide information on intratubular T concentrations which, in turn, is known to influence spermatogenesis. In relation to the identification of factors useful for understanding who should be prescribed FSH therapy, most studies consider FSH value within the range (<12 mIU/mL or, more often, <8 mIU/mL) as determining factor in the choice to prescribe the therapy. Although there is a rationale behind this, it must be taken into account that there are no studies demonstrating the lack of efficacy for serum FSH values above the range. Patients having *FSHR* polymorphisms associated with a raise in FSH levels may benefit from FSH stimulation, as well as monorchidic patients, where the raise in FSH does not imply a maturation arrest of spermatogenesis. The only study, very old, which analyzes the data according to the serum FSH levels (identifying three groups: 5–15 mIU/mL, 16–25 mIU/mL, >25 mIU/mL), demonstrates the efficacy of the therapy even in the groups with high FSH values [53]. Some authors have even administered gonadotropins after gonadotropin-releasing-hormone agonist in patients with high FSH levels, finding an improvement in sperm parameters [95]. These therapeutic approaches have been poorly explored so far. Understanding whether there is a margin of use of gonadotropins in patients with high serum FSH levels should be investigated.

Finally, SERMs and AIs are notoriously cheaper than FSH and therefore more used in the empirical therapy of idiopathic infertility on a large scale. Studies are needed that compare the benefits of FSH therapy (in terms of conventional sperm parameters, sperm DNA fragmentation and pregnancy rate) with those obtained with SERMs/AIs in a population with the same characteristics, to understand where it is best to direct research in the coming years.

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ORCID

Rossella Cannarella http://orcid.org/0000-0003-4599-8487
Rosita A. Condorelli http://orcid.org/0000-0002-5217-9343
Antonio Aversa http://orcid.org/0000-0002-2989-2618
Aldo E. Calogero http://orcid.org/0000-0001-6950-335X
Sandro La Vignera http://orcid.org/0000-0002-7113-2372

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