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ARTICLE

APHRODITE criteria: addressing male patients with hypogonadism and/or infertility owing to altered idiopathic testicular function

BIOGRAPHY

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KEY MESSAGE

The proposed APHRODITE criteria offer a standardized approach to classify patients with male infertility, to improve communication and clinical management among andrologists, urologists and ART experts. Furthermore, they may highlight areas lacking data and promote clinical research to discover new pharmacological treatment options for male infertility.

ABSTRACT

Research question: Can a novel classification system of the infertile male – 'APHRODITE' (Addressing male Patients with Hypogonadism and/or infeRtility Owing to altereD, Idiopathic TEsticular function) – stratify different subgroups of male infertility to help scientists to design clinical trials on the hormonal treatment of male infertility, and clinicians to counsel and treat the endocrinological imbalances in men and, ultimately, increase the chances of natural and assisted conception?

Design: A collaboration between andrologists, reproductive urologists and gynaecologists, with specialization in reproductive medicine and expertise in male infertility, led to the development of the APHRODITE criteria through an iterative consensus process based on clinical patient descriptions and the results of routine laboratory tests, including semen analysis and hormonal testing.

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KEY WORDS

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Results: Five patient groups were delineated according to the APHRODITE criteria; (1) Hypogonadotrophic hypogonadism (acquired and congenital); (2) Idiopathic male infertility with lowered semen analysis parameters, normal serum FSH and normal serum total testosterone concentrations; (3) A hypogonadal state with lowered semen analysis parameters, normal FSH and reduced total testosterone concentrations; (4) Lowered semen analysis parameters, elevated FSH concentrations and reduced or normal total testosterone concentrations; and (5) Unexplained male infertility in the context of unexplained couple infertility.

Conclusion: The APHRODITE criteria offer a novel and standardized patient stratification system for male infertility independent of aetiology and/or altered spermatogenesis, facilitating communication among clinicians, researchers and patients to improve reproductive outcomes following hormonal therapy. APHRODITE is proposed as a basis for future trials of the hormonal treatment of male infertility.

APHRODITE (Greek Mythology): a goddess of fertility, sexuality and procreation who is the female counterpart of POSEIDON, the male god for women's fertility. One of her origin stories describes her being born from the castrated genitals of the Sky god, Ouranos. She is often depicted as the creator of glorious offspring.

INTRODUCTION

Infertility, defined as the inability to conceive after 1 year of unprotected intercourse (*Boivin et al. 2007*), is estimated to affect 187 million couples worldwide (around 1 in 6 couples of reproductive age) (*Agarwal et al., 2020b*; *WHO, 2023b*). Male factors account for approximately 50% of infertility cases and represent an important contributory factor in about 20% (*Agarwal et al., 2020b*). Although multiple factors impairing male fertility are recognized (*Mazzilli et al., 2023*), in many cases the cause is unknown (idiopathic male infertility); furthermore, many men facing infertility do not seek medical assistance, even in developed countries, leading to an underestimation of its global impact (*Agarwal et al., 2015*).

Male infertility is a complex clinical condition, with a wide range of non-mutually exclusive causes and contributing factors that are often not thoroughly investigated, managed or treated with optimal effectiveness (*Esteves and Humaidan, 2023*). Even when the male partner is evaluated, clinicians and patients often feel frustrated due to the lack of progress in the diagnosis and management of male patients with reduced spermatogenesis. In this regard, the diagnostic workup needs to consider several variables, from congenital and genetic factors to anatomical disorders, hormonal disturbances, ejaculatory dysfunction and inadequate lifestyle habits.

In addition, a diverse range of healthcare specialists are involved in the management of male factor infertility, including andrologists, endocrinologists, urologists and gynaecologists specialized in reproductive medicine/assisted reproductive technology (ART) (*Zegers-Hochschild et al., 2017*). This heterogeneity results in the lack of a cohesive treatment strategy and a high proportion of men going untreated or receiving empirical treatment.

Fertility societies have now begun to recognize the societal challenge of male infertility. The European Society of Human Reproduction and Embryology has supported the Male Reproductive Health Initiative, a global collaboration dedicated to advance the science and practice of male reproductive medicine (*ESHRE, 2023*). The European Academy of Andrology also provides a website for laypeople, to improve awareness on infertility and other andrological problems (*European Academy of Andrology, 2023*). Furthermore, the World Health Organization (WHO) is engaging with countries to address infertility within an enabling legal and policy environment, to support the generation of data on the burden of infertility (*WHO 2023a*). Other societies, such as the American Urological Association/American Society for Reproductive Medicine (*Schlegel et al., 2021*) and the European Association of Urology (*Minhas et al., 2021*), have also developed guidelines for male infertility diagnosis and treatment.

Clinically, semen quantity and quality are typically used as measures of male fecundity through the conventional analysis of sperm parameters (*Esteves, 2022a*), including sperm count, morphology, motility and volume, as defined in the sixth edition (2021) of the WHO laboratory manual for the examination and processing of human semen (*WHO, 2021*). To establish the diagnosis and plan treatment strategies, further investigations in addition to semen analysis should be undertaken to identify the underlying cause of infertility based on the factors that can impact male fertility (*Esteves, 2022a*; *Esteves and Humaidan, 2023*) in line with published guidelines and algorithms (*Minhas et al., 2021*; *Schlegel et al., 2021*) However, the assessment of male infertility frequently falls short, and is typically limited to basic semen analysis (*Pozzi et al., 2021*). This approach overlooks critical dimensions encompassing factors such as paternal age, endocrine function, quality of sperm DNA, lifestyle influences and environmental determinants (*Esteves, 2022a*; *Murugesu et al., 2022*).

While assessing the infertile male is clinically relevant, the diagnostic and therapeutic strategies available remain controversial, and there is a prevailing notion that intracytoplasmic sperm injection (ICSI) may provide the couple with a baby without the need to explain the nature or cause of the underlying male infertility (*Esteves, 2022c*). This is despite an increasing number of studies suggesting that therapeutic interventions can improve sperm quantity/quality and overall male health, ultimately resulting in better reproductive outcomes, even when ICSI is the only option (*Bian et al., 2022*; *Esteves, 2022c*; *Esteves et al., 2016*; *Esteves et al., 2020*, *Faure et al., 2014*; *Humaidan et al., 2022*; *Kirby et al., 2016*; *Lira Neto et al., 2021*; *Omar et al., 2019*; *Persad et al., 2021*; *Ricci et al., 2018*; *Salas-Huetos et al., 2017*; *Samplaski et al., 2017*; *Santi et al., 2018*; *Vanegas et al., 2017*). Of particular concern is the lack of efficient diagnosis for male patients with idiopathic infertility who have subtle endocrinological imbalances, despite the well-established knowledge that normal spermatogenesis requires the action of both FSH and LH on the testes (The Endocrine *Society, 2023*; *Oduwole et al., 2021*).

The potential therapeutic role of hormonal therapy in male infertility is widely demonstrated in the case of hypogonadotropic hypogonadism (*Santi and Corona, 2017*) whereby the exogenous hormonal replacement of gonadotrophin-driven physiological stimulation of the pituitary gland on the gonads is known to be efficient at restoring spermatogenesis (*Kliesch et al., 1995*; *Rastrelli et al., 2014*). Hormonal therapy has been suggested as a potential treatment to enhance fertility in men with idiopathic oligozoospermia or non-obstructive azoospermia (NOA), aiming to replicate the therapeutic approach used for hypogonadotrophic hypogonadism (*Santi et al., 2015*, *Laursen et al., 2022*).

However, the evidence regarding the effectiveness of hormonal therapy for male patients with idiopathic oligozoospermia is mixed, and there are few randomized controlled trials, as reviewed by Esteves and colleagues (*Esteves et al., 2023*). Additionally, the therapeutic challenges lie in the limited knowledge about the aetiology of idiopathic infertility, a condition that affects a substantial portion of men undergoing evaluation (*Punab et al., 2017*; *Ventimiglia et al., 2021*). Compounding these challenges are the potential adverse effects and insufficiently researched reproductive outcomes associated with empirical pharmacological interventions, as discussed by Esteves and colleagues (*Esteves et al., 2023*). These factors collectively impede the comprehensive utilization of treatments that do not primarily target eliminating the underlying cause, while in women undergoing ovarian stimulation for ART gonadotrophins are applied independently of the cause of infertility with the sole aim of obtaining a surplus of gametes.

In summary, there is a huge unmet need in the treatment of male infertility, which is usually empirically addressed by clinicians. To address these challenges, and inspired by the POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) concept, which was developed to stratify infertile women undergoing ART (*Humaidan et al., 2016*), an international working group proposes a new approach to stratify men seeking paternity and who may be candidates for hormonal therapy.

The novel classification system of infertile male patients, namely, 'APHRODITE' – Addressing male Patients with Hypogonadism and/or infeRtility Owing to altereD, Idiopathic TEsticular function – stratifies eligible patients into five different subgroups. For each subgroup, specific suggestions are given for medical handling using hormonal therapy to improve sperm quantity and/or quality. The goal of the stratification system is to help scientists to design standardized clinical studies and clinicians to counsel and treat infertile men and, ultimately, increase the chances of notonly natural and also assisted conception. Finally, the APHRODITE criteria aim to improve communication between the key players in research and clinical care by proposing a standardized approach to patient stratification for future clinical trials.

DEVELOPMENT OF THE CRITERIA

A group of experts comprising andrologists, reproductive urologists and gynaecologists with sub-specialization in reproductive medicine and expertise in male infertility collaborated to develop the APHRODITE criteria. The group's steering committee (authors S.C.E., P.H., C.A., F. M.U., D.S. and M.S.) worked with clinical experts in the field (authors C.L.R.B., A.A. P., L.A., N.J. and H.M.B.) to create the criteria. The focus of the criteria is primarily infertile male patients who desire to have children and might benefit from hormonal therapy to improve semen quality/quantity for natural conception or as part of their ART treatment. These novel criteria were developed based on those variables collected during routine workup. In particular, these criteria were created considering conventional semen analysis parameters, serum gonadotrophin (FSH) concentrations and serum testosterone concentrations.

The APHRODITE stratification system was developed through an iterative consensus process. The critical drivers for its elaboration were the following questions. (i) How can male infertility patients, mainly those with unknown causes to explain their condition, be classified to guide therapeutical management? (ii) What is the importance of hormonal treatment for male infertility? (iii) What are the current options and outcomes/limitations of hormonal therapy?

First, the most commonly used terms to describe male infertility phenotypes based on semen and hormone parameters were collected, primarily from the International Glossary on Infertility and Fertility Care 2017 (*Zegers-Hochschild et al., 2017*). These terms were discussed among the experts, and a consensus was reached on the most reasonable terms for the purpose (**TABLE 1**). Second, the new stratification system was created based on clinical phenotypes and built on semen and reproductive hormone parameters. Several group discussions were conducted to reach the final version approved by all the authors.

The ultimate goals of the APHRODITE criteria are to record patients' phenotypes more precisely, to facilitate communication between andrologists, urologists and ART experts, to provide suggestions for unified clinical management (including tailored hormonal treatment and regimens based on patient characteristics) and to improve clinical and reproductive outcomes. Lastly, the APHRODITE criteria aim to identify areas where data are lacking in published guidelines, (*Minhas et al., 2021*; *Schlegel et al., 2021*) to encourage clinical research in this area and promote the discovery of novel pharmacological treatment options.

CLINICAL PARAMETERS

The terms relevant for the APHRODITE criteria are defined in **TABLE 1**.

Clinical history and physical examination

The clinical history of male patients who seek medical assistance due to infertility can provide answers or clues about possible underlying causes (*Minhas et al., 2021*; *Schlegel et al., 2021*). It should include enquiring about concomitant diseases/malformations, cryptorchidism, the onset of puberty, habitual sex life, lifestyle (e.g. smoking) and medication use. In addition, a physical examination, both general and andrological, should be an essential component of the assessment process. In particular, the general physical examination must be focused on body composition and obesity, a recognized risk factor for hypogonadism (*Pallotti et al., 2022*). The andrological physical examination should pay attention to the general signs of hypoandrogenization, virilization and abnormalities in scrotal content (*Minhas et al., 2021*; *Pallotti et al., 2022*; *Schlegel et al., 2021*).

TABLE 1 TERMS RELEVANT TO THE APHRODITE CRITERIA

Term	Definition	Reference(s)
Male infertility	Infertility caused primarily by male factors encompassing abnormal semen parameters and/or abnormal sperm function; anatomical, endocrine, genetic, functional or immunological abnormalities of the reproductive system; chronic illness; and sexual conditions incompatible with the ability to deposit semen in the vagina. Inadequate lifestyle, exposure to toxicants and advanced paternal age are critical factors acting alone or exacerbating the impact of known causative factors	*Zegers-Hochschild et al. (2017)*, *Minhas et al. (2021)*, *Schlegel et al. (2021)*, *Esteves and Humaidan (2023)*
Hypogonadotrophic hypogonadism	Gonadal failure associated with reduced gametogenesis and reduced gonadal steroid production due to reduced gonadotrophin production or action	*Zegers-Hochschild et al. (2017)*
Reduced FSH concentrations	There is no consensus on clinically relevant reference values for FSH serum concentrations. In the context of the APHRODITE criteria, FSH concentrations are grouped as reduced, normal or elevated, based on the clinician's assessment of the respective laboratory values	N/A
Biochemical hypogonadism	Serum total testosterone concentrations below the lower reference limit	*Bhasin et al. (2011)*, *Giagulli et al. (2021)*
Idiopathic infertility	Semen analysis reveals pathological findings without any demonstrable cause of infertility. These men present with no previous history of diseases affecting fertility and have normal findings on physical examination and endocrine, genetic and biochemical laboratory testing, suggestive of a functioning hypothalamus–pituitary–gonadal axis	*Agarwal et al. (2021)*, *Minhas et al. (2021)*
Unexplained infertility	Infertility in couples with apparently normal ovarian function, Fallopian tubes, uterus, cervix and pelvis and with adequate coital frequency; and apparently normal testicular function, genitourinary anatomy and a semen analysis with parameters within reference ranges. The potential for this diagnosis is dependent upon the methods used and/or methods available	*Zegers-Hochschild et al. (2017)*
Lowered semen analysis parameters	Reduced percentages of motile and/or morphologically normal spermatozoa, or a low concentration of spermatozoa in the ejaculate (e.g. below the 5th percentile of the data from the WHO semen analysis manual reference limits). Azoospermia: absence of spermatozoa in the ejaculate after examination of the centrifuged pellet	*Zegers-Hochschild et al. (2017)*, *WHO (2021)*, *Andrade et al. (2021)*
Sperm DNA fragmentation	Sperm DNA fragmentation refers to a change in the bases or a physical break in one or both of the DNA strands of the sperm chromatin. Types of DNA damage include mismatch of bases, loss of base (abasic site), base modifications, DNA adducts and cross-links, pyrimidine dimers and single-strand and double-strand breaks. Any of these alterations can induce sperm DNA fragmentation and may compromise natural conception or ART outcomes	*Agarwal et al. (2020a)*, *Esteves et al. (2021)*
Quality of life	Refers to a multidimensional concept that encompasses an individual's overall well-being and satisfaction with various aspects of their life. It includes physical health, mental and emotional well-being, social relationships, level of independence, access to resources and opportunities, and overall life satisfaction. The assessment of quality of life is often based on self-reported measures and can vary across different populations and cultural contexts. This parameter is rarely included in clinical studies, despite being considered highly relevant as an indirect end-point	*Mousavi et al. (2013)*
Azoospermia	Obstructive azoospermia: absence of spermatozoa in the ejaculate due to occlusion of the ductal system, which can often be treated through reconstructive surgery or using sperm retrieval and ART	*Zegers-Hochschild et al. (2017)*, *Esteves (2015)*
	NOA: absence of spermatozoa in the ejaculate due to severely reduced or absent production of mature spermatozoa. The aetiology includes genetic and congenital abnormalities, post-infectious testicular damage, exposure to gonadotoxins (e.g. radiotherapy/chemotherapy) and testicular trauma; yet the aetiology cannot be determined in all cases (idiopathic NOA). NOA is unrelated to any degree of male reproductive tract obstruction and cannot be treated through reconstructive surgery; however, male patients with NOA may be candidates for sperm retrieval and ART	

ART, assisted reproductive technology; ICMART, International Committee for Monitoring Assisted Reproductive Technologies; N/A, not applicable; NOA, non-obstructive azoospermia; WHO, World Health Organization.

Semen analysis

Basic semen analysis is commonly used as the initial classifier of male infertility phenotypes. Based on semen parameters (e.g. sperm concentration, sperm motility, sperm morphology), patients can be classified into two major categories: *lowered* (values outside the reference limit) or *normal*, according to the 5th percentile of the data from the *WHO 2021* reference ranges (*WHO 2021*). The *lowered* category could be further refined as azoospermia

(absence of spermatozoa in the ejaculate), oligozoospermia (reduced sperm concentration), asthenozoospermia (reduced percentages of motile spermatozoa), teratozoospermia (reduced percentages of morphologically normal spermatozoa) or a combination of these, although these terms are not supported in the *WHO 2021* standards. Despite its limitations (i.e. the lack of accuracy), standard semen analysis remains the most used approach to identify common phenotypes associated with decreased male fertility, guide clinical management and monitor treatment response (*AUA/ASRM, 2020*; *Esteves, 2022a*; *Minhas et al., 2021*; *WHO, 2021*).

Hormone concentrations

Spermatogenesis occurs within the testicular seminiferous tubules in a stepwise fashion, requiring autocrine, paracrine and endocrine stimuli that are controlled primarily by the actions of both FSH and LH (*Jan et al., 2012*, *Oduwole et al., 2018a*; *Oduwole et al., 2018b*). The action of FSH is mediated through its receptor (FSHR) (*Kangasniemi et al., 1990*), expressed only on the Sertoli cells located at the base of the seminiferous tubules of the testes, which creates an environment in which the spermatogonia can proliferate and mature (*Mruk and Cheng, 2004*; *Mruk and Cheng, 2015*; *Sharpe, 2012*). Conversely, LH acts on Leydig cells, stimulating testosterone production through the interaction with its specific receptor, the LH/choriogonadotrophin receptor (*Roth et al., 2010*).

Human spermatogenesis is physiologically regulated by the combined and synergic action of FSH- and LH-dependent intratesticular testosterone (ITT), which, in turn, acts on Sertoli cells through the androgen nuclear receptor. These mechanisms are exerted through the activation of similar, partially overlapping, mechanisms, since decreased concentrations of either gonadotrophin do not necessarily lead to azoospermia in humans (*Casarini et al., 2015*) and both are needed to obtain qualitatively and quantitatively adequate spermatogenesis (*Dabaja and Schlegel, 2014*). In particular, FSH acts mainly in the first step of spermatogenesis (from spermatogonia to spermatocytes), whereas ITT acts on the last steps of sperm maturation (*Santi et al., 2020*). The concentration of ITT is up to 100 times higher than serum total testosterone concentrations, and ITT concentrations similar to circulating total testosterone concentrations are not sufficient to maintain spermatogenesis (*Coviello et al., 2004*). However, ITT is not routinely measured and the hormonal workup of infertility relies on the assessment of FSH and total testosterone serum concentrations.

FSH

Apart from hypogonadotrophic hypogonadism, there is generally an inverse relationship between FSH concentrations and spermatogonial quantity (*Ishikawa et al., 2004*; *Martin-du-Pan and Bischof, 1995*). In the presence of normal testosterone concentrations, when spermatogonia are absent or their number is remarkably reduced, FSH concentrations typically increase; when spermatogonial number is normal, FSH concentrations tend to be within normal ranges. Therefore, FSH can be considered a marker of the spermatogonial situation. Additionally, reduced FSH concentrations, combined with reduced LH and testosterone concentrations and decreased testicular volume, indicate hypogonadotrophic hypogonadism. FSH serum concentrations are only partially able to discriminate between normal and lowered sperm production (*Bergmann et al., 1994*). While elevated FSH concentrations, typically above 9–12 IU/l by most assays, are associated with spermatogenic defects, normal FSH concentrations do not exclude such defects (*Hung et al., 2007*).

When considering the parameters to include in the APHRODITE criteria, a pragmatic approach was adopted when it came to defining cut-off values for FSH, in which the ranges defined by each assessing laboratory were referred to. An observational study conducted in Australia, including 124 healthy men (age 21–35 years) exhibiting semen parameters within the reference ranges, evaluated the consistency of reproductive hormone concentrations, including FSH, as determined by different automated immunoassays (*Sikaris et al., 2005*). In this study, all the participating clinical laboratories had an elite performance in the national immunoassay quality assurance programme. The authors found that the variation among laboratories for FSH and LH results was negligible. The smaller quantitative discrepancies allowed the assignment of consensus reference intervals for serum FSH (1.3–8.4 IU/l) and LH (1.6–8.0 IU/l), although these differed from the manufacturers' currently quoted expected values. The characteristics and reference ranges of commonly used chemoluminescent immunoassays for measuring serum FSH concentrations are provided in **TABLE 2**.

Testosterone

Serum total testosterone concentration, measured in a morning fasting blood sample (*Corona et al., 2023*), is often used as a proxy for ITT because obtaining testicular tissue for ITT determination is invasive (*Jarow et al., 2001*). Human chorionic gonadotrophin (HCG) stimulates Leydig cells to produce ITT, and adequate ITT concentrations support optimal spermatogenesis (*O'Donnell and McLachlan, 2012*). In eugonadal men, the concentrations of both hormones are highly correlated (*Roth et al., 2010*). Therefore, circulating testosterone concentrations rather than ITT (for the

TABLE 2 CHARACTERISTICS AND REFERENCE RANGES OF COMMONLY USED CHEMILUMINESCENT IMMUNOASSAYS FOR MEASURING SERUM FSH CONCENTRATIONS

Platform	Reference values (IU/l)
Cobas e (Roche Diagnostics, Mannheim, Germany)[a]	1.5–10.0
Architect i2000 (Abbott Laboratories, Chicago, USA)[b]	0.95–11.95
ADVIA ACS-180 (Bayer Diagnostics, Tarrytown, USA)[c]	1.4–18.1
ACCESS (Beckman Coulter, Fullerton, USA)[d]	1.27–19.26
DPC Immulite 2000 (Diagnostic Products Corp., Los Angeles, USA)[e]	0.7–11.1

[a] Available from Roche Product Care Immunology, http://labogids.sintmaria.be/sites/default/files/files/.
[b] Available from https://www.ilexmedical.com/files/PDF/FSH_ARC.pdf.
[c] Available from https://content.doclib.siemens-healthineers.com/rest/v1/view?document-id=981948.
[d] Available from https://www.brighamandwomens.org/assets/BWH/research/brigham-research-assay-core/pdfs/BC004_FSH_(Access).pdf.
[e] Available from http://www.dpcweb.com/documents/medical/reference_ranges/ZB197-A.pdf.

TABLE 3 LABORATORY TESTS AND RESULTS INTERPRETATION IN THE CONTEXT OF THE APHRODITE CRITERIA

Laboratory test	Interpretation of results
Semen analysis parameters	
Normal	Percentages of motile and morphologically normal spermatozoa, and a concentration of spermatozoa in the ejaculate that is equal or above the 5th percentile of the data from the 2021 WHO semen analysis manual reference limits (*WHO, 2021*)
Lowered	Reduced percentages of motile and morphologically normal spermatozoa, and a concentration of spermatozoa in the ejaculate that is lower than the 5th percentile of the data from the 2021 WHO semen analysis manual reference limits (*WHO, 2021*)
Azoospermia	Absence of spermatozoa in the ejaculate[a]
FSH concentration	
Normal	FSH within the normal range of the assessing laboratory
Reduced	FSH below the lower limit of normal range of the assessing laboratory
Elevated	FSH above the upper limit of normal range of the assessing laboratory
Testosterone concentration	
Normal	Testosterone within the normal range of the assessing laboratory
Reduced	Testosterone below the lower limit of normal range of the assessing laboratory

Additional hormone parameters that could also be measured, on a case-by-case basis, include LH, prolactin, inhibin B, thyroid-stimulating hormone, thyroxin and oestradiol.
[a] After examination of the centrifuged pellet.
WHO, World Health Organization.

reasons already explained) can be used to determine the effect of HCG therapy as an increase in ITT after HCG therapy results in an elevation of circulation testosterone concentrations.

However, it is important to note that ITT concentrations, although difficult to measure, are typically much higher (up to 100 times) than circulating total testosterone concentrations. Therefore, direct comparisons between ITT and circulating total testosterone concentrations may not be accurate. In addition, some studies have suggested the use of serum 17-hydroxyprogesterone as a potential biomarker for ITT, which merits further investigation (*Amory et al., 2008*, *Lima et al., 2020*, *Mouzannar et al., 2019*).

The laboratory tests and interpretation of results included as the basis of the APHRODITE criteria are summarized in TABLE 3.

TABLE 4 displays the testosterone threshold concentrations used by different scientific societies to diagnose biochemical hypogonadism. The most commonly used threshold for testosterone concentrations is 350 ng/dl (12 mol/l), which is based on a study of 456 healthy, non-obese men (the Framingham Heart Study Generation 3) (*Bhasin et al., 2011*). In this study, the median (quartile) testosterone concentration was 698.7 ng/dl (296.5 ng/dl), and testosterone concentrations below the 2.5th percentile (348.3 ng/dl) of the reference sample were considered low.

Current clinical laboratory assays for testosterone include immunoassay and mass spectrometry platforms; however, owing to the reported limited comparability among the different assays (*Herati et al., 2016*), a threshold below the lower limit of the normal range is recommended for the assessing laboratory to classify biochemical hypogonadism in the APHRODITE criteria, with the indispensable requirement that the normal range is calculated using sufficient samples from healthy men (*Travison et al., 2017*).

TABLE 4 CUT-OFF FOR INTERPRETATION OF TOTAL TESTOSTERONE AND FREE TESTOSTERONE SERUM CONCENTRATIONS AS RECOMMENDED BY DIFFERENT PROFESSIONAL MEDICAL SOCIETIES

Society	Total testosterone		Free testosterone	
	nmol/l	dg/dl	pmol/l	pg/ml
American Urological Association (*Mulhall et al., 2018*)	10.4	300	–	–
British Society for Sexual Medicine (*Hackett et al., 2017*)	12	346	225	65
Canadian Medical Association (*Morales et al., 2015*)	Local laboratory ranges	–	–	–
Endocrine Society (*Bhasin et al., 2018*)	9.2	264	–	–
Endocrine Society of Australia (*Yeap et al., 2016*)	7.4 (young); 6.6 (men older than 60 years)	216/190	–	–
European Academy of Andrology (*Corona et al., 2020*)	12	350	–	–
European Association of Urology (*Salonia et al., 2021*)	12	350	226	65
International Consultation for Sexual Medicine (*Morgentaler et al., 2019*)	12.1	350	225–347	65–100
International Society for the Study of the Aging Male (*Lunenfeld et al., 2015*)	12	350	225–243	65–70

APHRODITE CRITERIA

The patient groups according to the APHRODITE criteria are depicted in FIGURE 1.

APHRODITE Group 1: hypogonadotrophic hypogonadism (acquired and congenital)

Hypogonadotrophic hypogonadism is a medical condition that results from congenital or acquired disorders affecting the function of the hypothalamic–pituitary–gonadal axis. The congenital forms of hypogonadotrophic hypogonadism include anosmic hypogonadotrophic hypogonadism (Kallmann syndrome) and normosmic isolated hypogonadotrophic hypogonadism (idiopathic hypogonadotrophic hypogonadism) (*Santi and Corona, 2017*). Acquired hypogonadotrophic hypogonadism can result from various causes, including the use of certain drugs (e.g. anabolic steroids, opioids and testosterone replacement therapy), infectious or infiltrative pituitary lesions, pituitary tumours, hyperprolactinaemia, encephalic trauma, pituitary/brain radiation, excessive exercise, substance abuse involving alcohol or illicit drugs, and systemic diseases such as haemochromatosis, sarcoidosis and histiocytosis X (*Santi and Corona, 2017*).

Hypogonadotrophic hypogonadism is rare in the general population of patients seeking medical advice for infertility, with estimates indicating that 1.9% of azoospermic male patients (*Chiba et al., 2016*) and 1.6% of male infertility cases (*Jungwirth et al., 2012*) have hypogonadotrophic hypogonadism. APHRODITE Group 1 is characterized by gonadal failure associated with reduced gametogenesis and reduced gonadal steroid production due to reduced gonadotrophin production or action, which is the current, accepted definition of hypogonadotrophic hypogonadism (*Zegers-Hochschild et al., 2017*). Specifically, laboratory parameters include reduced FSH and LH serum concentrations (based on the clinician's assessment according to their respective laboratory values), reduced testosterone concentrations (below the lower limit of the normal range of the assessing laboratory) and lowered semen analysis parameters (e.g. semen analysis parameters below the WHO reference limits) (TABLE 3).

For men with hypogonadotrophic hypogonadism who wish to father a child, therapeutic options include gonadotrophin-releasing hormone pumps or exogenous gonadotrophins containing

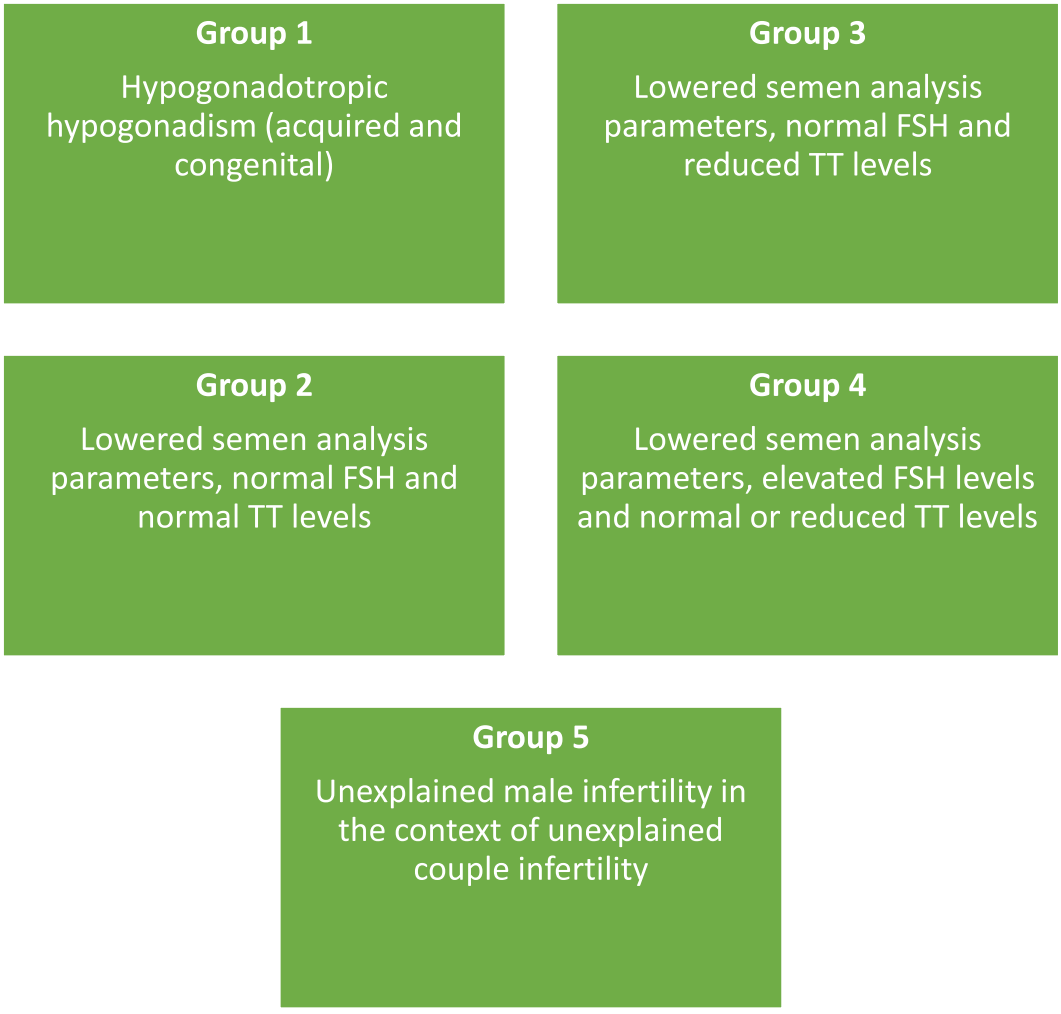

FIGURE 1 Patient groups according to the APHRODITE criteria. TT, total testosterone.

LH activity (*Lee et al., 2022*). Several studies have evaluated the administration of gonadotrophin therapy with HCG alone or combined with human menopausal gonadotrophin, urinary FSH or recombinant FSH, with significant improvements in spermatogenesis (to varying degrees) in up to 90% of patients (*Lee et al., 2022*). Moreover, in a cohort of 81 patients with idiopathic hypogonadotrophic hypogonadism treated with exogenous gonadotrophins, 77.8% of patients achieved pregnancy, either naturally (66% of pregnant couples) or assisted (34% of pregnant couples) (*Yılmazel et al., 2021*). The rationale for using HCG therapy (as a surrogate for LH) for treating male infertility conditions associated with hypogonadotrophic hypogonadism relies on the fact that low ITT concentrations disrupt spermatogenesis (*Lee and Ramasamy, 2018*). In rodents, reductions of more than 75% of the ITT concentration are incompatible with sperm maturation (*Ahmad et al., 1973*; *Cunningham and Huckins, 1979*; *Zirkin et al., 1989*).

Therapeutic regimens vary but typically begin with 1000–2500 IU of HCG twice weekly for 8–12 weeks (*Behre, 2019*; *Fraietta et al., 2013*). This initial phase is critical to increase ITT concentrations (*Coviello et al., 2005*). HCG alone appears to be able to restore spermatogenesis, especially in adult-onset hypogonadotrophic hypogonadism (*Coviello et al., 2005*). However, for patients with congenital hypogonadotrophic hypogonadism or adult-onset hypogonadotrophic hypogonadism who lack adequate concentrations of endogenous FSH (as determined prior to HCG treatment) treatment must involve administering FSH 150–300 IU two or three times weekly for up to 18 months (*Rastrelli et al., 2014*). The combination of HCG and FSH has been shown to achieve a better therapeutic effect than HCG alone for improving sperm concentration (*Behre, 2019*; *Yang et al., 2012*). However, this result is based on limited evidence in the literature and no clinical trials exist comparing different drugs and regimens.

During treatment, patients are monitored in terms of hormonal analysis and semen analysis, and sperm banking should be considered for patients who respond to therapy (*Behre, 2019*; *Fraietta et al., 2013*; *Yang et al., 2012*).

APHRODITE Group 2: lowered semen analysis parameters, normal serum FSH and normal serum total testosterone concentrations

This group consists mainly of patients with idiopathic male infertility, in whom the incidence could not be accurately calculated but was estimated to range from 15% to 60% of patients undergoing infertility evaluation (*Minhas et al., 2021*; *Olesen et al., 2017*; *Punab et al., 2017*; *Ventimiglia et al., 2021*) and also includes NOA patients with normal FSH and testosterone concentrations (*Esteves et al., 2023*; *Hung et al., 2007*). Patients with idiopathic male infertility have abnormal semen analyses but no history of diseases affecting fertility, and their physical examination and laboratory tests are normal.

The authors speculate that these patients might have 'relative' hypogonadism due to reduced gonadotrophin action, as stated in the ICMART (International Committee for Monitoring Assisted Reproductive Technologies) criteria (*Zegers-Hochschild et al., 2017*) and evidenced by lowered semen analysis parameters and reduced sperm concentration in particular, despite normal FSH and testosterone concentrations (**TABLE 3**). In other words, their normal serum hormone concentrations seem to be functionally insufficient to support normal spermatogenesis independently from the unknown, underlying cause. In this setting, there is little evidence demonstrating the treatment efficacy in real clinical practice.

However, the situation in Italy allows us to evaluate the potential FSH effectiveness in this condition, since the Italian Medicines Agency allows exogenous FSH prescription to men with altered semen analysis and normal FSH serum concentrations when these are below 8 IU/L (*AIFA, 2010*). A recent real-world data analysis performed on a cohort of 194 men with idiopathic male infertility treated with FSH reported one pregnancy for every four patients treated (*Romeo et al., 2023*). While this study is not a randomized controlled trial (RCT), it represents the first report of the potential clinical efficacy in a real-world setting of this treatment in patients corresponding to APHRODITE Groups 2 and 3; however, the authors acknowledge that this study only represents the real-world situation in one country (i.e. Italy). Although the definition of normal FSH serum concentration threshold represents a real clinical challenge, these findings suggest that this approach might be effective in a substantial subset of men with idiopathic infertility receiving a relatively low extra dose of FSH, corresponding to the 'substitutive' dose given to patients with hypogonadotrophic hypogonadism.

Empirical hormonal therapy, utilizing supraphysiological FSH stimulation, has been used in idiopathic oligozoospermic patients exhibiting FSH and testosterone concentrations within reference ranges as well as in normogonadotrophic NOA males (Supplementary Tables 1 and 2). Four meta-analyses published so far indicate that FSH therapy in male patients with idiopathic infertility was associated with increased sperm concentration, decreased sperm DNA fragmentation and improved pregnancy rates (*Attia et al., 2007*; *Attia et al., 2013*; *Cannarella et al., 2020*; *Santi et al., 2015*).

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 achieving pregnancy naturally (odds ratio [OR] 4.5, 95% confidence interval [CI] 2.17–9.33) or by ART (OR 1.60, 95% CI 1.08–2.37) (*Santi et al., 2015*). In this study, sperm concentration also improved, with a mean difference of 2.66 million/ml (95% CI 0.47–4.84, 11 studies), favouring the group receiving FSH therapy.

In a 2023 review by Esteves and colleagues, couples with male partners exhibiting idiopathic oligozoospermia who received gonadotrophin therapy had significantly higher natural or assisted pregnancy rates (30.7%, 205/667) compared with those who did not receive gonadotrophin therapy (15.4%, 62/403; $P < 0.0001$) (*Esteves et al., 2023*). The analysis revealed an odds ratio of 2.440 (95% CI 1.778–3.349; $P < 0.0001$) for pregnancy in favour of gonadotrophin therapy. On average, seven patients with idiopathic oligozoospermia (95% CI 4.9–9.6) need to be treated with gonadotrophins to achieve one additional pregnancy.

Other studies also confirmed the significant improvement in both semen analysis parameters and pregnancy rate in a dose-dependent fashion (between 150–300 IU every other day (*Cannarella et al., 2020*; *Ding et al., 2015*) in these patients. This effect may be due to the surplus of exogenous gonadotrophin compensating for the insufficient action of endogenous gonadotrophins and/or

stimulating a 'weak' (for any reason) spermatogenesis activity over the baseline levels.

However, the existing studies are heterogeneous regarding participant numbers, gonadotrophin therapy regimens, treatment duration and follow-up. Most trials included few participants, reported pre- and post-treatment semen outcomes based on a single semen analysis and had a short follow-up. Therefore, more research is needed to accurately assess the beneficial effect of gonadotrophin therapy in this patient population. This is particularly true considering that semen parameters significantly influence ART success, as recently detected in 22,013 fresh cycles in a single centre (*Villani et al., 2022*).

Gonadotrophin therapy has also been used to stimulate spermatogenesis in eugonadal NOA patients with normal FSH concentrations (*Esteves et al., 2023*; *Tharakan et al., 2022*). In NOA patients, although the deficient sperm production precludes spermatozoa from appearing in the ejaculate, spermatozoa can be retrieved from the testes in 30–60% of affected patients and used for ICSI (*Esteves 2022b*).

Gonadotrophin therapy was suggested to be useful for NOA male patients with spermatogenic maturation arrest (*Kobori et al., 2015*; *Laursen et al., 2022*) but this requires further study. The rationale for such attempts is that exogenous gonadotrophins might be able to maximally stimulate clones of 'good' spermatogonia that are capable of successfully surpassing the arrest point. The goals of this therapy are to induce the recovery of spermatozoa in the ejaculate or improve surgical sperm retrieval rates in the context of treatment with medically assisted reproduction.

A study involving 20 patients with failed sperm retrieval and who received gonadotrophin therapy before a second sperm retrieval demonstrated that spermatogonial and primary spermatocyte DNA synthesis, assessed by the expression of proliferating cell nuclear antigen in testicular biopsies, was increased after hormonal therapy with recombinant FSH and HCG (*Shinjo et al., 2013*). The authors showed that spermatozoa were successfully retrieved from three (15%) men treated with hormonal therapy before the second microdissection testicular sperm extraction. However, large-scale, well-designed, high-powered studies evaluating a fixed treatment regimen and sperm quality as primary end-points are needed to confirm the utility of hormonal therapy in these patients.

Idiopathic male factor infertility is mainly diagnosed in patients looking to start a family who attend medically assisted reproduction centres that normally proceed with ICSI. As discussed already, gonadotrophin treatment in such patients before ICSI could be beneficial for improving efficiency and time to live birth (*CDC, 2016*; *Chamayou et al., 2023*; *Romeo et al., 2023*; *Villani et al., 2022*). For some male patients with NOA, hormonal therapy might improve spermatogenesis, either to allow the appearance of spermatozoa in ejaculates for ICSI to be carried out (i.e. with ejaculated spermatozoa, thus avoiding sperm retrieval, which is an invasive procedure) or to increase sperm retrieval rates.

APHRODITE Group 3: lowered semen analysis parameters, normal FSH and reduced total testosterone concentrations

Group 3 is a variation of Group 2 and comprises patients with lowered semen analysis parameters, normal FSH concentrations and biochemical hypogonadism (**TABLE 3**). The authors propose that these patients could meet the definition of hypogonadotrophic hypogonadism according to ICMART, which defines it as 'gonadal failure associated with reduced gametogenesis and reduced gonadal steroid production due to reduced gonadotrophin production or action' (*Zegers-Hochschild et al., 2017*).

As APHRODITE Group 3 comprises patients exhibiting lowered spermatogenesis and reduced testosterone concentrations, this indicates a possible relative reduction of gonadotrophin action. It has been suggested that the response to FSH therapy might depend on the genetic background of the affected men. In one study, Simoni and co-workers found that semen quality improvement, assessed by sperm chromatin damage, was more pronounced in carriers of the homozygous N polymorphism of the FSHR (p.N680S) compared with those with the S allele (p.N680S) (*Simoni et al., 2016*). Apparently, the single-nucleotide polymorphisms *FSHB* −211G>T genotype modulated the observed effect, as patients with this genotype were the most responsive to therapy. In another report, Selice and colleagues observed that the use of FSH therapy only conferred a statistically significant improvement in the sperm parameters of men with idiopathic oligozoospermia who had common allelic variants in the *FSHR* gene, particularly Ala307-Ser680/Ala307-Ser680 homozygosis or Thr307-Asn680/Ala307-Ser680 heterozygosis (*Selice et al., 2011*).

Since ITT plays a crucial role in spermatogenesis, reduced circulating testosterone concentrations might indicate reduced LH activity on the testes due to some local hindrance. Thus, it is plausible that these patients may benefit from a hormonal therapy containing both FSH and LH activity. However, limited data are available on this combination, particularly in patients with NOA.

Using real-world data, following a mean FSH treatment duration of 9.1 ± 7.1 months, 43 pregnancies (22 natural and 21 after assisted reproduction) were recorded in 194 men with idiopathic infertility (around 1 in 4 of the total cohort), as well as a significant improvement in sperm quality in both natural and ART cycles (*AIFA, 2010*). In this cohort of men with idiopathic infertility treated with FSH, 20% of cases (n = 39) showed total testosterone serum concentrations below the reference ranges, together with altered semen analysis, before treatment (*Romeo et al., 2023*). This result confirms that in idiopathic infertility the reduction in sperm quality and quantity could be related to a reduced hormonal production (*Romeo et al., 2023*). As these findings are based on real-world data from a single country, they should be considered as a starting point for future research into this topic.

Although a clear threshold of serum testosterone concentrations facilitating optimal spermatogenesis has yet to be established, a positive relationship exists between testosterone serum concentrations and the likelihood of successful sperm retrieval in patients with NOA (*Guo et al., 2020*; *Mehmood et al., 2019*). The review by Esteves and colleagues on the use of hormonal therapy in NOA patients, mostly hypogonadal, found that patients who received hormonal therapy had a higher success rate for sperm retrieval by testicular sperm extraction than untreated subjects, with an odds ratio of 1.295 (95% CI 1.115–1.505; P = 0.0007) for a positive surgical sperm acquisition (*Esteves et al., 2023*). On

average, 17 patients (95% CI 10.5–39.3) needed to be treated to achieve an additional positive sperm retrieval outcome. The treatment was also associated with the return of spermatozoa to the ejaculate in 6.2% of NOA patients who received gonadotrophin therapy (*Esteves et al., 2023*).

In this review, however, studies on patients with either normal or elevated FSH concentrations were included, precluding an in-depth analysis on the effect of hormonal therapy according to FSH concentrations. Notably, in the Esteves review, the primary drug used was HCG, given its availability and positive effect on ITT production and spermatogonial DNA synthesis. The most common gonadotrophin regimens used to treat NOA males included urinary HCG, used alone or combined with FSH, administered subcutaneously two or three times a week for 3 months or longer, in varying doses titrated to keep the endogenous FSH and testosterone concentrations at optimal levels.

Additionally, the authors found that patients with either biochemical hypogonadism or histopathology showing maturation arrest or hypospermatogenesis were the best candidates for hormonal therapy (*Esteves and Humaidan, 2023*). However, the reasons why some individuals responded to treatment while others did not remain unknown and are probably related to the heterogeneous, albeit unknown, aetiology. It should be noted that even patients with a proven genetic cause of NOA could be candidates for gonadotrophin therapy because they may still have some tubules with normal spermatogenesis, as occurs in Klinefelter syndrome (*Sciurano et al., 2009*). The studies on gonadotrophin therapy in patients with NOA are highly heterogeneous in design, population, number of participants, regimens, treatment duration and sperm retrieval methods. In addition, many studies lack pregnancy data, and no RCT have been published; as such, further research is needed in this area.

APHRODITE Group 4: lowered semen analysis parameters, elevated FSH concentrations and reduced or normal total testosterone concentrations

Group 4 consists of patients with lowered semen analysis parameters, elevated FSH concentrations (according to the ranges for the analysing laboratory), and reduced or normal total testosterone concentrations (**TABLE 3**). Most patients in this group are NOA males with hypergonadotrophic hypogonadism.

In NOA males with elevated concentrations of endogenous FSH and LH, endogenous gonadotrophins might be ineffective in adequately stimulating the Leydig and Sertoli cells (*Shiraishi and Matsuyama, 2014*). In addition, about half of these patients also have low concentrations of circulating testosterone (*Bobjer et al., 2012*; *Reifsnyder et al., 2012*), indicating concurrent Leydig cell insufficiency. Gonadotrophin therapy, using HCG and FSH, has been shown to promote spermatogonial proliferation and DNA synthesis in these patients (*Shinjo et al., 2013*). ITT concentrations also increase in NOA patients receiving HCG therapy (*Shinjo et al., 2013*).

In one study involving NOA males with Klinefelter syndrome, patients with low baseline testosterone who responded to hormonal therapy with a resultant testosterone concentration of greater than 250 ng/dl had a higher chance of sperm retrieval (77% versus 55%) than their counterparts who did not respond to therapy (*Ramasamy et al., 2009b*). These findings suggest that the exogenous administration of gonadotrophins might stimulate Sertoli and Leydig cells, even under hypergonadotrophic hypogonadal conditions, providing an extra stimulus to the few tubules containing proliferating spermatogonia.

A meta-analysis conducted in 2022 analysed 10 controlled studies involving 985 participants who received various types of hormonal stimulation (*Tharakan et al., 2022*). The results showed that pre-treatment with hormonal therapy increased sperm retrieval rates (OR 1.96, 95% CI 1.08–3.56; $P = 0.03$; low-quality evidence). However, subgroup analyses based on the type of NOA patient showed that a significant improvement in sperm retrieval rates was observed only in normogonadotrophic men (five studies, OR 2.13, 95% CI 1.10–4.14; $P = 0.02$), but not in hypergonadotrophic men (four studies, OR 1.73, 95% CI 0.44–6.77; $P = 0.43$). The baseline FSH concentration that distinguished these populations was 12 mIU/ml.

Despite this, some studies have reported improved success in retrieving spermatozoa from hypergonadotrophic hypogonadal NOA patients who underwent hormonal therapy before sperm retrieval (*Amer et al., 2019*; *Andrabi et al., 2022*; *Kobori et al., 2015*; *Majzoub et al., 2016*; *Ramasamy et al., 2009a*; *Schiff et al., 2005*; *Shiraishi and Matsuyama, 2014*; *Shiraishi et al., 2012*). However, this evidence overwhelmingly stems from small retrospective cohorts and case series. Thus, the current available evidence is mixed, underscoring the need for further research to elucidate the precise impact of hormonal therapy on individuals with NOA. In this context, utilizing the APHRODITE classification could improve patient stratification for future research initiatives.

APHRODITE Group 5: unexplained male infertility

This group comprises men with FSH concentrations within the normal range, total testosterone concentrations also within the normal range, and normal semen analysis parameters. From the male standpoint, these patients have unexplained infertility, as they are characterized by no history of diseases affecting fertility and normal findings on physical examination and laboratory tests, including genetic analysis.

Currently, these patients are not individually treated since alterations are not detected in semen analysis or in the reproductive hormonal profile. Accordingly, their female partners are often treated in the context of unexplained infertility with low-dose ovarian stimulation, with or without intrauterine insemination, or with high-dose ovarian stimulation in the context of ART treatment. However, this group of male patients could benefit from FSH stimulation, if a 'stimulatory' rather than 'substitutive' therapeutic approach is applied (*Simoni and Santi, 2019*). Indeed, there is evidence that, physiologically, spermatogenesis does not run at its maximum capacity, and extra FSH might boost it further, resulting in increased sperm quantity/quality, which could increase pregnancy rates and reduce time to live birth (*Simoni and Santi, 2019*).

A more proactive approach is needed to explore this testicular overstimulation approach, starting with trials of higher FSH dosages over longer periods. Boosting spermatogenesis above baseline should be regarded as something similar to ovarian stimulation, defined as pharmacological treatment with the intention of inducing the development of multiple ovarian

follicles independently of the cause of the infertility, either for timed intercourse, for insemination or in ART, to obtain multiple oocytes at follicular aspiration (*Zegers-Hochschild et al., 2017*).

This latter condition is not completely free from side effects (i.e. ovarian hyperstimulation syndrome), but a similar complication has not been demonstrated in the male setting so far. The authors reason that if FSH stimulation can induce multiple follicular growth and dominance in the ovary, it should also, from an endocrinological perspective, be able to increase spermatogenesis in the testes. Otherwise, it would represent a paradoxical scenario wherein FSH is the only hormone unable to induce biological overstimulation effects in both genders.

Although the authors acknowledge that ovarian stimulation is not without side effects, no or minimal side effects have been reported in studies using FSH stimulation for male patients with idiopathic oligozoospermia, as reviewed by Esteves and Humaidan (*Esteves and Humaidan, 2023*). Moreover, a hypothetical FSH overstimulation is expected to be safe since no adverse events have been described so far in men with elevated endogenous FSH serum concentrations. The increase in testicular volume in men with FSH-secreting pituitary tumours in the absence of other hormonal effects (*Dahlqvist et al., 2010*; *Heseltine et al., 1989*) speaks for the efficacy and safety of pharmacological FSH stimulation on spermatogenesis. Despite this, the safety of supraphysiological FSH stimulation in men with unexplained male infertility must be determined, as spermatogenesis optimization would not be precisely determined until after one or more spermatogenic cycles.

TABLE 5 summarizes the characteristics of the five APHRODITE groups, their estimated prevalence, suggested hormonal therapy with gonadotrophins, and treatment end-points (primary and secondary).

DISCUSSION

The APHRODITE criteria propose a novel stratification system for infertile patients with various forms of testicular dysfunction, for whom fertility and pregnancy prospects may improve after hormonal therapy. These criteria are not designed for men with other confirmed infertility diagnoses, such as infection or obstruction, who would not primarily benefit from hormonal treatment.

To the authors' knowledge, APHRODITE is the first system to classify patients with male factor infertility using well-defined clinical criteria independent of aetiology and provide suggestions for hormonal therapy for each group. Under APHRODITE, patients are classified based on clinical patient descriptions (i.e. using the history and physical examination) and the results of routine laboratory tests. The groups are fully defined and characterized, with suggested therapeutic management and relevant end-points. The criteria have simple key objectives relevant to male patients: first, to improve semen parameters for natural conception and, second, to improve semen quantity and/or quality as part of the couple's ART treatment.

Germ cells may lack proper stimulation because of deficits in the production or action of FSH and/or LH, including at the FSHR and/or LH receptor level. In men with hypogonadism, for example, Leydig cells do not secrete enough testosterone even if circulating LH concentrations are adequate (*Ventimiglia et al., 2017*). Causes of hypogonadism are many and include testicular pathologies, systemic diseases, infections, congenital and genetic abnormalities, ageing and obesity, among others (*Dandona and Rosenberg, 2010*). However, although the biology of spermatogenesis is relatively well understood, there is currently a lack of standardized diagnostic and management approaches, particularly for infertile male patients with an unknown aetiology and a partner with no known reproductive health issues.

Despite the fact that current guidelines for diagnosing and treating male infertility recommend investigating both partners simultaneously (*Minhas et al., 2021*; *Schlegel et al., 2021*), the reliance on semen quality as the primary surrogate marker of male fecundity frequently results in the neglect of further investigations to identify the underlying cause, such as genetic or hormonal assessment. In fact, in real-world IVF settings, male infertility evaluation is often suboptimal, with only a basic semen analysis being performed in an analysis of data from a single centre (*Pozzi et al., 2021*). As a result, until now, limited progress has been made in the clinical management of men with reduced fertility and reduced spermatogenesis. In light of these deficits, the authors hope that the APHRODITE criteria will also lead to further basic and clinical research into the diagnostic and management approaches.

In the APHRODITE criteria, clinical patient descriptions (e.g. medical history and physical examination), basic semen parameters and FSH and testosterone serum concentrations are considered as the components of the workup panel that can provide a more comprehensive picture of specific patient profiles. By providing a framework for an expedited workup and clinical decision making, the APHRODITE criteria may support and assist healthcare professionals, thus adding to existing societies' guidelines and algorithms.

Indeed, an extended investigation of causative factors for infertility in couples could improve ART efficiency and reduce cost (*Esteves and Humaidan, 2023*). For example, the end of one full hormonal treatment cycle to improve sperm quantity and/or quality over 3 months could be timed to coincide with the culmination of the preparatory workup for the female partner's first ART cycle; this would increase the chances of achieving a 'one-and-done' ART cycle, with the option for male treatment to continue for a further 3 months in case a second cycle is needed.

The evidence referenced throughout this paper suggests that hormonal therapy may allow these men to achieve biological fatherhood by 'converting' the oligozoospermic male patient from IVF/ICSI treatment to intrauterine insemination or even natural conception, and those with NOA to ICSI. However, even when hormonal treatment is considered, options are often empirical and not based on a precise patient classification (*Foran et al., 2023*).

In this context, by stratifying patients into well-defined groups, the APHRODITE criteria could help better understand which patients might benefit from hormonal treatment and how, by both exploring the analysis of real-world data and stimulating the development of prospective trials. This collaborative approach could also empower the male and female partners and decrease the number of patients who decide not to continue their treatment. However,

TABLE 5 CHARACTERISTICS OF THE FIVE APHRODITE GROUPS

Classification	Definition[a]	Prevalence	Suggested gonadotrophin regimen	End-points[a]
Group 1 Hypogonadotrophic hypogonadism (acquired and congenital)	Gonadal failure associated with reduced gametogenesis and reduced gonadal steroid production due to reduced gonadotrophin production or action (*Zegers-Hochschild et al., 2017*) FSH concentrations below the reference range and reduced LH concentrations, reduced total testosterone concentrations (below the lower limit of the normal range of the assessing laboratory) and lowered semen analysis parameters (e.g. OAT or azoospermia)	1.9% of azoospermia cases (*Chiba et al., 2016*) and 1.6% of male infertility cases (*Jungwirth et al., 2012*) overall	HCG (± FSH[b,c])	Semen parameters/ sperm retrieval rates, total testosterone concentrations, QoL, pregnancy rates
Group 2 Lowered semen analysis parameters, normal serum FSH and normal serum total testosterone	Functional hypogonadism with reduced gonadotrophin action Lowered semen analysis parameters, including NOA FSH concentrations within the reference range and total testosterone concentrations within the normal range of the assessing laboratory	Idiopathic male infertility: 15–60% of patients undergoing infertility evaluation (*Minhas et al., 2021*; *Olesen et al., 2017*; *Punab et al., 2017*; *Ventimiglia et al., 2021*)	FSH alone[c,d]	Semen parameters /sperm retrieval rates, SDF rates, QoL, pregnancy rates
Group 3 Lowered semen analysis parameters, normal FSH and reduced total testosterone concentrations	Functional hypogonadism with reduced gonadotrophin action and reduced testosterone production Lowered semen analysis parameters, including NOA FSH concentrations within the reference range and reduced total testosterone (below the lower limit of the normal range of the assessing laboratory)	Around 20% of the total idiopathic male infertility population treated with hormonal therapy (*Romeo et al., 2023*)	FSH[c,d] (± HCG)	Semen parameters/ sperm retrieval rates, total testosterone concentrations, SDF rates, QoL, pregnancy rates
Group 4 Lowered semen analysis parameters, elevated FSH concentrations and normal or reduced total testosterone concentrations	Functional hypergonadotrophic hypogonadism Lowered semen analysis parameters, mainly NOA FSH concentrations above the upper limit of the reference range and normal or reduced total testosterone concentrations (excluding genetic causes)	Up to 10% (*Cocuzza et al., 2013*)	HCG (± FSH[d])	Semen parameters/ sperm retrieval rates, total testosterone concentrations, SDF rates, QoL, pregnancy rates
Group 5 Unexplained male infertility in the context of unexplained couple infertility	FSH concentrations within the reference range, testosterone concentrations within the normal range and normal semen analysis parameters	15% of couples presenting with unexplained infertility, and unexplained male infertility in 6–27% (*Esteves et al., 2015*)	FSH alone[e]	SDF rates, pregnancy rates

[a] Sperm parameters are the primary end-points as they are the main outcome measures of hormonal treatment.
[b] The regimen can be tailored according to the congenital or acquired forms of hypogonadotrophic hypogonadism.
[c] Groups 1, 2 (Italy only) and 3 (Italy only) meet the label for FSH treatment. Groups 4 and 5 represent an off-label indication.
[d] FSH treatment might improve DNA fragmentation and sperm quality; however, indiscriminate use of FSH will reset the parameters.
[e] The suggestion for FSH alone is based on empirical evidence and the opinion of the authors, and will need to be updated as more data become available. If FSH concentrations are low, treatment with exogenous FSH can be considered.
HCG, human chorionic gonadotrophin; NOA, non-obstructive azoospermia; OAT, oligoasthenoteratospermia; QoL, quality of life; SDF, sperm DNA fragmentation.

finances, time cost, treatment access and the need for several monitoring visits are important considerations that may influence the provision of treatment for male infertility and will need to be taken into account. This paper is proposing a classification of infertile men according to phenotype; as this is not intended as a guideline, it is not within the remit of the paper to include cost-effectiveness evaluations with the evidence available.

For patients with hypogonadotrophic hypogonadism (APHRODITE Group 1), in whom gonadal failure is associated with reduced gonadotrophin production or action, gonadotrophin-releasing hormone or exogenous gonadotrophin treatment with HCG in combination with FSH (human menopausal gonadotrophin, highly purified-FSH or recombinant humanFSH) has proven success and is the only indication for which exogenous gonadotrophin treatment has been generally accepted by the scientific community and health authorities (*Boehm*

et al., 2015; Boeri et al., 2021; Casarini et al., 2020).

Patients categorized as APHRODITE Group 2, who have a normal hormonal profile but lowered semen parameters, consist mainly of patients with idiopathic male infertility, including selected cases of NOA, in whom it can be presumed that there is a functional form of hypogonadism due to insufficient or inefficient gonadotrophin action. Indeed, it has been suggested that men with idiopathic oligozoospermia who exhibit low-to-normal circulating FSH concentrations may be FSH deficient because of reduced FSH activity, which depends on the amount of circulating FSH, its glycosylation and the genetically determined expression levels and function of the FSHR (Benson et al., 2013; Casarini and Simoni, 2021; Grigorova et al., 2008; Simoni et al., 1997; Simoni et al., 2016; Tüttelmann et al., 2012).

Notably, the FSH gene transcriptional activity is primarily modulated by the presence of a single-nucleotide variation (SNV) falling within the promoter of the *FSHB* gene (Benson et al., 2013), characterized by a G to T nucleotide change (211 G>T; rs10835638). This SNV is associated with reduced serum FSH concentrations, decreased sperm concentration and reduced testicular volume; patients carrying the T allele may not adequately up-regulate circulating concentrations of FSH to achieve full spermatogenesis (Grigorova et al., 2008). Additionally, the amplitude of the Sertoli cell response to FSH is modulated by a common *FSHR* SNV, characterized by the A to G nucleotide change at position 2039 of the transcription start site, resulting in an asparagine to serine change at position 680 of the protein chain (Simoni et al., 1997). Some evidence indicates that men who are homozygous for the serine receptor phenotype are less sensitive to FSH (Simoni et al., 2016). On this basis, hormonal therapy with exogenous FSH might help improve sperm parameters, particularly in carriers of the *FSHB* or *FSHR* SNV (Tüttelmann et al., 2012).

As reported in the recent meta-analysis by Cannarella and colleagues, exogenous treatment, regardless of origin, showed a dose-dependent effect on sperm parameters after at least 3 months of treatment, with the highest doses of FSH (700–1050 IU per week) leading to increased sperm concentration, total sperm count and progressive mobility, and a trend of improved sperm morphology (Cannarella et al., 2020). However, despite the evidence indicating a significant increase in pregnancy rates (Attia et al., 2007; Attia et al., 2013; Santi et al., 2015) and sperm concentration (Cannarella et al., 2020) in men with idiopathic infertility, reported in four meta-analyses (21 trials) and using real-world data (Romeo et al., 2023), the use of FSH in these patients is still experimental.

Other therapeutic modalities apart from gonadotrophins could be used to modulate the reproductive hormones, including selective oestrogen-receptor modulators and aromatase inhibitors. In a meta-analysis of eight studies (616 patients), treatment with clomiphene led to a significant improvement in sperm concentration compared with placebo or pretreatment values ($P < 0.00001$) (Puia and Pricop 2022). In a meta-analysis of the safety and efficacy of letrozole and anastrozole in men with a low testosterone to oestradiol ratio (10 studies, 666 patients), both treatments significantly increased sperm concentration, total sperm count, serum LH, FSH and testosterone concentrations, and testosterone-to-oestradiol ratio, whereas oestradiol concentrations were significantly reduced compared with baseline values. However, compared with selective oestrogen receptor modulators or HCG, neither drug showed superiority for improving sperm concentration, motility or morphology (Guo et al., 2022). Lastly, in a meta-analysis of eight articles, anastrozole, letrozole and testolactone were found to improve all evaluated hormonal (testosterone, oestradiol and testosterone to oestradiol ratio) and seminal (sperm concentration, total sperm count and sperm motility) parameters (Del Giudice et al., 2020).

Elevated oestradiol concentrations, which are frequently seen in overweight and obese patients, mainly due to excessive aromatization of testosterone to oestradiol in the adipocytes, might exert an inhibitory effect on the hypothalamic–pituitary–gonadal axis and negatively impact sperm production (Salas-Huetos et al., 2021). For this specific population, there might be a role for aromatase inhibitors in the therapeutical regimen, but future RCTs are needed to determine the efficacy of these medications in such patients. Furthermore, there are currently no head-to-head trials of these treatments compared with gonadotrophins, and patients with primary hypogonadotrophic hypogonadism will not respond to clomiphene or anastrozole, owing to the absence or lack of action of endogenous gonadotrophin.

Finally, while these drugs lead to an increase in serum gonadotrophin concentrations, these do not usually reach the level of overstimulation that would be achieved with gonadotrophins. Moreover, treatment with FSH in Group 2 patients may have advantages over anti-oestrogenic hormonal treatments, such as tamoxifen and letrozole, owing to the pivotal and multifaceted testicular response to FSH (Carreau et al., 2012; Dostalova et al., 2017; Kula et al., 2001; Luo et al., 2021; Rochira V, 2000; Stocco, 2008).

Patients classified in APHRODITE Group 3 are estimated to make up around 20% of all men with idiopathic infertility (Romeo et al., 2023). This group differs from Group 2 by having reduced total testosterone serum concentrations, suggesting insufficient or inefficient LH activity on the testes, and making it possible to potentially define these patients as having hypogonadotrophic hypogonadism according to the ICMART definition (Zegers-Hochschild et al., 2017). As such, these patients may benefit from gonadotrophin therapy with both FSH and LH activity, although data on this therapy are limited.

Italy is currently the only country where FSH treatment is indicated (and reimbursed) for patients with idiopathic infertility and normal gonadotrophin concentrations (e.g. APHRODITE groups 2 and 3), providing the opportunity to study the benefits of therapy in this patient group. Thus, the application of real-world data analysis in the Italian setting represents a relevant opportunity to evaluate FSH effectiveness in clinical practice. Obviously, the Italian scenario does not reflect other regions, raising the issue of equity regarding access to care, but it represents a starting point for future studies. Indeed, in this real-world setting, with a mean FSH treatment duration of 9.1 ± 7.1 months, 43 pregnancies (22 natural and 21 after assisted reproduction) were recorded in 194 men with idiopathic infertility (around 1 in 4 of the total cohort), as well as a significant improvement in sperm quality in both natural and ART cycles (AIFA,

2010). The validation of the efficacy and effectiveness of hormonal therapy in APHRODITE Group 2 and 3 patients, as assessed by independent investigators is fundamental for supporting the case for change.

Patients included in Group 4 show alterations in most or all parameters used for classification (lowered semen analysis parameters, elevated FSH concentrations and normal or reduced total testosterone concentrations) and predominantly comprise men with NOA exhibiting hypergonadotrophic hypogonadism. While the concentrations of endogenous FSH and LH are high in this group, there is an obvious lack of effective stimulation of the Leydig and Sertoli cells. As such, treatment with exogenous gonadotrophins may be of benefit, although the results of a systematic review and meta-analysis report that treatment success seems to be dependent on the patients' hormone profile (*Tharakan et al., 2022*).

Finally, men who are categorized in Group 5 have unexplained infertility (i.e. no history of diseases that affect fertility, and normal findings on physical examination and laboratory tests) and might benefit from stimulatory FSH treatment to raise concentrations above their normal baseline. However, owing to the paucity of data on this, clinical trials should be instigated to investigate the efficacy of treatment in this group.

When considering the parameters included in the APHRODITE criteria, a pragmatic approach was adopted when it came to defining cut-off values for FSH, whereby the ranges defined by each assessing laboratory are referred to. Inconsistency in the literature and among countries about what constitutes the normal range for FSH and variation in the sensitivity and calibrators of the different analytical methods used to measure FSH are key reasons why FSH cut-off concentrations were not specified. However, it is acknowledged that in Italy FSH (150 IU three times a week for 4 months, with an extension up to 18 months in the case of clinical benefits) can be prescribed to men with idiopathic infertility when serum FSH concentrations are <8 IU/l (*AIFA, 2010*).

Current guidelines and algorithms mainly provide classification and management recommendations for patients with hypogonadotrophic hypogonadism. With regard to the management of idiopathic oligozoospermia and NOA, current guidelines predominantly state that hormonal therapy is not recommended. The APHRODITE criteria provide evidence supporting the utility of hormonal therapy in specific groups of patients, which were well characterized in the newly proposed system, and make a call for action regarding research studies to fill the existing knowledge gaps. The proposal set out in this opinion paper will help direct further studies in this field.

The APHRODITE criteria offer a clear and well-defined patient stratification that can facilitate communication among clinicians, researchers and patients. This approach can potentially standardize patient management, provide a better understanding of the prevalence of different groups of patients and allow for comparisons between treatments and outcomes. Clinicians may benefit from the criteria by identifying specific patient groups and using targeted interventions to improve fertility. Additionally, the criteria may help researchers discover new pharmacological interventions and promote a detailed analysis of the proposed patient groups to identify novel causes of male infertility.

This opinion paper introduces an innovative proposal that is being shared with the scientific community to encourage further discussion and research. We welcome feedback from a wide range of stakeholders, including global fertility associations and societies. This collaborative approach will help a full exploration of the potential of the APHRODITE criteria by creating a globally aligned research strategy that can be subsequently implemented in carefully planned clinical trials. Such trials could achieve several goals. In the first instance they could address the use of FSH in male idiopathic infertility (APHRODITE Group 2) in which FSH concentrations are within the normal range and the underlying cause of infertility may be related to an insufficient action of FSH. The real-world benefits of FSH treatment seen in this group (*Esteves et al., 2023*; *Romeo et al., 2023*) should now be confirmed through well-designed clinical trials that have an improvement of sperm quantity and quality and/or pregnancy rates as primary end-points. As sperm quantity and quality are associated with the likelihood of achieving a pregnancy as well as the time to pregnancy (*Keihani et al., 2021*; *Romero Herrera et al., 2021*), these metrics could also serve as the basis for attaining regulatory approval for hormonal treatment for this indication.

Furthermore, trials could confirm the benefit of treatment with HCG associated with FSH in APHRODITE Group 3, which comprises patients having a form of hypogonadism that presents as altered gamete quantity/quality, insufficient gonadotrophin action and reduced steroid production. Conducting such trials using the APHRODITE criteria rather than less specific and/or heterogeneous definitions of idiopathic infertility could help to gain a clearer picture of the benefits of therapy. Furthermore, such trials could validate the real-world data already reported (*Romeo et al., 2023*), wherein FSH treatment resulted in pregnancy in 1 in 4 men. This, in turn, might prompt a reappraisal of the cost–benefit ratio.

The authors acknowledge that developing a perfect stratification system for male infertility patients is challenging. However, the proposed APHRODITE criteria offer a practical and valuable approach to improve patient care and research in this area. By using these criteria and collecting data, new insights will be gained that can lead to adaptations in the existing patient groups and further improvements in reproductive outcomes.

CONCLUSIONS

The proposed APHRODITE criteria offer a standardized approach to classify patients with male infertility. They can improve communication and clinical management among andrologists, urologists and ART experts. Furthermore, they may highlight areas lacking data and promote clinical research to discover new pharmacological treatment options for male infertility.

DATA AVAILABILITY

No data was used for the research described in the article.

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AUTHOR CONTRIBUTIONS

All authors contributed to the development of the APHRODITE criteria. All authors were involved in the writing and critical review and revision of the manuscript. All authors approved the manuscript for submission.

ETHICS STATEMENT

As this article is based on published data and expert opinion, no ethics approval is required.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.rbmo.2023.103647.

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