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Refining the Clinical Utility of the Testicular Index for Pregnancy Prediction in FSH-Treated Idiopathic Male Infertility

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ABSTRACT

Background and aim: Follicle-stimulating hormone (FSH) therapy is widely used for idiopathic male infertility (IMI), but high inter-individual variability in treatment efficacy complicates its management. The aim of this study was to refine the clinical utility of a multiparametric Testicular Index (TI) to predict pregnancy achievement in men with IMI undergoing empirical FSH therapy.

Materials and methods: A retrospective, observational, real-world study was conducted at the University Hospital of Modena, Italy, enrolling 228 infertile couples whose male partners were treated with FSH for IMI. Clinical, seminal, and endocrine data were collected at baseline (V0) and post-treatment (V1). Patients were also stratified according to the APHRODITE criteria. The TI was calculated as (FSH + Total testosterone) / Bi-testicular volume. The primary clinical outcome was the achievement of a clinical pregnancy.

Results: A total of 68 clinical pregnancies (29.8%) were recorded following an average treatment duration of 8.7 ± 5.0 months. Significant improvements in sperm concentration, total sperm number, and motility parameters were observed following FSH administration. Men whose partners achieved pregnancy were significantly younger (36.2 ± 4.7 vs 38.6 ± 6.4 years; $p = 0.007$), had larger baseline bi-testicular volumes ($p = 0.003$), and exhibited lower baseline FSH ($p = 0.020$) and TI values ($p = 0.002$). Multivariable logistic regression identified male age (OR 1.148, $p = 0.011$), bi-testicular volume (OR 1.091, $p = 0.043$), and the baseline TI (OR 0.334, $p = 0.001$) as independent predictors of pregnancy success. Furthermore, the TI demonstrated significant discriminative ability within APHRODITE Group 3 (AUC 0.673), unmasking potential responders within borderline-hypogonadal phenotypes.

Conclusions: This real-world study confirms the TI as a reliable clinical tool that synthesizes endocrine drive and morphological reserve. A lower baseline TI correlates with a higher probability of achieving pregnancy through exogenous FSH stimulation. Integrating the TI within structured phenotypic frameworks, such as APHRODITE, facilitates patient selection and supports phenotype-driven therapeutic strategies for male factor infertility.

INTRODUCTION

1.1 Infertility: definitions, global burden and temporal trends

1.1.1 Clinical definitions and the couple-based approach

Infertility is defined by the World Health Organization (WHO) and the International Committee for Monitoring Assisted Reproductive Technology as a disease of the reproductive system characterized by the failure to achieve a clinical pregnancy after 12 months or more of regular, unprotected sexual intercourse[1]. Thus, the term 'infertility' should refer to the couple. In routine practice, primary infertility refers to couples who have never attained a prior conception, whereas secondary infertility is characterized by the inability to achieve a new pregnancy following at least one documented gestation[2].

Couple infertility is recognized as the result of the interaction of various factors, potentially impairing the fertility potential of both partners. In this framework, the age of the female partner remains the most critical independent variable affecting reproductive outcomes. However, this criterion is not sufficient on its own to explain all cases of couple infertility. Thus, the evaluation of couple infertility should be viewed within the context of the couple-based approach[2]. This process generally starts after one year of unsuccessful unprotected sexual intercourse, although for women aged 35 or older, the diagnostic window is appropriately shortened to 6 months[3].

However, emerging evidence suggests that optimizing the male fertility potential may partially attenuate the prognostic impact of advanced maternal age[4], a concept further explored in Section 1.6.2.

1.1.2 Epidemiology of the male component of couple infertility

On a global scale, infertility represents a major public health concern, affecting up to 15% of couples of reproductive age[2,5]. The male factor is considered to be solely responsible for 20-30% of cases and contributing to approximately 50% of infertility cases overall[2,5]. Recent epidemiological data indicate a substantial increase in the prevalence of male infertility worldwide[6]. In this scenario, the introduction of assisted reproductive technology (ART) in daily practice significantly improved the potential

treatments in case of couple infertility[7]. In Italy, according to the regulatory framework established by Law 40/2004 on ART, children that are born through these techniques account for a growing proportion of total births, reaching 4.5% in 2023[8].

This rising demand shows the need for reliable predictive markers to optimize and personalize ART pathways, ensuring both therapeutic efficacy and economic sustainability of reproductive medicine interventions[9]. Many studies have focused on improving therapeutic approaches for female partners in infertile couples. In contrast, male infertility remains largely understudied, and scientific knowledge regarding its treatment is still considerably less advanced compared with that of female infertility[4,5].

1.1.3 The global decline in semen quality and environmental drivers

The increasing prevalence and incidence of male infertility may reflect the suggested global decline in sperm quality. A landmark meta-analysis[10] confirmed a significant and accelerating decrease in both sperm concentration and total sperm number across all continents between 1973 and 2018, noting that the percentage of decline per year actually doubled post-2000.

This trend is increasingly attributed to the "testicular dysgenesis syndrome" (TDS) hypothesis, which posits that environmental factors, particularly endocrine-disrupting chemicals (EDCs) such as phthalates, bisphenol A, and various pesticides interfere with fetal testicular development[11,12]. This disruption can manifest clinically as a spectrum of disorders, including cryptorchidism, hypospadias, testicular germ cell cancer, and impaired spermatogenesis in adulthood[2,11,12]. EDCs act as estrogen mimetics or androgen antagonists, directly altering the signaling within the hypothalamic-pituitary-testicular (HPT) axis and compromising the structural integrity of the blood-testis barrier[13].

Moreover, the rising incidence of obesity and high body mass index (BMI) in the reproductive-age population contributes to an unfavorable endocrine milieu. Adipose tissue does not merely function as passive energy storage, but acts as an active endocrine organ, expressing high levels of the aromatase enzyme (*CYP19A1*)[14,15]. Excess adiposity promotes the peripheral aromatization of androgens, specifically driving the conversion of circulating testosterone into 17β-estradiol (E2)[2,14]. This hyperestrogenic

state contributes to a functional hypogonadotropic status, chronic systemic inflammation, and increased oxidative stress within the spermatogenic niche[14,15].

Notably, lifestyle factors such as maternal obesity, are also regarded as significant risk factors for impaired testicular development and TDS symptoms in offspring[11,16]. These factors, congenital, environmental, and metabolic, collectively impair Sertoli and Leydig cell functions, increase reactive oxygen species (ROS) production, and induce epigenetic DNA damage[13,17].

This multifaceted etiology of male infertility underscores the inadequacy of current diagnostic protocols, which often fail to account for the cumulative impact of these systemic stressors on male reproductive potential[12,13].

1.1.4 The diagnostic work-up of male infertility: the role and limitations of semen analysis

The clinical assessment of the male partner fundamentally relies on semen analysis[2]. According to international guidelines[2,18], it serves as the primary starting point for the evaluation of male reproductive health, focusing on a combination of macroscopic characteristics, such as volume and pH, and microscopic variables, primarily sperm concentration, motility, and morphology.

However, despite its role as the gold standard, semen analysis has several limitations in its diagnostic accuracy[19,20]. Pivotal clinical studies demonstrate a substantial overlap in these parameters between fertile and infertile populations, making it difficult to establish a clear-cut distinction based only on these metrics[20,21]. Consequently, a "normal" semen analysis does not inherently guarantee fertility, nor do seminal alterations strictly preclude the possibility of natural conception[18,21]. This creates a diagnostic gray zone where conventional semen analysis, while remaining an indispensable tool, proves insufficient to fully characterize an individual's reproductive potential, necessitating a more comprehensive clinical and physiological evaluation[2,18,20].

This diagnostic ambiguity is deeply rooted in the statistical nature of current andrological standards; indeed, according to the WHO 6th edition (2021), reference values represent merely the 5th centile of a fertile population and must therefore be interpreted as a statistical probability of the "lower limit" rather than a definitive threshold of biological fertility[19]. This conceptual shift from a dichotomous 'normal *versus*

abnormal' perspective toward a percentile-based assessment is crucial: it demonstrates that normozoospermia does not necessarily translate into functional fertility[20,22], just as a minor deviation from the 5th centile does not strictly imply infertility[19,21,23].

1.1.5 Endocrine assessment and the integrated evaluation of gonadal function

Beyond semen analysis, which quantifies just the sperm production of the testes, evaluating total gonadal function necessitates a thorough endocrine assessment of the steroidogenic compartment and the integrity of the HPT axis. In line with the guidelines from the Italian Society of Andrology and Sexual Medicine (SIAMS), the American Urological Association (AUA), and the American Society for Reproductive Medicine (ASRM), a first-level hormonal screening is strongly recommended in patients presenting with altered semen parameters, including any degree of oligo-astheno-teratozoospermia or azoospermia[18,24].

This baseline endocrine panel relies on the simultaneous measurement of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone (TT), which must be collected in the fasting morning state to account for circadian fluctuations[18,25]. Conditions altering sex hormone-binding globulin (SHBG), including obesity, type 2 diabetes, hepatopathy, or thyroid dysfunction, can produce discordance between total and free testosterone levels. In such cases, SHBG measurement and determination of the unbound fraction[26,27] are warranted to refine the interpretation of borderline TT values[25,28].

From this triad, a fundamental pathogenetic distinction can be drawn:

- **Primary spermatogenic failure (hypergonadotropic status):** elevated serum FSH serves as a marker of seminiferous tubule damage[24], typically associated with Sertoli cell-only syndrome, meiotic arrest, or Klinefelter syndrome[24,26]. Concomitant elevation of FSH and LH alongside low testosterone indicates global testicular failure, probably affecting both spermatogenic and steroidogenic compartments[26,27].
- **Secondary testicular failure (hypogonadotropic status):** low testosterone combined with low or inappropriately normal gonadotropins points toward a pre-testicular etiology, specifically hypogonadotropic hypogonadism (HH)[26].

In these circumstances, the work-up must expand to explore global pituitary function[24,26]. Measurement of serum prolactin is indicated to rule out hyperprolactinemia. This diagnostic path should be generally completed with sellar magnetic resonance imaging to exclude pituitary or hypothalamic lesions[25,29].

- **Isolated spermatogenic failure:** a distinct pattern of normal testosterone and LH with selectively elevated FSH indicates isolated damage to the seminiferous epithelium with preserved Leydig cell function[26–28].

These hormonal findings must be integrated with clinical findings, semen parameters, metabolic variables (such as BMI), and instrumental evaluation, such as by testicular ultrasound[24].

1.2 Physiology of the hypothalamic-pituitary-testicular axis

Male reproductive function is regulated by the HPT axis, a complex endocrine system characterized by a closed-loop feedback mechanism. Any disruption within this finely regulated axis can compromise fertility potential[2].

The hierarchical control of the axis begins upstream of the gonadotropin releasing hormone (GnRH) neurons, where kisspeptin, a neuropeptide produced in the arcuate nucleus, acts as the essential "gatekeeper" of reproductive function integrating metabolic and environmental signals to trigger and modulate the pulsatile release of GnRH[30]. In sexually mature men, GnRH is secreted intermittently at intervals ranging from 60 to 120 minutes. The pattern and the frequency of this pulsatility are mandatory for preserving pituitary sensitivity and ensuring the proper secretion of LH and FSH[31].

Within the testicular microenvironment, these gonadotropins engage specific cellular populations through high-affinity receptor interactions:

- LH stimulates Leydig cells to promote steroidogenesis and local testosterone synthesis. Spermatogenesis requires intra-testicular testosterone (ITT) concentrations above the 70 nM threshold to fully support the process[32].

- FSH binds to its receptors (FSHR) on Sertoli cells, triggering signaling cascades, including the expression of cyclic adenosine monophosphate (cAMP)

response element modulator isoforms, essential for germ cell maturation and survival[32]. This signaling is a prerequisite for maintaining overall testicular homeostasis[9] and the structural integrity of the blood-testis barrier[32]. Sertoli cell functions are multiple and fundamentally critical during the first steps of spermatogenesis, from spermatogonia to secondary spermatocytes, where they entirely surround the developing germ cells[33] and where the stimulatory action of FSH is especially exerted[12].

The axis is regulated by negative feedback loops: testosterone and its derivative, E2 (produced via aromatization by the enzyme aromatase in testicular and peripheral adipose tissues)[2], inhibit GnRH and LH secretion, while inhibin B acts as the primary selective inhibitor of pituitary FSH secretion[34].

Biochemically, inhibin B is a heterodimeric glycoprotein belonging to the TGF-β superfamily[35]. Composed of an α and a β_B subunit[36], it is a direct secretory product of the Sertoli cells, exhibiting a robust inverse correlation with serum FSH and a positive correlation with both sperm concentration and total testicular volume (TTV). Additionally, gonadotrope function and GnRH pulsatility are fine-tuned by other systemic hormones, including prolactin, whose homeostasis is secondary to lactotroph regulation[37] but deeply intertwined with HPT axis stability[12,38] (Figure 1).

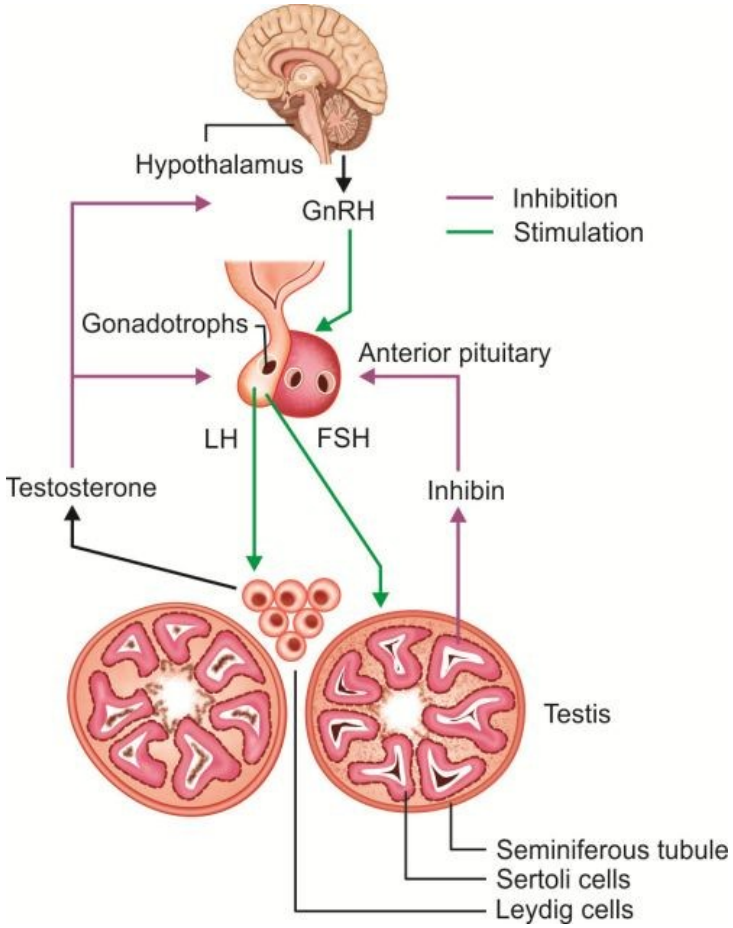

Figure 1: Schematic representation of the Hypothalamic-Pituitary-Testicular (HPT) axis.[38] *The diagram illustrates the hierarchical control of the hypothalamus over the gonads, detailing the cell populations and the main feedback mechanisms. Notes: FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone.*

1.3 Etiology of male infertility

The diagnostic evaluation of the infertile male requires a systematic taxonomic approach. According to the European Association of Urology Guidelines (2026) and recent literature, identifiable causes of male infertility are classified based on their primary site of impact along the HPT axis or the seminal tract[2,12].

1.3.1 Pre-testicular: endocrine and systemic disorders

This category encompasses hypothalamic-pituitary disturbances leading to secondary testicular failure[12]:

- **Hypogonadotropic hypogonadism (HH):** congenital or acquired deficiencies in GnRH pulsatility or LH and FSH secretion[2].

- **Hyperprolactinemia:** pathological increases in prolactin levels that interfere with the endocrine drive of the HPT axis[2,12].
- **Metabolic factors:** obesity contributes to a hyperestrogenic state (Section 1.1.3), clinically evidenced by an altered testosterone/estradiol ratio[2].

1.3.2 Testicular: primary gonadal failure

Direct damage to the seminiferous epithelium or the interstitial compartment represents the most common clinical finding[12]:

- **Congenital and developmental:** as a manifestation of TDS, cryptorchidism remains a major risk factor, often resulting in reduced TTV and a compromised germ cell niche[2,39].
- **Genetic factors:** including Klinefelter syndrome, Y-chromosome microdeletions (AZF region), and specific pharmacogenetic variants (e.g., *FSHB* and *FSHR* polymorphisms)[2,40].
- **Acquired insults:** conditions such as varicocele, testicular torsion, and infections (e.g., mumps orchitis). The severity of tubular damage is often signaled by a compensatory rise in baseline FSH[2,12].

1.3.3 Post-testicular: transport and accessory gland dysfunction

Conditions that impair the maturation, transport, or ejection of spermatozoa[12]:

- **Obstruction:** including congenital bilateral absence of the vas deferens or acquired blockages resulting from infection or surgery[2].
- **Inflammation and accessory gland dysfunction:** evaluation of semen pH, volume, and inflammatory markers helps identify states in the prostate or seminal vesicles that compromise sperm quality via oxidative stress[2,41]. In this context, serum prostate-specific antigen (PSA) serves as a surrogate marker of prostatic inflammation, as epithelial barrier disruption causes the antigen to leak into the systemic circulation[42]. Concurrently, alterations in seminal PSA concentration or its inactivation by anti-PSA antibodies are associated with hyperviscosity and defective liquefaction, further impairing sperm transit and functional integrity[43].

1.4 Idiopathic male infertility

When all identifiable anatomical, genetic, and obstructive factors are ruled out, the patient receives a diagnosis of idiopathic male infertility (IMI)[2,40]. This category, representing the most common presentation (accounting for 30-50% of cases)[9], marks the transition from a known etiological category to a more complex diagnostic challenge, which requires a deeper characterization as discussed in the following sections[12,40]. While it represents the largest subgroup of infertile men, the management of IMI is often hindered by the inherent limitations of standard diagnostic protocols[9]. Consequently, it is essential to establish a precise classification that accounts for both clinical definitions[22] and the statistical nature of current reference values[23].

1.4.1 Clinical definitions: idiopathic *versus* unexplained

A rigorous taxonomic distinction is required to differentiate "idiopathic" from "unexplained" cases, as they represent distinct pathophysiological entities[22].

IMI is defined by the presence of altered semen parameters, in the absence of any identifiable cause during a standard diagnostic work-up[22].

On the other hand, unexplained male infertility (UMI) refers to cases where a man is infertile despite a completely normal semen analysis and workup, and only after ruling out any identifiable female-factor cause[44].

1.4.2 The diagnostic challenge of the idiopathic phenotype

The therapeutic management of male infertility is frequently complicated by a discrepancy between conventional semen parameters and actual fertilizing potential[21,23]. This challenge arises because traditional semen analysis, based on the WHO reference centiles, provides only a quantitative snapshot of the testicular output rather than a comprehensive assessment of the qualitative and structural integrity of the spermatozoa[23].

This diagnostic gap inherent in standard protocols is primarily attributed to underlying cellular and molecular damage, which remains occult during routine microscopic examination. Key mechanisms of occult sperm damage include:

- Oxidative stress and membrane integrity: an imbalance between ROS and antioxidant defenses leads to the peroxidation of the sperm plasma membrane's

polyunsaturated fatty acids. This process compromises the acrosome reaction and the ability of the sperm to penetrate the oocyte[45].

- Sperm DNA fragmentation (SDF): high levels of SDF, resulting from impaired chromatin packaging during spermiogenesis or post-testicular oxidative stress, correlates negatively with both natural and assisted conception rates[7]. Crucially, significant DNA damage is frequently observed in men classified as "normozoospermic", potentially explaining many cases of unexplained infertility[22,46].

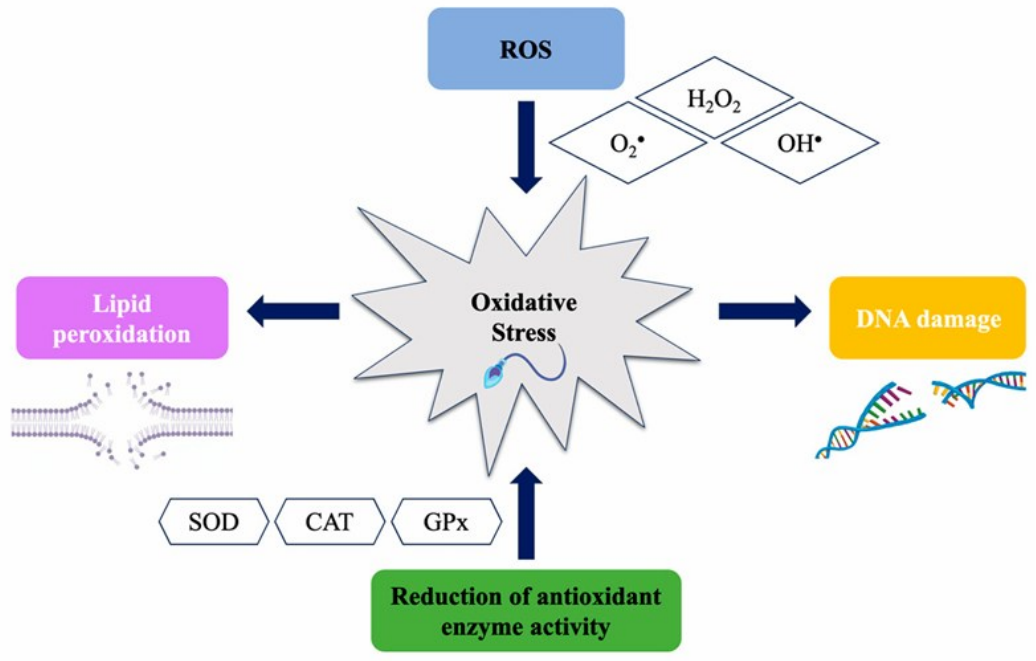

Figure 2: Cellular pathways of oxidative stress in spermatozoa.[18]
The illustration shows the biochemical imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, which leads to lipid peroxidation and compromises the integrity of sperm DNA. Notes: CAT = catalase; GPx = glutathione peroxidase; SOD = superoxide dismutase

Ultimately, this diagnostic challenge underscores that the secretory end-product of the testis (i.e., what is evaluated in the conventional semen analysis) is an insufficient proxy for the true functional integrity of the seminiferous epithelium[9,23].

1.5 Empirical FSH treatment

IMI represents a substantial proportion of male factor cases, leaving couples without targeted etiologic options[4,9,12]. To overcome this limitation, the empirical

administration of exogenous FSH is widely utilized to stimulate testicular function and maximize semen production[40].

1.5.1 Biological rationale and mechanism of FSH action on Sertoli cells

The therapeutic approach targets the complex regulation of the spermatogenic niche[9], operating through an "over-stimulatory" strategy[4,47]. By maximizing Sertoli cell recruitment and enhancing metabolic and structural support for developing germ cells[40,47], this intervention aims to boost spermatogenesis beyond baseline physiological thresholds. This pharmacological drive acts in synergy with endogenous ITT to optimize germ cell differentiation, particularly promoting up to the round spermatid stage[32,47].

1.5.2 Evidence for a functional reserve: the full spermatogenic potential

This stimulatory strategy is predicated on the evidence that the adult testis does not normally operate at its maximum capacity under baseline conditions[48]. Both experimental models and clinical observations suggest that physiological FSH concentrations leave a latent functional reserve that can be successfully recruited through exogenous gonadotropin stimulation[49].

Non-human primate models, specifically the adult rhesus monkey (*Macaca mulatta)*, demonstrate that unilateral orchidectomy triggers a robust adaptive response in the remaining gonad, characterized by a marked expansion of mature germ cell generations[50]. While the absolute number of adult Sertoli cells remains stable, unilateral orchidectomy induces a significantly tighter Sertoli cell-to-germ cell correlation, rising from $r = 0.44$ to $r = 0.92$[50], demonstrating an enhanced spermatogenic efficiency per cellular unit. This adaptive mechanism is driven by an elevation in endogenous FSH secondary to an abrupt decline in circulating inhibin B (approximately 50%)[50,51]. Testicular clamp models further confirm that a selective elevation in FSH, but not in LH, amplifies the mitotic pool by expanding the growth fraction of undifferentiated (A_p) spermatogonia entering the cycle[52].

Similar homeostatic adjustments occur in humans. Following unilateral orchidectomy for testicular cancer, mean sperm concentrations show a significant recovery from an early post-operative baseline of 26 million/mL (measured at 3 weeks) to 39

million/mL, with approximately 75% of subjects re-establishing normospermia within the first post-operative year[53]. This functional rebound highlights the capacity of the contralateral testis to scale up production under an intensified gonadotropin drive, a response that is most prominent when baseline FSH levels reside within the physiological range[53]. However, this reserve is ultimately constrained by the finite, non-renewable pool of Sertoli cells. In a cohort of 89 boys with monorchidism, this compensatory capacity exhibited a clear cellular dissociation: while Leydig cell function was largely preserved, Sertoli cell function remained deficient, as evidenced by lower serum anti-Müllerian hormone (AMH) and higher FSH[54]. This implies that chronic gonadotropin elevation can only partially offset the loss of the contralateral gland.

Further confirmation of this recruitable reserve is provided by cases of FSH-secreting pituitary macroadenomas. Affected patients often exhibit macroorchidism caused by chronic FSH hypersecretion, which results from an increase in seminiferous tubule length rather than diameter[55]. Notably, in the original series, three out of four patients had fathered children and all maintained normal sexual function. Furthermore, testicular volume and inhibin B levels regressed only after surgical normalization of FSH hypersecretion[55]. This pattern, corroborated across different age groups[56,57], confirms a selective tubular response to gonadotropin excess, reinforcing the hypothesis that therapeutic hyperstimulation can successfully unlock this underutilized spermatogenic potential.

1.5.3 Clinical efficacy on seminal parameters

Extensive evidence demonstrates that exogenous FSH administration significantly improves both quantitative and qualitative sperm parameters[40]. Beyond dose-dependent increases in sperm concentration and motility[40], FSH therapy effectively enhances genomic integrity by mitigating SDF, specifically decreasing double-strand breaks[7].

1.5.4 The therapeutic challenge: variability of response and resource optimization

Despite its proven biological potential, empirical FSH therapy is limited by significant inter-individual variability, whereby around 50% of patients are identified as "non-responders"[40]. A landmark 2015 meta-analysis established that the number needed to treat (NNT) ranges between 10 and 18 patients to achieve a single pregnancy,

accounting for both spontaneous and ART conceptions[58]. Although recent real-world data suggest a more favorable scenario, with pregnancy rates reaching approximately 25% (NNT ≈ 4)[59], the overall success rate remains suboptimal.

In daily practice, the human spermatogenic cycle requires 72 to 90 days to complete, necessitating 3 to 6 months of uninterrupted treatment before assessing efficacy[47]. The prolonged need for treatment, together with the high cost of the drug, makes it mandatory to optimize the efficacy of this therapy.

Consequently, transitioning from a generalized therapeutic model to a precision-medicine approach is imperative to direct pharmacological efforts exclusively toward patients in whom a measurable reproductive benefit can be expected[9,40].

1.5.5 Baseline phenotypic stratification: the APHRODITE framework

A fundamental prerequisite for the personalization of empirical hormonal treatments in male infertility is the systematic classification of patients who potentially could benefit from hormonal stimulation based on the integration of baseline seminal and endocrine parameters[60]. This stratified approach has been successfully established in female reproductive medicine through the POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) criteria[61]. These criteria stratify low-prognosis women undergoing ART into clinically distinct subgroups based on female age, ovarian reserve biomarkers, and expected ovarian response to gonadotropin stimulation, thereby enabling tailored management strategies[61]. Following this conceptual model, the APHRODITE (Addressing male Patients with Hypogonadism and/or infertility Owing to altereD, Idiopathic Testicular function) system was developed through an international consensus process[60]. This framework provides a standardized, phenotype-based stratification of infertile males independent of the underlying etiology, utilizing routine diagnostic laboratory variables to guide therapeutic interventions to optimize outcomes[60]. Specifically, it categorizes individuals into five distinct phenotypes based on their baseline endocrine and spermatogenic profile:

- **Group 1 (hypogonadotropic hypogonadism):** characterized by congenital or acquired HPT axis failure, presenting with reduced serum FSH and LH concentrations, low TT levels, and lowered semen analysis parameters[60].

- **Group 2 (normogonadic infertility):** defined by lowered semen analysis parameters in the presence of normal serum concentrations of both FSH and TT, representing a state where baseline circulating hormones appear insufficient to support optimal spermatogenesis. This condition could represent the 'true idiopathic' infertility[60].
- **Group 3 (normogonadic hypogonadism):** marked by lowered semen analysis parameters and normal serum FSH levels, accompanied by low-normal TT concentrations signifying a state of functional hypogonadism[60].
- **Group 4 (hypergonadotropic hypogonadism):** comprises patients with lowered semen analysis parameters and elevated serum FSH levels, presenting with either normal or reduced TT concentrations[60].
- **Group 5 (unexplained male infertility):** consists of men presenting with normal semen analysis parameters, normal serum FSH, and normal TT concentrations, evaluated within the clinical context of unexplained couple infertility[60].

1.6 The search for predictive markers: state of the art

1.6.1 The prognostic failure of baseline semen parameters

Considering the diagnostic limitations established in Section 1.1.4, current evidence indicates that baseline sperm concentration, motility, and morphology are poor predictors of therapeutic success[9].

In the real-world study by Santi et al. (2025), no significant differences were found in baseline (V_0) prevalence of semen analysis alterations between men who subsequently achieved pregnancy and those who did not[9]. Specifically, the prevalence of oligozoospermia or asthenozoospermia at baseline was comparable between responders (those achieving pregnancy) and non-responders, suggesting that the initial degree of seminal impairment does not dictate the regenerative capacity of the spermatogenic niche under FSH stimulation[9].

This lack of correlation emphasizes that the quantitative output at V_0 represents a static endpoint rather than a dynamic indicator of the testis's regenerative capacity,

necessitating the search for a predictive marker that reflects the functional reserve (the HPT axis and testicular volume), rather than the secretory output (spermatozoa)[9].

1.6.2 Endocrine evaluation of the HPT axis and accessory gland function

The biochemical evaluation of the HPT axis remains a cornerstone in the diagnostic workup of male infertility. This assessment relies on basal measurement of FSH, LH and TT. Isolated and static hormonal measurements, however, often fail to reflect the dynamic sensitivity of the germinal epithelium, leading to significant uncertainty in managing therapeutic strategies[48].

To date, attempts to identify reliable baseline biochemical markers capable of predicting the qualitative or quantitative response to exogenous FSH stimulation have been largely unsuccessful. The prognostic significance of the parameters investigated can be summarized as follows:

- **Basal FSH:** serum FSH levels indirectly reflect the functional status of the germinal epithelium *via* negative feedback mechanisms[36]. While elevated FSH points toward primary testicular failure and a normal level suggests an obstructive etiology, this differentiation is often oversimplified, as normal FSH values can coexist with secretory defects (e.g., maturation arrest or focal spermatogenesis)[36]. From a therapeutic perspective, basal FSH serves to guide clinical decision-making rather than predict outcomes: markedly elevated levels rule out the rationale for exogenous FSH administration, as sustained high FSH concentrations induce homologous down-regulation of FSH receptor gene expression and binding capacity[62]. Conversely, normal or low-normal levels justify a trial of testicular overstimulation[48,63]; however, a definitive cut-off value for candidate selection has not been established, and empirical thresholds lack an evidence-based foundation regarding the actual response to treatment[9,64].
- **Basal LH:** reflecting the pituitary drive on Leydig cells, basal LH assessment is indispensable for evaluating the steroidogenic component of the HPT axis[63]. When interpreted alongside serum TT, it allows clinicians to differentiate between hypergonadotropic hypogonadism (testicular failure) and hypogonadotropic hypogonadism (central failure). While crucial for the baseline diagnostic

characterization of the patient, isolated LH levels hold no independent predictive capacity regarding spermatogenic recovery or response to FSH therapies.

- **Inhibin B:** synthesized directly by Sertoli cells, this glycoprotein provides a direct feedback signal for FSH production and closely correlates with TTV and total sperm number[36,65]. Although receiver operating characteristic analysis highlights its high diagnostic accuracy in differentiating competent from impaired spermatogenesis, its routine clinical use remains limited due to the lack of universally established reference ranges, variability across immunoassay methodologies, and limited clinical relevance for individual patient management[65,66]. Furthermore, its isolated baseline predictive power regarding the efficacy of FSH therapy remains clinically incomplete[64,65].

Collectively, these markers provide a comprehensive endocrine and metabolic profile, but their isolated prognostic weight remains strictly limited. A specific hormonal level, indeed, does not reflect the dynamic response capacity of the seminiferous epithelium when considered without the context of the total anatomical mass it is intended to stimulate[63].

Beyond the endocrine feedback of the HPT axis, the diagnostic workup incorporates the evaluation of accessory gland function, primarily through the measurement of PSA[67]. In the andrological context, PSA is investigated as a marker of prostatic secretory activity[43]. As an androgen-dependent glycoprotein, low serum PSA levels in infertile patients may highlight a state of functional hypoandrogenism within the reproductive tract[67]. Conversely, elevated PSA levels serve as an indicator of localized inflammatory or infectious processes, such as prostatitis or male accessory gland infections[2,43]. These conditions can significantly impair overall semen quality by inducing oxidative stress and leukocytospermia, thus representing an independent confounding factor in the evaluation of idiopathic subfertility[2].

1.6.3 Pharmacogenetics frontier: *FSHR* and *FSHB* polymorphisms

The inter-individual variability in response to FSH therapy is significantly influenced by genetic determinants governing hormone biosynthesis, receptor expression, and downstream molecular signaling[48,68], explaining why clinically identical patients often exhibit divergent outcomes following the same pharmacological stimulation[69].

- ***FSHR* p.N680S polymorphism:** this single-nucleotide polymorphism (SNP) in the *FSHR* gene modulates intracellular signaling kinetics, specifically affecting the activation of the cAMP/CREB and ERK1/2 pathways[69]. The presence of the serine (S) allele at position 680 is associated with a higher threshold for FSH action, suggesting a relative resistance that may require increased exogenous doses to achieve a biological response[70]. Clinical evidence and recent reports confirm the successful translation of this pharmacogenomic approach, demonstrating that men homozygous for the asparagine (N) allele exhibit greater reductions in SDF and more favorable ART outcomes following recombinant FSH administration, compared to S-allele carriers[69,71].
- ***FSHB* -211G>T polymorphism:** located in the promoter region of the *FSHB* gene, this SNP impairs gene expression by disrupting the binding site for key transcription factors[70], which multi-gene analysis has associated with attenuated circulating FSH, reduced testicular volumes, and diminished sperm number[68,72]. However, phenotypic characterization across different populations reveals critical nuances; a pivotal study on an Italian cohort, demonstrated that while the -211 TT genotype is strongly associated with lower serum FSH concentrations, baseline conventional semen parameters, including sperm number, motility, and morphology, do not differ statistically from GG or GT carriers, displaying only a downward trend[73]. Notably, men homozygous for the T-allele often present a "hypogonadotropic-like" phenotype of idiopathic infertility, making them highly sensitive responders to exogenous FSH administration, which acts as a targeted pharmacological rescue of their constitutional biosynthetic deficit[73]. Specifically, prospective data show that standard-dose recombinant human FSH therapy (150 IU three times per week) induces a therapeutic benefit in TT homozygotes, yielding a 100% response rate (defined as a doubling of total sperm number from baseline)[73]. Furthermore, 66.7% of these patients achieve full normozoospermia post-treatment, a pharmacogenetic response profile significantly superior to that observed in G-allele carriers[73].

Evidence suggests that the impact of these genetic variants is not merely additive but synergistic[48,72]. In this integrated genetic framework, the *FSHB* variant exerts a distinct dosage effect on serum FSH levels, while the *FSHR* counterpart modulates the

downstream gonadal response, providing a far more accurate prediction of reproductive parameters than any single marker[72]. Patients carrying unfavorable alleles in both genes exhibit the lowest testicular volumes and sperm concentrations[72]. This combinatorial perspective is essential for identifying "true responders" who may benefit most from FSH stimulation[48,70], although individual single-locus evidence underscores that constitutional biosynthetic deficits, such as the *FSHB* -211 TT genotype, can be successfully managed through standard stimulatory or replacement-like regimens rather than aggressive over-stimulation[73].

1.6.4 Clinical gap: the need for integrated surrogate markers

The evidence analyzed in this chapter reveals a persistent diagnostic void in the management of IMI. Neither baseline seminal parameters, nor isolated biochemical markers, nor complex pharmacogenetic profiles have yet provided a universally accessible and low-cost tool to *a priori* predict FSH efficacy[9,70]. The limitation lies in the lack of an integrated metric capable of synthesizing dynamic endocrine signaling with the actual anatomical volume of the gonadal target[9].

To bridge this gap, a transition toward a multiparametric surrogate phenotype could be an option, one that can transform individual clinical variables into a comprehensive measure of testicular functional reserve, as detailed in the following section[9].

1.7 The Testicular Index (TI): A multiparametric tool for precision medicine

The limitations of single-parameter assessments and the current unstratified therapeutic model necessitate a transition toward personalized treatment strategies[9].

To fulfill the diagnostic need for precision medicine and address the aforementioned diagnostic gap (Section 1.6.4), the Testicular Index (TI) has been conceptualized as an applicative solution designed to synthesize endocrine and morphological data into a unified, low-cost predictive metric[9].

The TI is calculated using the following formula[9]:

$$TI = \frac{Basal\ FSH\ (IU/L) + Total\ Testosterone\ (ng/mL)}{Bi - testicular\ Volume\ (mL)}$$

This index aims to define the functional reserve of the testis by simultaneously evaluating three critical components of male reproductive health[9]:

- The interstitial compartment function (numerator): represented by serum TT concentrations[9], mirroring the secretory output of Leydig cells[14].
- The spermatogenic potential (numerator): represented by basal FSH concentrations[9], which reflect the pituitary drive and the feedback mechanism of the germinal epithelium[34,74].
- The target tissue amount (denominator): represented by bi-testicular volume. This serves as a proxy for the total mass of the seminiferous tubules, representing the global anatomical substrate available for sperm production and the primary target for exogenous FSH stimulation[9].

From a pathophysiological standpoint, the predictive power of this tool lies in its biological rationale. As demonstrated, a lower baseline TI suggests that for a given testicular volume, the endocrine drive is relatively diminished. This indicates a functional deficit with a preserved biological reserve rendering the germinal epithelium more responsive to exogenous pharmacological stimulation[9].

Recent real-world data confirm that a reduced TI is a reliable independent predictor of pregnancy success[9]. While traditional semen parameters may improve following treatment regardless of the final outcome, only the patient's age and the baseline TI have demonstrated the robust ability to discriminate between true "responders" and "non-responders" in terms of achieving clinical conception[9].

MATERIALS AND METHODS

2.1 Study design and population

This retrospective, observational, real-world study was conducted at the Unit of Andrology, Department of Endocrinology, University Hospital of Modena, Italy. Consecutive male partners of infertile couples treated with FSH for IMI were included. The clinical database was locked and updated for analysis on May 17, 2026.

Patients were eligible if they had undergone a complete andrological diagnostic work-up, including medical history, physical examination, testicular ultrasound, hormonal assessment, and semen analysis before FSH prescription. Idiopathic infertility was defined as the absence of identifiable causes of male infertility after standard diagnostic evaluation. Female causes of infertility were excluded according to routine couple assessment.

For each patient, two time points were considered: the baseline visit, before FSH treatment initiation (V0), and the post-treatment visit, corresponding to the last available follow-up after FSH administration or treatment discontinuation (V1). Patients without both V0 and V1 data were considered for cross-sectional baseline analyses when appropriate, whereas paired analyses were restricted to subjects with both visits available.

2.2 Clinical practice

The routine diagnostic and therapeutic pathway followed in clinical practice at the Unit of Modena has been described elsewhere[9,59,75] and was based on the position statement of the Italian Society of Andrology and Sexual Medicine on the topic[24]. Specifically, the diagnostic work-up included conventional semen analysis, hormonal assessment, genetic testing, and scrotal ultrasound examination. Only after the exclusion of all identifiable causes of male infertility, FSH empirical treatment was proposed to the patient.

2.3 Treatment

FSH treatment was prescribed according to clinical practice and Italian reimbursement criteria[76], following the fixed scheme of 150 IU three times weekly for four months, eventually renewed for up to 12 months of treatment or until pregnancy achievement. Treatment duration was expressed in months and recorded. The cumulative FSH dose administered during the study period was calculated for each patient.

2.4 Pregnancy

Pregnancy occurrence was collected during treatment or follow-up and coded as a binary outcome. When available, pregnancy modality was also recorded, distinguishing among spontaneous pregnancy, pregnancy obtained after ART, oocyte donation, miscarriage, and suspended cycle.

2.5 Data collection

The following baseline variables were collected: male partner age, female partner age, history of cryptorchidism, body weight, height, BMI, right and left testicular volume, bi-testicular volume, serum TT, LH, FSH, estradiol, prolactin, PSA, and the APHRODITE score[60]. In addition, treatment-related variables, including cumulative FSH dose, were systematically recorded.

Semen analysis parameters included semen volume, pH, sperm concentration, total sperm number, progressive motility, total motility, typical forms, and total motile sperm number. Changes in sperm concentration after treatment were calculated both as absolute variation (Δ sperm concentration) and percentage variation (%Δ sperm concentration) when paired data were available.

Data were collected at baseline (V0) and at FSH discontinuation (V1).

2.6 Candidate testicular indexes

The main objective of the study was to identify the best TI able to predict pregnancy achievement and/or the response to FSH treatment. Candidate indexes were generated

by combining routinely available markers reflecting different components of testicular function: endocrine stimulation, Leydig cell function, gonadotropin drive, and testicular volume[9].

The previously proposed index was recalculated as:

$$TI_1 = \frac{FSH + total\ testosterone}{Bi - testicular\ volume}$$

Additional candidate indexes were generated using different mathematical combinations of FSH, LH, TT, and testicular volume. Candidate formulas included, but were not limited to:

$$TI_2 = \frac{FSH \times total\ testosterone}{Bi - testicular\ volume}$$

$$TI_3 = \frac{FSH + LH + total\ testosterone}{Bi - testicular\ volume}$$

$$TI_4 = \frac{FSH \times LH \times total\ testosterone}{Bi - testicular\ volume}$$

Indexes including semen parameters were excluded, because semen analysis is highly variable and may limit the clinical robustness of a predictive tool[9].

2.7 APHRODITE-based patient stratification

Patients were retrospectively classified according to the APHRODITE criteria, a recently proposed stratification system for infertile men based on conventional semen parameters, serum FSH concentrations, and serum TT levels[60]. The APHRODITE system classifies patients into five groups, and this classification was performed using baseline data collected before FSH treatment initiation. Semen parameters were categorized as normal or lowered according to WHO reference limits[19], whereas FSH serum levels were classified as normal or elevated considering the threshold of 8 IU/L and serum testosterone levels were considered reduced or normal considering a threshold of 350 ng/dL[60].

2.8 Statistical analysis

Data distribution was assessed using the Kolmogorov-Smirnov test and by visual inspection of histograms and Q-Q plots. Continuous variables were reported as mean ± standard deviation or as median and interquartile range, according to their distribution. Categorical variables were reported as number and percentage.

The analysis was conducted stepwise:

- First, baseline characteristics of the cohort were described. The efficacy of FSH treatment was assessed by comparing V0 and V1 values in paired subjects, using the Wilcoxon signed-rank test.
- Second, patients whose partner obtained pregnancy were compared to those in the non-pregnant group, using the Mann-Whitney U-test for continuous variables.
- Third, each candidate TI was tested for biological plausibility. Correlations between baseline candidate indexes and baseline semen parameters were evaluated using Spearman's rank correlation coefficient. The association between each baseline candidate index and pregnancy achievement was assessed, comparing candidate indexes between the pregnancy and non-pregnancy groups. Univariable logistic regression models were then fitted using pregnancy achievement as the dependent variable and each candidate index as the independent variable[9].
- Fourth, multivariable logistic regression models were generated to identify independent predictors of pregnancy achievement. Covariates included clinically relevant baseline variables, such as male age, BMI, FSH, LH, TT, bi-testicular volume, baseline semen parameters and candidate TIs, as well as treatment-related factors (cumulative FSH dose and human chorionic gonadotropin therapy). This analysis was performed considering both baseline and post-treatment parameters.
- Fifth, the predictive performance of each candidate index was evaluated using receiver operating characteristic (ROC) curve analysis. Area under the curve (AUC), 95% confidence interval (CI), sensitivity, specificity, positive

predictive value, and negative predictive value were calculated. The best cut-off for each index was identified using Youden's index.

- Sixth, secondary analyses evaluated whether the selected index predicts improvement in semen parameters after FSH treatment. Absolute and percentage changes in sperm concentration and total motile sperm number were used as secondary outcomes[75].
- Seventh, APHRODITE-based subgroup analyses were performed. Patients were stratified according to APHRODITE groups and data were compared across these groups using the Kruskal-Wallis test or the Mann-Whitney U-test, where appropriate[60].
- Eighth, the efficacy of FSH treatment was assessed within each APHRODITE group by comparing V0 and V1 semen parameters using paired tests. Logistic regression models were used to evaluate whether the APHRODITE classification predicts pregnancy achievement.

Finally, the distribution and predictive performance of the selected TI were evaluated within each APHRODITE group. TI values were compared across APHRODITE groups and between patients achieving or not achieving pregnancy within each group. ROC curve analyses were repeated in the APHRODITE subgroups when the sample size allowed, to assess whether the discriminative ability of the selected index differs according to the patient phenotype.

A two-sided p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS.

RESULTS

A total of 228 infertile couples were finally enrolled, in which the male partner was treated with FSH in accordance with the Italian Medicines Agency (AIFA) Note 74 criteria for drug reimbursement[76].

3.1 Treatment efficacy

The average treatment duration was 8.7 ± 5.0 months (range: 2.4–12.0 months). Following the therapeutic regimen, a total of 73 biochemical pregnancies were recorded (32.0%). Among these, 5 early spontaneous miscarriages occurred, representing a miscarriage rate of 6.8% within the pregnant subcohort. Consequently, 68 confirmed clinical pregnancies were achieved (29.8%), including 52 following ART (22.8% of the total cohort) and 16 conceived naturally (7.0%).

Comparison of data collected at V0/V1 showed a significant increase after treatment in both sperm concentration (from 4.4 million/mL, IQR 1.2–9.4, to 6.46 million/mL, IQR 2.0–15.0, $Z=-5.723$, $p < 0.001$) and total sperm number (from 10.0 million, IQR 2.88–21.69, to 16.99 million, IQR 5.00–37.11, $Z=-5.652$, $p < 0.001$) (Table 1).

In detail, among the 224 patients who underwent the follow-up semen analysis at V1 (4 patients did not undergo the test), sperm concentration increased in 149 patients (66.5%), decreased in 62 (27.7%) and did not change in 13 (5.8%). Similarly, progressive and total motility significantly improved after FSH treatment. Median progressive motility increased from 10% (IQR 1–25) at V0 to 15% (IQR 3–27) at V1 ($Z=-2.009$, $p = 0.044$) (Table 1). Median total motility increased from 15% (IQR 5–30) to 20% (IQR 6–35) ($Z=-2.447$, $p = 0.014$) (Table 1). In contrast, no significant changes in sperm morphology were observed after treatment ($p = 0.907$) (Table 1).

Regarding hormonal parameters, serum TT levels showed a small but statistically significant increase after treatment (from 4.55 ng/mL, IQR 3.6–5.7, to 4.60 ng/mL, IQR 3.5–6.0, $Z=-2.716$, $p = 0.007$) (Table 1). Considering other hormones, FSH serum levels significantly increased after treatment (from 4.60 IU/L, IQR 2.95–6.00, to 7.15

IU/L, IQR 3.95–9.65, $Z=-5.027$, $p < 0.001$), although mean post-treatment FSH levels (7.1 ± 4.0 IU/L) remained well below 8 IU/L (Table 1).

***Table 1**. Patients' characteristics at baseline and after treatment with FSH of the male partner. The p-value was obtained using the Wilcoxon signed-rank test. [Notes: BMI = body mass index; FSH = follicle-stimulating hormone; LH = luteinizing hormone; PSA = prostate-specific antigen]*

	Baseline (V0)				**After Treatment (V1)**						
	Mean	**Std. Deviation**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**	**Number**	**%**	***p*-value**
Male Age (years)	37.9	6.0	23.3	59.5	-	-	-	-			
Treatment Duration (months)	-	-	-	-	8.7	5.0	2.4	12.0			
Pregnancy (biochemical)									73	32.0	
Pregnancy (clinical)									68	29.8	
After ART									52	22.8	
Natural conception									16	7.0	
Female Age (years)	33.5	4.4	20.0	46.0	-	-	-	-			
Cryptorchidism									17	7.4	
Testicular Volume Right (mL)	15.9	4.6	5.6	34.6	15.9	5.0	5.6	34.6			0.961
Testicular Volume Left (mL)	15.1	4.6	4.1	30.6	15.2	5.0	4.1	30.6			0.930
Bi-testicular Volume (mL)	31.0	8.6	12.5	62.8	31.1	9.0	12.5	62.8			0.942
Total Testosterone (ng/mL)	4.8	2.3	1.1	19.8	5.0	2.0	1.0	13.1			**0.007**
LH (IU/L)	3.6	1.4	0.7	9.5	3.5	2.0	0.1	8.2			0.758
FSH (IU/L)	4.5	1.9	0.9	10.2	7.1	4.0	0.9	15.7			**<0.001**
APHRODITE group											
1									0	0	
2									176	77.2	
3									52	22.8	
4									0	0	
5									0	0	

PSA (ng/mL)	2.2	2.0	0.2	9.1	2.5	2.5	0.3	13.2			0.313
Estradiol (pg/mL)	12.1	13.0	0.1	48.0	17.5	15.9	0.2	63.0			**0.008**
Prolactin (ng/mL)	12.8	5.3	4.3	30.9	10.5	4.1	4.8	20.2			-
Semen volume (mL)	2.8	1.6	0.3	11.5	3.0	1.7	0.5	15.5			0.291
pH	8.0	0.3	7.0	9.0	8.0	0.2	7.2	9.2			0.416
Sperm Concentration (million/mL)	7.8	13.6	0.0	113.0	13.8	23.9	0.0	219.0			**<0.001**
Delta Sperm Concentration	-	-	-	-	6.2	21.0	-32.5	215.5			
Delta Sperm Concentration (%)	-	-	-	-	338.0	1337.8	-100.0	14200.0			
Total Sperm Number (million)	20.8	49.3	0.0	519.8	36.9	67.2	0.0	527.0			**<0.001**
Progressive Sperm Motility (%)	15.1	15.1	0.0	73.0	18.6	25.8	0.0	313.0			**0.044**
Total Sperm Motility (%)	19.2	17.2	0.0	81.0	21.9	18.1	0.0	88.0			**0.014**
Typical Sperm Forms (%)	1.6	3.2	0.0	25.0	1.3	1.9	0.0	14.0			0.907
Total motile sperm number	3.6	9.1	0.0	85.8	8.9	20.2	0.0	164.6			**<0.001**
Weight (kg)	86.0	16.0	60.0	140.0	86.1	16.3	60.0	147.0			
Height (m)	1.8	0.1	1.6	2.0	1.8	0.1	1.6	2.0			
BMI (kg/m^2)	27.6	4.8	18.9	43.3	27.6	4.8	18.9	43.3			

3.2 Comparison according to pregnancy achievement

Patients whose partners achieved pregnancy were significantly younger than those who did not achieve pregnancy ($p = 0.007$) and showed, at baseline, lower serum FSH levels ($p = 0.020$), and higher bi-testicular volume ($p = 0.003$) (Table 2). After treatment, men whose partners conceived showed a significantly greater increase in sperm concentration, both in absolute and percentage delta values ($p < 0.001$ and $p = 0.012$, respectively).

When patients were stratified according to pregnancy outcome, significant improvements in sperm concentration and total sperm number were observed in both groups. Among patients whose partners achieved pregnancy, median total sperm number significantly increased (from 10.90 million, IQR 2.45–21.78, to 21.75 million, IQR 11.80–50.10, $Z=-4.866$, $p < 0.001$). Among patients whose partners did not achieve pregnancy, a significant increase was observed in sperm concentration (from 4.33 million/mL, IQR 1.2–10.0, to 5.80 million/mL, IQR 1.58–14.0, $Z=-3.293$, $p < 0.001$) and total sperm number (from 9.69 million, IQR 2.99–21.96, to 14.01 million, IQR 3.70–32.51, $Z=-3.293$, $p < 0.001$).

Regarding sperm motility, different patterns emerged according to pregnancy outcome. Among patients whose partners achieved pregnancy, both progressive and total motility significantly improved following treatment. Median progressive motility increased from 10% (IQR 5–24) at baseline to 15.5% (IQR 5–38) at follow-up ($Z=-2.893$, $p = 0.004$), while median total motility increased from 15% (IQR 10–30) to 25% (IQR 10–40) ($Z=-3.340$, $p < 0.001$). Conversely, among patients with unsuccessful outcomes, no significant changes were observed in either progressive or total motility after treatment ($p = 0.755$ and $p = 0.553$, respectively).

Regarding hormonal parameters, different trends were observed according to pregnancy outcome. Among patients whose partners achieved a pregnancy, serum TT levels significantly increased after treatment (from median 4.90 ng/mL, IQR 3.0–6.3, to 6.30 ng/mL, IQR 3.45–9.50, $Z=-3.430$, $p < 0.001$) (Table 2). Conversely, TT levels did not significantly change after treatment in men whose partner did not achieve a pregnancy ($p = 0.802$) (Table 2).

Furthermore, at post-treatment evaluation (V1), men whose partners achieved pregnancy exhibited a significantly higher percentage of typical sperm forms compared to the non-pregnant group ($p < 0.001$) (Table 2).

Table 2. *Patients' characteristics at baseline and after treatment with FSH of the male partner, dividing the cohort according to the pregnancy achievement. The p-value was obtained using the Wilcoxon signed-rank test when V1 was compared to V0 and with Mann-Whitney U-test when data were compared between the two groups. [Notes: BMI = body mass index; FSH = follicle-stimulating hormone; LH = luteinizing hormone]*

	Pregnancy achieved (n=68)			**Pregnancy not achieved (n=160)**			**V0 comparison**	**V1 comparison**
	Baseline	**After treatment**	***p*-value**	**Baseline**	**After treatment**	***p*-value**	***p*-value**	***p*-value**
Male Age (years)	36.2 ± 4.7	-	-	38.6 ± 6.4	-	-	**0.007**	-
Treatment Duration (months)	-	7.5 ± 3.2	-	-	8.1 ± 3.4	-	-	0.199
Female Age	32.6 ± 4.1	-	-	33.9 ± 4.5	-	-	0.056	-
Testicular Volume Right (mL)	16.9 ± 4.6	16.9 ± 4.5	0.963	15.4 ± 4.6	15.5 ± 4.6	0.936	**0.026**	**0.025**
Testicular Volume Left (mL)	16.7 ± 4.7	16.7 ± 4.6	0.956	14.4 ± 4.3	14.5 ± 4.3	0.884	**<0.001**	**<0.001**
Bi-testicular Volume (mL)	33.6 ± 8.9	33.7 ± 8.7	0.958	29.9 ± 8.3	30.0 ± 8.3	0.904	**0.003**	**0.003**
Total Testosterone (ng/mL)	4.6 ± 1.8	5.0 ± 2.7	**<0.001**	5.2 ± 2.4	4.7 ± 1.7	0.802	0.096	0.517
LH (IU/L)	3.6 ± 1.4	3.6 ± 1.4	0.945	3.6 ± 1.4	3.5 ± 2.0	0.760	0.981	0.924
FSH (IU/L)	4.1 ± 1.6	6.0 ± 3.3	**<0.001**	4.7 ± 1.9	7.4 ± 3.6	**<0.001**	**0.020**	0.166
Estradiol (pg/mL)	11.6 ± 12.0	17.6 ± 17.9	0.128	12.2 ± 13.4	17.4 ± 15.2	0.032	0.794	0.976
Prolactin (ng/mL)	12.8 ± 4.6	9.1 ± 4.7	0.037	12.7 ± 5.5	10.7 ± 4.4	0.116	0.984	0.381
Semen volume (mL)	3.0 ± 1.8	3.2 ± 2.0	0.592	2.7 ± 1.5	2.8 ± 1.4	0.396	0.144	0.116
pH	8.0 ± 0.2	8.0 ± 0.2	0.893	8.0 ± 0.3	8.0 ± 0.3	0.292	0.373	0.953
Sperm Concentration (million/mL)	5.3 ± 4.6	18.4 ± 30.3	**<0.001**	8.9 ± 15.9	11.8 ± 20.3	**<0.001**	0.071	0.058
Delta Sperm Concentration	-	13.2 ± 29.8	-	-	2.9 ± 15.0	-	-	**<0.001**
Delta Sperm Concentration (%)	-	706 ± 2068	-	-	192.3 ± 870.3	-	-	**0.012**
Total Sperm Number (million)	14.5 ± 14.1	50.5 ± 76.9	**<0.001**	23.5 ± 58.1	31.0 ± 61.8	**<0.001**	0.211	0.045
Progressive Sperm Motility (%)	14.4 ± 12.9	22.3 ± 18.4	**0.004**	15.4 ± 16.1	17.0 ± 29.2	0.755	0.665	0.161
Total Sperm Motility (%)	17.9 ± 14.3	27.5 ± 18.4	**<0.001**	19.8 ± 18.4	19.5 ± 16.8	0.553	0.440	**0.002**
Typical Sperm Forms (%)	1.9 ± 4.0	1.9 ± 2.5	0.970	1.5 ± 2.8	1.0 ± 1.4	0.075	0.323	**<0.001**
Total motile sperm number	2.5 ± 3.6	14.9 ± 28.3	**<0.001**	4.0 ± 10.6	6.3 ± 14.8	0.112	0.249	**0.003**
BMI (kg/m^2)	27.9 ± 4.7	-	-	27.5 ± 4.9	-	-	0.691	-

3.3 Testicular Index

Spearman's rank correlation analysis was performed to evaluate the biological plausibility of the proposed TIs by assessing their association with baseline semen parameters. TI2 and TI3 did not show significant correlations with sperm concentration, total sperm number, progressive motility, total motility, sperm morphology, or number of motile spermatozoa (Table 3). Similarly, TI4 was not significantly associated with any conventional semen parameters (Table 3). Conversely, TI1 showed a weak but statistically significant negative correlation with sperm concentration, total sperm number and progressive motility (Table 3).

Table 3. *Correlation analyses, performed with Spearman's Rho and evaluating the four testicular indexes (TIs) proposed and semen analysis parameters.*

Semen parameter	**TI1**	**TI2**	**TI3**	**TI4**
Sperm concentration	−0.114 (p=0.036)	−0.050 (p=0.453)	−0.050 (p=0.453)	−0.074 (p=0.295)
Total sperm number	−0.165 (p=0.019)	−0.120 (p=0.070)	−0.120 (p=0.070)	−0.123 (p=0.080)
Progressive motility	-0.103 (p=0.046)	0.043 (p=0.517)	0.043 (p=0.517)	0.056 (p=0.430)
Total motility	−0.007 (p=0.918)	0.052 (p=0.431)	0.052 (p=0.431)	0.077 (p=0.276)
Typical sperm forms	−0.057 (p=0.129)	−0.062 (p=0.352)	−0.062 (p=0.352)	−0.070 (p=0.323)
Number of motile sperm	−0.073 (p=0.302)	−0.020 (p=0.760)	−0.020 (p=0.760)	−0.020 (p=0.779)

Candidate TIs were compared between patients who achieved pregnancy and those who did not. At V0, all four indexes showed significant differences between the two outcome groups. Patients achieving pregnancy had significantly lower baseline values of TI1 ($Z=-3.157$, $p = 0.002$), TI2 ($Z=-3.157$, $p = 0.002$), TI3 ($Z=-2.952$, $p = 0.003$), and TI4 ($Z=-2.400$, $p = 0.016$) compared with patients who did not achieve pregnancy.

Univariable logistic regression analysis was performed to evaluate the association between candidate TIs and pregnancy achievement. At V0, TI1 was significantly associated with pregnancy outcome (OR 0.315, 95% CI 0.159–0.625, $p < 0.001$), indicating that higher baseline index values were associated with a lower likelihood of achieving pregnancy. The model showed a modest predictive performance, with a Nagelkerke R^2 of 0.086.

3.4 Predictive parameters of treatment efficacy

At V0, multivariable logistic regression analysis identified male age, bi-testicular volume, and TI as independent predictors of pregnancy achievement (Table 4). Conversely, in the post-treatment model (V1), no variable retained independent predictive significance, although progressive and total motility showed borderline associations with pregnancy outcome.

Table 4. *Multivariable logistic regression analyses evaluating independent predictors of pregnancy achievement using baseline (V0) and post-treatment (V1) clinical, hormonal, seminal, and testicular index (TI) parameters. Data are reported as odds ratios (ORs) with 95% confidence intervals (95% CIs). Significant p-values (<0.05) are highlighted in bold. V0 = baseline evaluation; V1 = post-treatment evaluation. OR = Odds Ratio*

Variable	Baseline model (V0) OR (95% CI)	*p*-value	Post-treatment model (V1) OR (95% CI)	*p*-value
Male age	**1.148 (1.032–1.278)**	**0.011**	—	—
Bi-testicular volume	**1.091 (1.003–1.187)**	**0.043**	—	—
Testosterone	0.534 (0.279–1.024)	0.059	0.819 (0.320–2.096)	0.677
LH	1.170 (0.768–1.783)	0.464	0.605 (0.256–1.431)	0.253
FSH	0.544 (0.276–1.072)	0.078	1.341 (0.633–2.842)	0.443
Sperm concentration	1.028 (0.985–1.073)	0.206	1.033 (0.883–1.209)	0.683
Progressive motility	0.968 (0.886–1.059)	0.478	1.476 (0.932–2.339)	0.097
Total motility	1.050 (0.972–1.136)	0.217	0.729 (0.506–1.050)	0.089
Typical sperm forms	0.824 (0.692–1.081)	0.129	0.755 (0.388–1.471)	0.409
Number of motile sperm	1.038 (0.937–1.150)	0.472	0.947 (0.884–1.014)	0.118
Testicular Index	**0.334 (0.173–0.643)**	**0.001**	—	—

3.5 Predictive performance of candidate testicular indexes

ROC curve analysis showed modest discriminative ability of the candidate TIs for pregnancy achievement. Because lower index values were associated with a higher probability of pregnancy, AUC values were interpreted after reversing the direction of the test.

TI1 and TI2 showed identical performance, with an AUC of 0.632 (95% CI 0.556–0.707). TI3 showed a slightly lower AUC of 0.631 (95% CI 0.551–0.712), whereas TI4 showed the lowest discriminative ability, with an AUC of 0.607 (95% CI 0.525–0.689) (Table 5) (Figure 3).

***Table 5**. Receiver operating characteristic (ROC) curve analysis of the candidate testicular indexes for prediction of pregnancy achievement. Area under the curve (AUC), 95% confidence intervals (95% CIs), optimal cut-off values identified by Youden's index, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are reported for each index. Since lower index values were associated with pregnancy achievement, ROC analyses were interpreted after reversing the direction of the test variables.*

Index	**AUC**	**95% CI**	***p*-value**	**Best cut-off**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
Testicular Index 1	0.632	0.556–0.707	0.001	≤1.037	89.9%	33.1%	36.7%	88.4%
Testicular Index 2	0.632	0.556–0.707	0.001	≤1.037	89.9%	33.1%	36.7%	88.4%
Testicular Index 3	0.631	0.551–0.712	0.001	≤0.454	71.7%	51.7%	38.4%	81.3%
Testicular Index 4	0.607	0.525–0.689	0.011	≤3.661	81.7%	39.9%	—	—

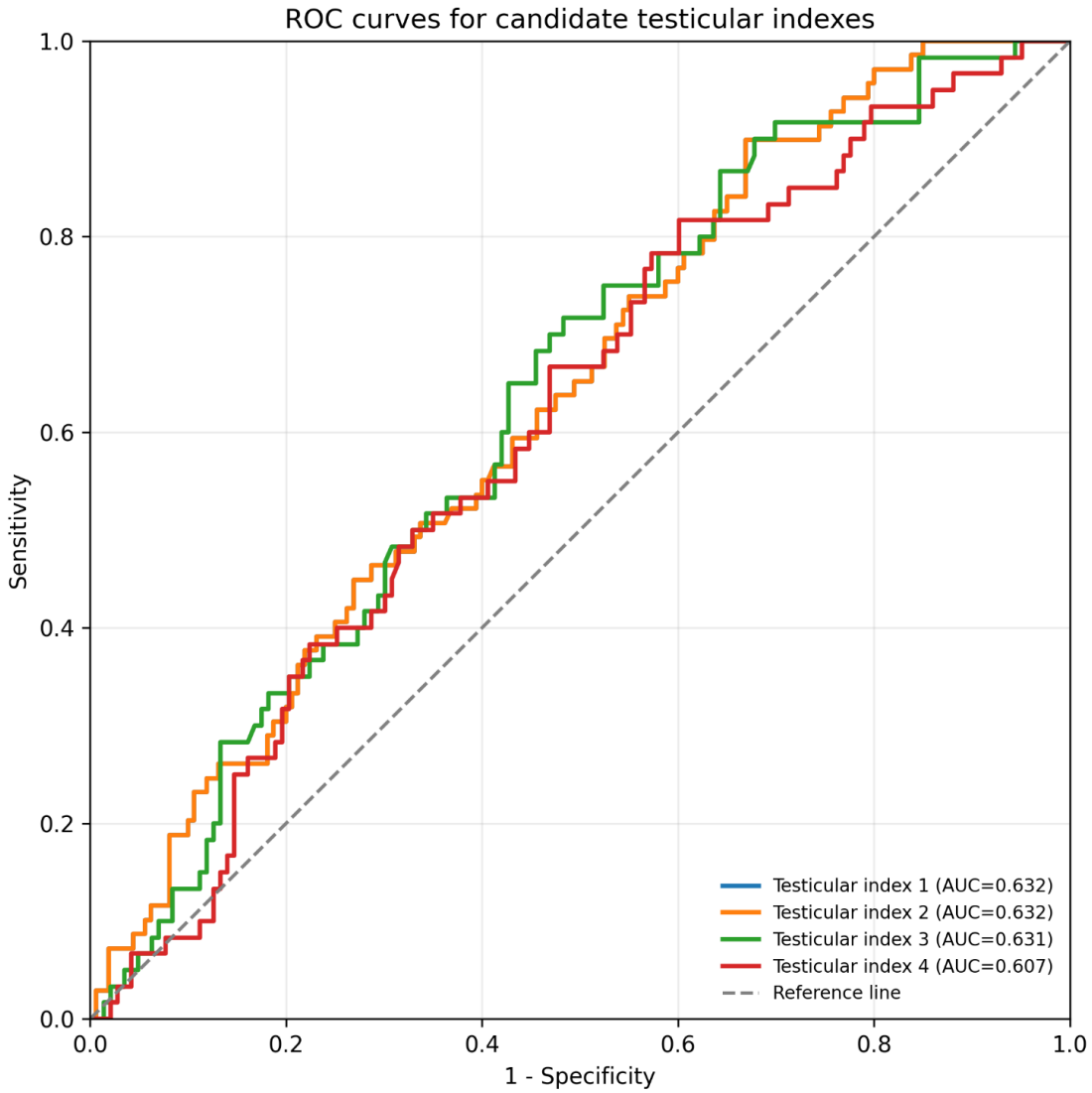

Figure 3. *Receiver operating characteristic (ROC) curves of the four candidate testicular indexes for prediction of pregnancy achievement. ROC curves were generated to evaluate the discriminative performance of each index in identifying patients achieving pregnancy. Testicular Index 1 and Testicular Index 2 showed overlapping ROC curves with identical diagnostic performance. The diagonal reference line indicates random classification (AUC = 0.50). AUC values were interpreted after reversing test direction because lower index values were associated with higher probability of pregnancy achievement.*

Secondary analyses were performed to evaluate whether the selected TI predicted improvement in semen parameters following FSH treatment[75]. Linear regression analysis using percentage change in sperm concentration as the dependent variable demonstrated no significant association between baseline TI1 values and treatment-related improvement in sperm concentration (β=0.054, B=95.140, 95% CI −146.078 to 336.358, p = 0.438). The overall predictive performance of the model was poor (R^2=0.003), indicating that the TI explained only a limited proportion of the variability in sperm concentration improvement after treatment.

3.6 APHRODITE criteria

Since the treatment was provided according to the Italian rules for drug reimbursement, no patients eligible for the therapy were included *a posteriori* in APHRODITE Groups 1, 4 and 5. Thus, patients treated were included in Group 2 (77.2%) and in Group 3 (22.8%) (Table 6)[59,60].

At V0, APHRODITE Group 3 patients showed significantly lower serum TT levels compared with Group 2 patients ($Z=-10.809$, $p < 0.001$), confirming the hormonal distinction between the two phenotypes[60]. Group 3 patients also showed lower LH levels ($Z=-2.342$, $p = 0.019$) (Table 6). No significant differences were observed between groups regarding age, testicular volume, FSH levels, semen volume, sperm concentration, total sperm number, motility parameters, morphology, or total motile sperm number (Table 6). Regarding TIs, all indexes showed lower baseline levels in Group 3 compared to Group 2 (Table 6).

At V1, APHRODITE Group 3 patients showed significantly lower sperm concentration ($Z=-2.545$, $p = 0.011$), lower total sperm number ($Z=-2.443$, $p = 0.015$), and smaller absolute and percentage improvements in sperm concentration following FSH treatment ($Z=-2.258$, $p = 0.024$ and $Z=-2.385$, $p = 0.017$, respectively) compared with Group 2 patients (Table 6). No significant differences were observed for progressive motility, total motility, morphology, or number of motile spermatozoa (Table 6).

Table 6. *Patients' characteristics at baseline and after treatment with FSH of the male partner, dividing the entire cohort according to APHRODITE criteria. The p-value was obtained using the Mann-Whitney U-test. [Notes: FSH = follicle-stimulating hormone; LH = luteinizing hormone]*

	Baseline					**After Treatment**				
	Aphrodite Group 2		**Aphrodite Group 3**			**Aphrodite Group 2**		**Aphrodite Group 3**		
	Mean	**Std. Deviation**	**Mean**	**Std. Deviation**	***p*-value**	**Mean**	**Std. Deviation**	**Mean**	**Std. Deviation**	***p*-value**
Age (years)	37.8	6.1	38.3	5.8	0.513	-	-	-	-	-
Treatment Duration (months)	-	-	-	-	-	7.6	3.3	8.8	3.3	0.024
Female Age (years)	33.3	4.5	34.5	4.0	0.085	-	-	-	-	-
Bi-testicular Volume (mL)	31.4	8.4	29.7	9.2	0.155	-	-	-	-	-
Testosterone (ng/mL)	5.6	2.2	2.9	0.6	**<0.001**	4.7	1.8	5.0	2.4	0.945
LH (IU/L)	3.7	1.4	3.2	1.5	**0.019**	3.6	1.7	3.3	2.2	0.581
FSH (IU/L)	4.5	1.8	4.7	2.1	0.712	7.1	3.5	6.9	3.8	0.686
Semen volume (mL)	2.8	1.7	2.9	1.5	0.628	2.9	1.7	3.0	1.5	0.698
pH	8.0	0.3	8.0	0.3	0.243	8.0	0.2	8.0	0.3	0.462
Sperm Concentration (million/mL)	7.7	13.1	8.3	15.3	0.984	15.1	25.6	9.3	16.2	**0.011**
Delta Sperm Concentration	-	-	-	-	-	6.7	21.9	3.2	16.8	**0.024**
Delta Sperm Concentration (%)	-	-	-	-	-	405.6	1493.9	80.0	202.1	**0.017**
Total Sperm Number (million)	21.3	54.3	19.1	26.7	0.679	41.3	73.3	22.3	38.7	**0.015**
Progressive Motility (%)	15.1	15.2	15.2	15.1	0.966	19.3	27.8	16.5	17.5	0.306
Total Motility (%)	19.5	17.5	18.3	16.4	0.760	22.8	17.7	19.2	19.2	0.096
Typical Forms (%)	1.7	3.6	1.2	1.4	0.544	1.3	1.8	1.4	2.1	0.917
Total motile sperm number (million)	3.3	8.7	4.7	10.4	0.570	9.5	21.2	7.2	16.6	0.070
Testicular Index 1	0.9	0.6	0.5	0.3	**<0.001**	-	-	-	-	-
Testicular Index 2	0.9	0.6	0.5	0.3	**<0.001**	-	-	-	-	-
Testicular Index 3	0.5	0.2	0.4	0.2	**0.010**	-	-	-	-	-
Testicular Index 4	3.7	3.5	1.8	1.6	**<0.001**	-	-	-	-	-

However, the efficacy of FSH treatment was further evaluated within APHRODITE subgroups by comparing V0 and V1 semen parameters using paired analyses.

In APHRODITE Group 2, FSH treatment was associated with significant improvements in sperm concentration ($Z=-5.952$, $p < 0.001$), total sperm number ($Z=-5.881$, $p < 0.001$), progressive motility ($Z=-1.971$, $p = 0.049$), total motility ($Z=-2.746$, $p = 0.006$), and number of motile spermatozoa ($Z=-6.022$, $p < 0.001$). No significant changes were observed in sperm morphology ($p = 0.891$). Conversely, among patients classified as APHRODITE Group 3, no significant improvements in semen parameters were observed following treatment. Specifically, sperm concentration ($p = 0.320$), total sperm number ($p = 0.327$), progressive motility ($p = 0.577$), total motility ($p = 0.900$), sperm morphology ($p = 0.912$), and number of motile spermatozoa ($p = 0.209$) remained substantially unchanged after FSH administration.

Considering pregnancy, no significant difference was observed in pregnancy frequency between the two groups (28.4% [n = 50] *versus* 34.6% [n = 18], $p = 0.301$). Accordingly, logistic regression analysis was performed to evaluate whether APHRODITE classification predicted pregnancy achievement, showing no significant association. Patients classified as APHRODITE Group 3 did not show significantly different odds of achieving pregnancy compared with Group 2 patients (OR 1.274, 95% CI 0.804–2.017, $p = 0.302$).

3.7 The testicular index within the APHRODITE classification

The distribution and predictive performance of the selected TI1, that best performed in previous analyses, were further evaluated within APHRODITE subgroups[60].

Within APHRODITE Group 2, patients whose partners achieved a pregnancy showed lower TI1 values compared with patients who did not achieve pregnancy, with a trend toward statistical significance (Mann-Whitney U test: $Z=-1.923$, $p = 0.054$). In contrast, within APHRODITE Group 3, TI1 was significantly lower among patients achieving pregnancy compared with non-pregnant patients ($Z=-2.155$, $p = 0.031$).

ROC curve analyses were subsequently repeated within APHRODITE groups to evaluate the discriminative ability of the TI1 according to patient phenotype. The AUC of

TI1 was slightly higher in APHRODITE Group 3 (0.673, 95% CI 0.536–0.810, $p = 0.013$) than in Group 2 (0.644, 95% CI 0.564–0.724, $p < 0.001$).

DISCUSSION

4.1 The efficacy of empirical FSH therapy and the discrepancy between quantitative and qualitative seminal responses

The empirical administration of exogenous FSH in men with idiopathic infertility remains a subject of ongoing debate[77,78]. Guidelines from several international scientific societies, such as the European Academy of Andrology (EAA) and the European Association of Urology (EAU), suggest, rather than firmly recommend, FSH as a therapeutic option, due to the lack of large-scale randomized controlled trials (RCTs)[2,41]. RCTs remain the gold standard for evaluating treatment efficacy; however, their strict inclusion and exclusion criteria may limit the generalizability of their findings to routine clinical practice. Furthermore, the complexity, duration, and resource requirements of RCTs often hinder the generation of large-scale evidence in many clinical settings. Consequently, real-world data analyses have become increasingly valuable as a complementary source of evidence, providing insights into treatment effectiveness in unselected patient populations. Our real-world cohort provides valuable evidence that integrates the rigorously controlled environments of RCTs with the actual dynamics of usual patient care[79].

In our study, the targeted FSH therapy enabled almost one-third of the treated couples to achieve pregnancy. This success rate compares favorably with recent literature. For instance, a similar real-world analysis reported a pregnancy rate of 27.7% in FSH-treated men[75], indicating that our findings are consistent with, and even slightly exceed, those of other contemporary cohorts. These results support the therapeutic value of FSH administration in an everyday scenario, justifying its use despite the cautious stance of current guidelines, which is primarily driven by the lack of robust RCTs.

A longitudinal analysis of seminal parameters provides a clearer understanding of the biological response of the germinal epithelium to hormonal stimulation. Following treatment, a statistically significant overall improvement was observed in almost all conventional semen parameters. However, stratifying these results according to pregnancy outcome reveals a divergence between quantitative and qualitative responses. While quantitative metrics, such as sperm concentration and total sperm number,

showed a marked improvement in both successful and unsuccessful treatment groups, qualitative functional features (i.e., progressive and total motility) demonstrated a clear enhancement only in the subgroup of patients who achieved pregnancy. This finding closely aligns with recent literature demonstrating that an increase in sperm concentration represents a key predictive parameter of FSH efficacy[75]. Moreover, the specific predictive role of sperm motility and morphology in guiding therapeutic decisions for assisted reproduction has been recently emphasized, confirming that qualitative seminal improvements are intrinsically tied to reproductive success[80].

The pathophysiological rationale behind this differential response lies in the testicular microenvironment. Exogenous FSH exerts its main mitotic and anti-apoptotic effects on spermatogonia and early primary spermatocytes via Sertoli cell stimulation, driving the quantitative expansion of the germ cell pool[81,82]. Conversely, the final stages of spermiogenesis and maturation, such as the acquisition of progressive motility during epididymal transit, are heavily dependent on optimal Leydig cell function and ITT production[83,84]. Additionally, proper sperm chromatin condensation requires the synergistic action of both ITT and FSH[81,85].

Our hormonal data support this paradigm. While the overall cohort showed a small but significant increase in systemic testosterone, this elevation was observed exclusively in the responder subgroup. Since Leydig cells lack FSH receptors, this phenomenon likely reflects an enhanced Sertoli-Leydig paracrine crosstalk in testes with a preserved functional reserve[60,86]. Although ITT is the principal driver of spermiogenesis, its direct measurement is unfeasible in routine diagnostic workups due to technical challenges. Consequently, circulating serum testosterone serves as a reliable proxy for HPT axis responsiveness, aiding in patient classification regarding their intra-testicular androgenic status. This approach is suggested by studies demonstrating a correlation between peripheral and intra-testicular androgen pools[60]. Although the evidence supporting this correlation is limited, it remains the only potential marker with practical utility in clinical practice.

Finally, the observation that mean post-treatment FSH levels remained within the physiological range provides a crucial pathophysiological insight, confirming the real rationale behind our current clinical approach. Historically, the rationale for the

administration of exogenous FSH in idiopathic infertility was translated directly from the management of hypogonadotropic hypogonadism, a condition characterized by a complete absence of endogenous gonadotropins where a replacement framework is highly effective in terms of both steroidogenesis and spermatogenesis repair. However, since idiopathic infertile men present with preserved and normal baseline FSH levels, the therapeutic objective must shift from mere replacement toward a targeted testicular over-stimulation approach. This conceptually mirrors the controlled ovarian hyper-stimulation protocols employed in female reproductive medicine to maximize gamete yield. Therefore, the evidence that post-treatment FSH levels remained within physiological bounds highlights that typical empirical dosing may often be insufficient to induce the supraphysiological state strictly required to overcome the functional indolence of the idiopathic infertile testis[48,60].

4.2 The prognostic limits of sperm morphology and the paradox of seminal predictability

The predictive value of sperm morphology in conventional semen analysis is frequently questioned in the modern andrology field[87,88]. Our findings confirm that sperm morphology does not respond to exogenous gonadotropin stimulation. The lack of longitudinal improvement from baseline to post-treatment in both responder and non-responder groups aligns with current literature questioning the diagnostic utility of morphological evaluation[87]. In fact, sperm morphology is an operator-dependent parameter, relying on the subjective judgment of the seminologist examining the slide under the microscope and deciding whether each spermatozoon meets the strict criteria for "normal" or "abnormal". Consequently, the metric suffers from well-documented poor intra- and inter-observer reproducibility, limiting its reliability as a clinical marker[89,90].

Although a cross-sectional comparison at the post-treatment evaluation (V1) revealed that men in the successful outcome group retained a higher percentage of typical forms compared to the non-pregnant group, this finding must be interpreted with caution. At baseline (V0), the two groups exhibited no significant differences in sperm morphology. Rather than reflecting a structural remodeling induced by FSH therapy, the V1 discrepancy is likely a statistical artifact driven by the baseline phenotypic stability of

the responder cohort (which remained unchanged), juxtaposed with a borderline morphological deterioration in the non-pregnant group. This highlights a diagnostic paradox: FSH enhances macroscopic spermatogenesis, yet this seminal improvement does not always translate into pregnancy. The non-pregnant group experienced significant increases in absolute sperm concentration and total number. This confirms that evaluating FSH efficacy through standard semen analysis is inadequate.

Conventional semen analysis fails to capture the molecular and functional competence of the spermatozoon, such as chromatin integrity. Scientific evidence suggests that FSH reduces SDF by preventing abortive apoptosis and reducing oxidative stress during epididymal transit, thereby improving fertilizing potential regardless of visible quantitative changes[69,91]. Routine seminal parameters do not provide direct evidence regarding the capacity of a spermatozoon to fertilize an oocyte and support early embryonic development[92]. Consequently, conventional semen analysis is only partially reliable in predicting a strong, dichotomous outcome such as pregnancy. This limitation reflects the fundamental physiology of reproduction: conception is not solely dependent on the male gamete, but requires a synergy between male and female factors. The achievement of pregnancy relies on the reproductive potential of the female partner, which remains a critical co-factor dictating the final outcome[92].

4.3 Baseline phenotypes and the clinical utility of the TI

Given the limitations of post-treatment seminal modifications in predicting reproductive success, identifying reliable baseline predictors is crucial[9,40]. Our data reveal a distinct phenotypic profile among responders (in terms of pregnancy achieved): patients whose partners conceived exhibited a younger age, lower pre-treatment FSH levels, and larger bi-testicular volumes.

These specific baseline variables provide actionable insights. Pre-treatment FSH is essential to evaluate the therapeutic window. Current research indicates a dose-dependent effect, as demonstrated by Cannarella et al.[64], where higher FSH doses induce improvements in semen parameters. Therefore, the widespread practice of prescribing a standard uniform dose to all patients limits the proportion of responders. Similarly,

testicular volume represents the target organ mass; a reduced volume of germinal tissue limits the capacity to respond to gonadotropin stimulation.

To quantify this phenotype, our results confirmed that the testicular index TI1 serves as a useful indicator[9]. Multivariable logistic regression identified male age, bi-testicular volume, and TI1 as independent initial predictors of pregnancy. The primary merit of the TI lies in its ability to act as a proxy for response potential, overcoming the poor performance of single parameters as isolated predictors.

4.4 APHRODITE classification and tailored therapeutic strategies

The applicative value of TI1 becomes evident when the cohort is stratified according to the APHRODITE criteria[60]. Following FSH treatment, patients in APHRODITE Group 2 exhibited improvements across major semen parameters, whereas those in Group 3 lacked seminal enhancements.

Biologically, such a discrepancy requires functional interpretation. The quantitative response observed in Group 2 is driven by the synergistic action of exogenous FSH and a preserved ITT pool. Since spermatogenesis relies on both hormones, the intact Leydig cell compartment in Group 2 provides the optimal androgenic background for FSH to maximize germ cell proliferation. Conversely, Group 3 patients present with lower baseline serum testosterone and lower LH levels, framing a phenotype of subtle secondary (central) hypogonadism or a sluggish HPT axis[60]. Moreover, the comparable BMI and baseline estradiol levels observed across the cohorts exclude major adiposity-related endocrine shifts as confounding factors. This confirms that the endocrine deficit in Group 3 represents a true functional trait of the interstitial compartment rather than a metabolic artifact. In these men, the lack of adequate ITT functionally limits the spermatogenic process. Because FSH monotherapy targets the tubular compartment but leaves the interstitial deficit unresolved, the germinal epithelium cannot undergo an optimal mitotic expansion, explaining the stagnant semen parameters[60].

However, despite this lack of improvement in Group 3, pregnancy rates were comparable between the two APHRODITE groups (28.4% vs 34.6%). This counterintuitive finding suggests that while the suboptimal ITT environment in Group 3 prevents a surge in sperm production, FSH still exerts a qualitative effect on the available germ

cells. By supporting chromatin maturation and reducing SDF, exogenous FSH may confer enhanced fertilizing competence to the spermatozoa, leading to conception despite numerically stable semen parameters[69,91]. This reinforces the concept that conventional seminal modifications are an inadequate surrogate endpoint for reproductive success[92]. Finally, TI1 emerged as a predictor of treatment response specifically within APHRODITE Group 3 (AUC 0.673), unmasking hidden responders within complex, borderline-hypogonadal phenotypes.

These results confirm that a uniform therapeutic approach is inadequate. The current Italian regulatory framework (AIFA Note 74), which dictates specific FSH protocols for all eligible patients, imposes a standardized management approach that may overlook individual phenotypic requirements. While this framework ensures uniform access, it often lacks an evidence base for the one-size-fits-all dosage (e.g., 150 IU three times a week for 4 months), suggesting that therapeutic protocols require revision, both to improve outcomes and from a pharmacoeconomic perspective.

A tailored strategy must also reconsider the role of treatment duration. Human spermatogenesis requires approximately 74 days[93], followed by an epididymal transit and maturation phase of up to two weeks[83,94]. While a standard 4-month prescription covers roughly 1.5 spermatogenic cycles[60], the literature suggests extending treatment beyond 5 months to achieve full qualitative remodeling[48]. Analysis of our sample demonstrated no difference in treatment duration between couples who achieved a documented conception and those who did not. Once FSH therapy extends beyond the minimal 4-month timeframe, time ceases to be the sole limiting factor. Instead, the primary determinants of reproductive success are the baseline biological reserve and the underlying endocrinological phenotype. For patients harboring a compensated Leydig cell impairment (e.g., APHRODITE Group 3), simply prolonging FSH monotherapy cannot overcome the functional deficit[60].

Therefore, therapeutic algorithms must pivot from a time-restricted approach to a phenotype-driven strategy. This requires integrating combination therapies, such as the addition of hCG to stimulate the interstitial compartment, when prolonged FSH monotherapy fails to yield a successful pregnancy[60,77].

In this scenario, real-world data derived from the Italian clinical context provide essential insights to complement conventional randomized controlled trials, supporting the shift toward a more personalized approach to male infertility[79].

4.5 Study limitations

Despite the real-world evidence provided, this study presents limitations.

First, the retrospective, observational design restricts the ability to establish definitive causal relationships between the intervention and the outcomes. A methodological constraint is the absence of a control group comprising untreated men with idiopathic infertility[9,59]. Consequently, the baseline spontaneous pregnancy rate cannot be isolated from the treatment effect.

Second, while semen analyses were conducted in a certified center adhering to WHO guidelines, the single-center nature of the study may limit the broad generalizability of these findings. Thus, there remains a need to confirm these results in an independent cohort.

Third, the nature of the study led to heterogeneous treatment durations (ranging from 2.4 to 12.0 months), although this reflects routine practice.

Finally, couple infertility is a dyadic issue. Although primary female causes of infertility were excluded prior to enrollment and female age was balanced between the successful and unsuccessful cohorts, the absence of granular data regarding female reproductive potential (such as AMH levels or detailed antral follicle counts) remains a limitation[9]. Moreover, the reliance on ART in a portion of our successful cohort (22.8% of total patients vs 7.0% natural conceptions) acts as a non-modifiable confounding factor when interpreting the primary outcome[92].

CONCLUSIONS

Male infertility remains a highly heterogeneous pathological entity and a persistent challenge for clinicians. While exogenous FSH stimulation is demonstrably effective, its real-world efficacy frequently falls short of its theoretical potential. Our findings strongly suggest that this limitation is, at least in part, attributable to the suboptimal *a priori* classification of patients. In this context, the systematic implementation of standardized phenotypic stratification tools, such as the APHRODITE criteria, is of paramount importance[60].

Moving forward, future research must continue to prioritize the identification of robust baseline predictors, such as the TI, to appropriately select candidates for hormonal intervention[9]. Ultimately, empirical treatments must transcend the one-size-fits-all paradigm, shifting towards increasingly personalized and phenotypically tailored strategies to maximize reproductive outcomes for infertile couples.

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