University of Padua

School of Medicine

Single-Cycle Master's Degree Course in Medicine and Surgery

Unit of Andrology and Reproduction Medicine
Director: Prof. Alberto Ferlin

THESIS

Redefining Male Factor Infertility: Toward a Novel Diagnostic and Therapeutic Classification- Preliminary Findings

Supervisor: Prof. Alberto Ferlin

Co-supervisor: Dr. Andrea Graziani

Graduand: Matilda Manetti

Academic Year 2024-2025

TABLE OF CONTENTS

ABSTRACT

Background

Male factor infertility (MFI) is estimated to contribute to 30-50% of cases of couple infertility. Although MFI impact on couple infertility is relevant, during infertility investigations it is often neglected or considered only through the examination of a single semen analysis. On the contrary, it is important to have a complete andrological assessment, beyond semen analysis, which is just a sign of an underlying disorder, and alone it is not sufficient for a correct diagnosis of MFI. This can only be achieved through a proper diagnostic pathway, aimed at identifying precise patho-physiological etiologies, reducing the idiopathic counterpart. Only following a precise diagnosis, a proper therapeutic pathway for fertility restoration can be initiated. The previous classification into pre-testicular, testicular and post-testicular causes, although serving research purposes, has limited clinical utility and does not specifically guide therapy. A new proposal of classification has been proposed, including the following six categories: I) Infection/inflammation; II) Congenital or acquired seminal pathway obstruction, including retrograde ejaculation; III) Primary testicular disease, further divided into IIIa whit hypergonadotropic testiculopathy and IIIb whit normogonadotropic testiculopathy; IV) Hypogonadotropic hypogonadism; V) Idiopathic infertility with seminal alterations; VI) Idiopathic infertility with normal semen analysis.

Aim of the study

This study aims to pragmatically analyze preliminary findings of the new diagnostic-therapeutic classification of MFI and evaluate its real-life diagnostic and therapeutic outcome.

Materials and Methods

We conducted a monocentric retrospective study including 505 male patients from the Unit of Andrology and Reproduction Medicine from 2023-2025. Specifically, only male patients with isolated MFI were included in the study, after the exclusion of patients with known female infertility factors or those with semen alterations but not trying to conceive. We classified patients according to the new diagnostic-therapeutic classification of MFI (category I-VI), adding category VII as well, for significant Varicocele, considering only high-grade varicoceles after the exclusion of other relevant causes of infertility. Furthermore, we conducted an evaluation on

FSH treatment on a selected population of 31 subjects of the whole cohort of 505 patients.

Results

We enrolled 505 patients with a mean age of 35.7 +/- 7.8 years and a mean age of the female partner of 35.1 +/- 5.2. From preliminary data of our evaluation, we found that, as a leading cause of MFI, the categories to be the most populated were category III (IIIa with 33.41% and IIIb with 28.54%) and category I (27.88%). Relevant differences were found considering hormonal profiles (especially FSH), seminal parameters and testicular volumes. Importantly, after our analysis, we found an overall condition of idiopathic infertility (category V and VI) in 2.77% of patients. In addition, varicocele was found to be significant only in 1.39% of patients. Finally, we showed that, when an appropriate selection of subjects (belonging to category IIIb without infections and sub-obstructions) is performed, FSH treatment can lead to meaningful improvement of semen parameters (> 100%) in 90.3% of patients evaluated.

Discussion and Conclusions

According to our results we understood that a large portion of MFI causes can be reversed or improved with specific treatment, when patients are properly categorized after a meticulous diagnostic pathway. We saw that the Idiopathic share among MFI is actually very low, as well as that of significant varicoceles. Too often the diagnosis of idiopathic infertility is made without a thorough diagnostic process, and it should be reserved as a real exclusion diagnosis and when a varicocele is found, other causes should not be overlooked, but on the contrary, investigations should continue. FSH treatment can lead to a very significant improvement in seminal parameters, importantly higher to the percentage of improvement found in literature (50%). This can only be obtained after an appropriate selection of patients excluding the presence of infections with sperm culture and sub-obstructions with ultrasound. This novel classification seems to be reliable, easily applicable in clinical practice and able to specifically guide treatment, compared to the previous one. A suitable classification of patients with MFI, gives better therapeutical chances.

INTRODUCTION

1. INFERTILITY

1.1Definitions

Fertility is the capacity to establish a clinical pregnancy in a certain timeframe (1). By contrast, infertility is defined as the inability to establish a clinical pregnancy after at least 12 months of regular (at least twice per week), unprotected sexual intercourse (1) (2). It is a retrospective diagnosis, but although the threshold is 12 months, the timing of the initial investigation can be anticipated when already known risk factors, as advanced maternal age, are present (3).

It is important to distinguish infertility from two other conditions: subfertility and sterility. Subfertility indicates a situation characterized by a reduced form or grade of fertility, in a couple with unsuccessful attempts of conception. It is still possible to achieve a spontaneous pregnancy, but with lower probability compared to a fertile couple. It is the only term which can be interchangeably used with infertility(1).
Whereas sterility, is a permanent state of infertility (1), associated with the inability of spontaneous conception. It is due to irreversible and unmodifiable factors like complete closure of the Fallopian tubes or gonadectomy.
It must be underlined though, that, in some situations, there could be the possibility to establish a clinical pregnancy via assisting-reproductive techniques (ARTs).

Infertility can be further described as either primary or secondary (2). The former occurs when neither of the components of the couple have ever experienced a clinical pregnancy in the context of a previous relationship. The latter instead, applies to a couple with current inability to conceive, where either the woman or the man have established a clinical pregnancy with a previous partner in the past.

An essential component of infertility is that it is a condition which always involves the couple, implying that it should always be referred to as “couple infertility”. It should not be considered as an issue purely associated to the woman or purely associated to the man. Indeed, the preferable approach is to distinguish between

“male factors” and “female factors”, which are equally represented, either in combination or alone.

Frequently, the attention is focused only on one partner, usually the female, mostly due to socio-cultural reasons. This occurs also because there is an erroneous conception believing that full medical investigation for infertile men is not necessary (3). However, male factors are responsible for about half of the cases and should be properly investigated and addressed.

Moreover, individual medical conditions can deeply affect the approach and the management of the couple. For instance, a male with oligozoospermia in a couple with a young partner, lacking risk factors impacting fertility, could be compatible with natural conception. On the other hand, the same male circumstance, together with a partner having a reduced fertility potential, could be a cofactor of couple infertility.

As a result, the diagnostic and therapeutic approach should proceed in parallel, and the results should be contextualized within the setting of that particular couple.

1.2 Epidemiology and relevance

Infertility is a medical condition with an important social and economic impact worldwide. It affects over 70 million people globally (4). Approximately, one in every six people of reproductive age worldwide experience infertility in their lifetime (5). It is estimated by WHO that 9% of couples around the world struggle with fertility issues. In Western countries around 15 to 20% of couples are infertile. Male factor infertility accounts for 50% of causes within couple infertility (6).

Reproductive health, together with sexual health, refers to a complete physical, mental and social well-being in matters related to the reproductive system and its functions (5).

Every human being has the right to the enjoyment of the highest standard of physical and mental health.

Experiencing sexual and reproductive health means, in everyday life, that people are able to have a safe and satisfying sex life, to have healthy pregnancies and births.

Infertility can deny the fulfillment of these essential human rights.
Infertility is not only related to reproductive health, but has psychological, economic and medical implications (7). It has a significant social impact on the lives of infertile couples, frequently leading to emotional stress, depression, anxiety, low self-esteem, social stigma and divorce, particularly in societies where there is a culture putting strong emphasis on child-bearing (8) (9).

To effectively be addressed, infertility should be recognized by health policies as a condition that can often be prevented, alleviating the need for accessible treatments.
Fertility awareness should be incorporated by governments, through policies and interventions with national comprehensive sexuality and reproduction education programs, promoting healthy lifestyles to reduce behavioral risks.

2. ETIOLOGY

Infertility is a complex medical condition where different elements are involved. Male and female factors interact, influencing couple fertility. There are several risk factors impacting conception outcome, either specific to one gender or shared among them. In the table below, there are some examples.

Both genders	Female	Male
Family history of infertility	Age >35 years	Cryptorchidism
Recurrent abortions	Reduced ovarian reserve (AMH and/or AFC count)	Testicular hypotrophy
Obesity	Ovulation Disorders	Testicular cancer
Using of anabolic steroids	Infertility >3 years	Known genetic factors
Lifestyles	Menstrual disorders	Varicocele
Environmental/occupational factors	Endometriosis	Testicular trauma
Systemic and/or endocrine diseases	Family history of POF	Testicular torsion
Iatrogenic factors	PID (consequent to infectious diseases)	Puberty disorders
Infertility with previous partners		Aging
Cystic fibrosis		Testicular microlithiasis
Infection		Unhealthy diet and obesity
		Pollution
		Cigarette smoking

AFC, antral follicle count; AMH, anti-müllerian hormone; BMI, body max index; PID, pelvic inflammatory disease; POF, primary ovarian insufficiency.

Figure 1. Female and male infertility risk factors.

Other conditions that can affect both genders include the following:

- Hypogonadotropic-hypogonadism (Kallman)
- Hyperprolactinemia
- Disorders of the ciliary function
- Cystic fibrosis
- Infection
- Systemic disease
- Lifestyle-related

2.1 Female factors

According to the National registry of ARTs, the main causes of female factor infertility in couples seeking medical attention include:

- Reduced ovarian reserve (13,3%)
- Female multiple factors (5,6%)
- Tubal factor (4,7%)
- Endometriosis (4,3%)
- Endocrine ovulatory infertility (2,6%)
- Uterine factor (0,4%)

The first etiology is mainly associated with increased maternal age, while the others are disease related.

Although not further explored in this thesis, as it is not its aim, these risk factors require an appropriate multi-step diagnostic approach with allocated suitable exams.

2.2 Male factors

Male factor is causative of infertility in 50% of couples, 20% of which is in combination with female infertility and in 30% of cases alone (10).

Physiology

The testis is composed by germ cells and somatic cells, which are divided into two main cell populations: Sertoli cells and Leydig cells. The formers are located along

the course of the seminiferous tubules and are responsible for spermatogenesis and they provide structural and functional support to germ cells. Leydig cells, on the other hand, are located within the stroma surrounding the seminiferous tubules, and synthetize testosterone.

Spermatogonia, the testicular germ cells, during spermatogenesis become mature spermatozoa via 3 different phases; firstly, they divide by meiosis into spermatocytes, secondly, they undergo meiosis I and II in order to form spermatids. Spermatids then develop into spermatozoa after cytodifferentiation.

These testicular functions are dependent on the hypothalamic-pituitary-testicular (HPT) axis. Pulsatile secretion of gonadotropin-releasing hormone (GnRH) by the neurons of the supraoptic and paraventricular nuclei stimulate follicle-stimulation hormone (FSH) and luteinizing hormone (LH) secretion form the anterior pituitary. FSH, in turn, stimulates Sertoli cell function and spermatogenesis, LH induce the production of testosterone by Leydig cells (11) Sertoli and Leydig cells produce, respectively, inhibin B and testosterone, which have a negative feedback effect on the pituitary and on the hypothalamus (12).

Abnormalities in any of these steps, can lead to testicular dysfunction and male infertility.

Primary: testicular dysfunction

Genetic

There can be chromosomal abnormalities, either numerical or structural, affecting both sex and autosomal chromosomes that can impact fertility potential (6)

Klinefelter syndrome is the most common aneuploidy of testicular failure in azoospermic and oligospermic men. It is a numerical chromosomal abnormality affecting sex chromosomes, either in non-mosaic 47XXY (80-90%) or mosaic 47 XXY/46 XY (5-10%) (6). The classical habitus is that of a tall man with long arms, firm hypotrophic testes and gynecomastia (13). However, the phenotype can be heterogeneous, ranging from a fully virilized man to one with androgen deficiency. The endocrine profile is characterized by hypergonadotropic hypogonadism, with high FHS and high LH, low-normal testosterone.

Robertsonian translocation and inversions are the most common structural abnormalities, involving rearrangement and "crossing over" of chromosomes. They can be balanced, when there is a deviation from normal chromosomal structure, but no net loss or gain of genetic material, or unbalanced, when there is indeed a net gain or loss of genetic material, which is usually incompatible with life (6). Balanced ones are usually associated to spermatogenic failure with oligospermia or azoospermia.

Y-chromosome microdeletions can be found in men with severe oligospermia and azoospermia (14). They are deletions of specific regions of the Y chromosome, either AZFa, AZFb and AZFc, containing genes responsible of spermatogenesis (14). AZFc microdeletions are the most common ones (65-70%) and are associated to low levels of spermatozoa in the ejaculate or testicular biopsies (15).

46 XX male syndrome, also called "de la Chapelle" syndrome, is a disorder of sex development distinguished by the translocation of a portion of the Y chromosome, including the SRY (sex determining region) tract, to the X chromosome during paternal meiosis (6). These individuals present completely differentiated male external and internal genitalia although affected by infertility, as they lack all of the AZF regions necessary for spermatogenesis.

Monogenic causes of male infertility comprehend a variety of genes important for testicular function. The cystic fibrosis transmembrane conductance regulator (CFTR) is responsible for the formation of the vas deferens, the ejaculatory duct, the seminal vesicles and a portion of the epididymis. TEX11 is an X-linked gene, encoding a protein crucial for chromosomal synapsis and male germ cell meiotic DNA recombination. Mutation analysis of INSL3/RXFP2 has been associated with history of cryptorchidism. Other specific gene mutations (dynein genes, DNAI1, DNAH5, DNAH11, DPY19L2, AURK) can be a rare cause of male infertility (3).

Developmental anomalies like cryptorchidism and undescended testis are associated to impaired spermatogenesis and testicular germ cell tumors. Cryptorchidism is the unilateral or bilateral absence of the testes from the scrotum, because of failure of physiological descent from the genital ridge through the

external inguinal ring (13). In this condition there is a deterioration of testicular structure, associated to the loss of germ cells (8).
Treatment with orchidopexy is available and recommended between 6 and 18 months of age. These changes in testicular composition are highly impacting and observed primarily when the testis remains undescended. Although surgery corrects the inappropriate temperature, it may not reverse the detrimental changes occurred. Damage and testicular atrophy can anyway be found.

Acquired

Genitourinary infections are a common finding among males undergoing investigations for infertility, accounting for 10% to 15% of cases. They are associated to different types of pathogens, ranging from bacteria and viruses to protozoa, and they are potentially curable. These infections can affect different sites of the male reproductive tract, including the testis, epididymis and male accessory sex glands (16). They can have an acute or a chronic course.
Chronic or inadequately treated infections have a broader association to infertility than acute ones, although many times the precise etiological agent remains unknown (17).

Damage to spermatozoa can occur at several levels of their development, maturation and transport (16).

Mechanisms of harm are several, such as increased levels of reactive-oxygen species (ROS) due to neutrophils' activation, cross-reactivity, adherence to spermatozoa, formation of anti-sperm antibodies, DNA damage and obstruction of the seminal tract.
Microorganisms detected in the semen have been associated with poor sperm quality, decreased motility and sperm concentration, changes in morphology, viability, pH, viscosity and biochemical composition (17).
Many infections of the male genitourinary tract are asymptomatic, leading to diagnostic delays and facilitated spread to the female partner, potentially affecting also her fertility potential.
For the diagnosis of the correct pathogens the methods used include sperm culture and PCR assays, together with an antibiogram when needed.

For some pathogens the causal relationship with infertility is clear, while for others it is still not completely understood whether their impact can actually cause genital disease and infertility or if they act as mere cofactors (16).

In the table below there is a summary of the main microorganisms involved and their fertility impact within the couple.

Microorganism	Female infertility			Male infertiliy		
	Cervical/vaginal	Uterine	Tubal/pelvic	Testes/epididimus [a]	Prostate/accessory glands	Semen alterations/sperm damage
Bacteria						
C. trachomatis	Definite	Definite	Definite and very common	Definite	Doubtful	Possible
N. gonorrhoae	Definite, but less studied	Definite	Definite	Definite	Probable	Probable
M. hominis	Probable	Possible	Still to be defined	Doubtful	Doubtful	Doubtful
U. urealyticum	Probable	Possible	Still to be defined	Doubtful	Doubtful	Doubtful
M. genitalium	Probable	Possible	Most probable	Doubtful	Doubtful	Attaches to human sperm
Bacteria associated with vaginosis	Possible	Possible	Probable; no associations with specific organisms	Doubtful	Doubtful	Doubtful
Escherichia coli	Doubtful	Possible	Possible	Definite, common	Definite, common	Possible
Yeasts						
Candida spp.	Doubtful	Doubtful	Highly improbable	Doubtful	Doubtful	Rare cases
Protozoa						
T. vaginalis	Possible cofactor	Doubtful	Possible cofactor	Doubtful	Doubtful	Probable under specific conditions
Viruses						
Human papilloma virus	Defined through CIN	Defined through CIN	Improbable	Doubtful	Doubtful	Association needing further investigations
Herpes simplex virus	Doubtful	Doubtful	Association needing further investigations	Doubtful	Doubtful	Probable

a The total frequency of this condition is quite low.

Figure 2. Summary of possible associations between some microbiological agents and infertility.

Cancer, together with its treatment, is associated to male infertility during reproductive years. The most common associated cancers are leukemia, Hodgkin's lymphoma and testicular germ cell tumors. A portion of patients affected by this last type of tumors, present with azoospermia prior to any cancer treatment. Damage occurs due to systemic effects, endocrine changes, possible autoimmune effects, intrinsic testicular damage and eventual congenital abnormalities in testicular maturation (8).

Sperm cryopreservation is offered to patients before the beginning of gonadotoxic chemo-radiotherapy or pelvic surgery and it is the only effective prevention of male infertility (18).

Medications can cause different types of testicular adverse effects, ranging from sexual dysfunction and ejaculatory failure to direct spermatogenic impairment and antiandrogenic action. These medications are numerous (e.g. Chemotherapeutic agents, calcium channel blockers, Colchicine, Spironolactone, Alpha blockers, serotonin- uptake inhibitors) and can be dose- and type-dependent. There usually is an improvement in sperm parameters after therapy cessation, except for a few exceptions, like alkylating agents as cyclophosphamide (6).

Endocrine disruptors (EDs) are natural or artificial chemical substances that can mimic or interfere with the body's hormones (19). They can interfere with testicular function in many ways, they can act as antiandrogens, can exert an estrogenic effect or can directly cause testicular toxicity. Their action can affect different developmental stages, starting from in utero exposure, to neonatal and adult life. Examples of these substances include bisphenol A, phthalates and pesticides. They can cause heterogenous effects including cryptorchidism, hypospadias and poor semen quality, but can also predispose to testicular germ cell cancers.
However, nowadays clinical relevance still remains limited, due to synergistic effects after exposure to multiple EDs and confounding factors including the lifestyle.

Varicocele is an abnormal dilation of the veins of the pampiniform plexus within the scrotum. It is a common medical condition that may occur in up to 15% of the normal male population. It is usually located on the left side and can be found bilaterally only when severe. Large varicoceles, clinically visible and palpable, can impair spermatogenesis via several mechanisms including compromised testicular cooling, hypoxia, increased ROS and damage of sperm DNA.
Its repair is a debated option for male infertility. It is surely not recommended in men with normal semen parameters, unless causing relevant symptoms impairing the quality of life of the patient, or with subclinical varicoceles, being those only detectable by imaging like Doppler US.
Treatment is suggested in infertile couples where the female partner has normal fertility or a potentially treatable condition, and the male partner has abnormal semen parameter presumably caused by a high-grade varicocele (20). On the other

hand, subclinical varicocele should only be monitored, as no significant post-operative fertility improvement has been detected (20).
Although varicocele represents a common clinical condition, it is a rare cause of male factor infertility.

Lifestyle factors meaning adverse health behaviors, are associated with reduced fertility. These include excessive alcohol intake, heavy smoking, use of recreational drugs and poor physical activity (21). The exposure to these risk factors can lead to lower sperm motility, increased morphological sperm defects, high DNA fragmentation in sperm, lower sperm concentration and reduced fertility index. Some substances, like opioids can both cause direct testicular failure, as well as secondary hypogonadism by inhibiting neuronal activity via binding to kisspeptin-neurokinin B-dynorphin (KNDy).
In reality, exposure to these risk factors occurs simultaneously and not individually, as a result, there could be an underestimation of each factor to male infertility in the general population.

Obesity is a major risk factor for male infertility, and its prevalence is rising more and more worldwide. It is associated with hormonal changes including low sex-hormone binding globulin (SHBG), low total testosterone, with normal LH and FSH, secondary to excess of adipose tissue. There is a positive relationship between increasing body-mass index (BMI) and risk of oligospermia and azoospermia (22). Associated findings include increased semen ROS levels and DNA fragmentation, abnormal morphology and reduced mitochondrial membrane potential (23).
On the other hand, combined diet and physical activity, were showed to be associated with increased sperm count, semen volume and testosterone (24).

Other acquired causes of male factor infertility include trauma, testicular torsion and orchitis (2) .

Secondary

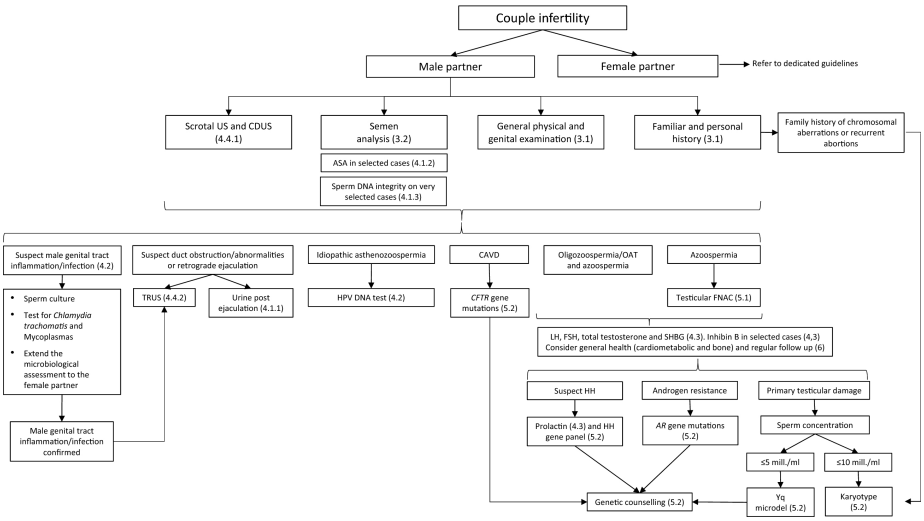
Hypogonadotropic hypogonadism is a medical condition resulting in inadequate gonadal stimulation by LH and FSH, either due to insufficient stimulation of GnRH or pituitary problems. Failure of migration of the GnRH secretory neurons

to the forebrain represent the principal etiology of GnRH deficiency. When associated to anosmia, it is called Kallman syndrome, otherwise it is referred as normosmic idiopathic hypothalamic hypogonadism.

Hyperprolactinemia inhibits gonadotropin secretion leading to low serum testosterone levels, infertility and sexual dysfunction (2). It can be due to a prolactin secreting pituitary adenoma that can be assessed via imaging.

Post-testicular impairment involves either obstruction to sperm delivery or ejaculatory dysfunctions. Obstruction can be congenital or acquired and can be located in different parts of the seminal tract as the epididymis, being the most common, the vas deferens and the ejaculatory duct. Among the acquired ones, infections are considered the principal cause, but vasectomy and inguinal hernia repair represent other triggers. Post-inflammatory obstruction of the ejaculatory duct is usually secondary to urethral prostatitis.
On the other hand, congenital bilateral absence of the vas deferens (CBAVD) is found in most men with cystic fibrosis (2).

3. DIAGNOSTIC PATHWAY



Diagnostic flowchart for male factor infertility. Numbers indicate the corresponding recommendations in the text. *AR* androgen receptor, *ASA* antisperm antibodies, *CAVD* congenital absence of vas deferens, *CDUS* color Doppler ultrasound, *CFTR* cystic fibrosis transmembrane conductance regulator, *FNAC* fine needle aspiration cytology, *FSH* follicle-stimulating hormone, *HH* hypogonadotropic hypogonadism, *HPV* Human Papillomavirus, *LH* luteinizing hormone, *OAT* oligo-astheno-teratozoospermia, *SHBG* sex-hormone-binding globulin, *TRUS* trans-rectal ultrasound, *US* ultrasound

Figure 3. Management of MFI.

Male factor infertility remains a significant challenge for clinicians in everyday practice. There is no general consensus regarding the diagnosis and the best workup for the evaluation of fertility potential and risk factors (3).
The clinical approach to the male partner is not uniformly standardized across international guidelines. The diagnostic approach is often limited to semen analysis (25), with the couple being directly referred to ART.

When should a couple begin the diagnostic pathway for infertility? Generally, after 12 months, but if there are known risk factors, as advanced maternal age, or HPV infection for instance, it can be started even after 6 months.

The medical approach should be complete, couple-centered and should involve reproductive specialists, respectively gynecologists and andrologists, who should proceed jointly (25). The diagnostic and therapeutic process should proceed in parallel for both partners (26).

3.1 First-step analysis

Risk factor assessment: medical history and physical examination

A thorough history should be performed as a starting point in all patients seeking medical attention for infertility, before starting any therapeutical procedure or second-level examination(27). It should be aimed at understanding both the general health of the patient, including his lifestyle and comorbidities, and the andrological health. It should be focused on finding possible causes and risk factors for infertility, like history of cryptorchidism or testicular trauma. It should investigate as well the possible sexual problems involving libido, erection and ejaculation (3).
A general physical examination should be carried out, together with an andrological examination, evaluating secondary sex characteristics, with palpation of testes to assess their volume, but also to gain information related to the epididymis and the eventual presence of abnormalities.

Endocrine assessment

A hormonal profile with gonadotropins (FSH & LH), total testosterone (TT) and sex hormone-binding globulin (SHBG) can rule out or rule in the presence and the

type of hypogonadism. In addition, prolactin (PRL), vitamin D, albumin, thyroid stimulating hormone (TSH) and estradiol can amplify the endocrine assessment for disease impacting on fertility (27).

This step represents an essential part of the process as it allows the distinction between primary and secondary spermatogenic failure and the recognition of hypogonadism. It contributes to the treatment choice, even for empirical approaches. As any other step, hormonal assessment alone does not allow an etiological diagnosis, but it should always be interpreted together with other data emerged from investigations, in order to have a complete picture of the patient (20).

Sperm analysis

Firstly, it is crucial clarifying that semen analysis is an essential component during investigations of an infertile couple, but it is not an absolute indicator of fertility. It is necessary, but not sufficient to obtain the final diagnosis. It is rather a sign of an underlying disorder, deriving from different pathophysiological mechanisms.

It differs from the clinical diagnosis, and it should always be combined with a comprehensive evaluation including adequate medical history, physical examination, endocrine assessment and ultrasound examination (25). No therapy should be initiated only based on semen analysis alone (3).

It is a tool to better understand the physiology of the reproductive organs and the causes of dysfunction.

It provides information on the functional status of the entire seminal tract, including the seminiferous tubules, epididymis and accessory sex glands (28). It reflects the production of spermatozoa in the testes, the patency of the duct system and the glandular secretory activity (6).

Sperm analysis is a descriptive exam and is not a direct marker of sperm function (6).

Semen analysis should be performed by highly experienced personnel, in qualified laboratories and should follow strict internal and external quality control programs. Manuals released by the World Health Organization should be used as a source of standard methods. During interpretation pre-analytical, analytical and post-analytical factor should be considered, since they could interfere with the reliability of the analysis (3).

Reference values depicted by WHO guidelines are expressed as percentiles and the lower limit is represented by the fifth percentile (5).

It must be clarified that, although WHO guidelines represent reference values, they cannot be used as a universal cutoff to limit fertile and infertile men in a dichotomous manner (29). Fertility must be considered a continuum, as there is a substantial overlap between the results of semen examination of fertile and infertile populations (28).

Even though WHO thresholds are based on the 5th percentile, natural conception is entirely possible below this reference value (6). On the other hand, parameters in the 95% confidence interval do not guarantee fertility (3).

In addition, there is not only interindividual variability, but biologic variability even within the same individual. Therefore, the results from at least 2, and preferably 3, separate seminal analyses have to be obtained before a conclusion can be drawn (29).

Parameter	Lower Reference Limit
Semen volume (mL)	1.5 (1.4–1.7)
Total sperm number (million per ejaculate)	39 (33–46)
Sperm concentration (million per mL)	15 (12–16)
Total motility (progressive + non-progressive, %)	40 (38–42)
Progressive motility (%)	32 (31–34)
Vitality	58 (55–63)
Sperm morphology (normal forms, %)	4 (3.0–4.0)
Other reference values	
pH	≥7.2
Peroxidase-positive leukocytes (million per mL)	<1.0

Figure 4. Lower reference limits (5th percentiles and their 95% confidence intervals) for semen parameters.

Seminal characteristics

The ejaculate is a heterogeneous mixture of secretions and cells. The fluid volume reflects the secretory activity of the accessory glands, being the seminal vesicles, the prostate and bulbourethral (Cowper's) glands, and the smooth muscle contraction following the autonomic nervous stimuli after sexual arousal. The cellular portion is mainly made by spermatozoa, whose number reflects testicular production, patency of the ductal system and the efficacy of smooth muscle

contraction (5). The nature of spermatozoa and the fluid composition are important for sperm function.

Volume and pH

The normal seminal volume is between 1.5 to 5.0 mL and the pH usually spans from 7.4 to 8. Low volume (<1.5 mL) usually reflects incomplete collection or ejaculatory duct obstruction, but it can also occur due to hypogonadism or retrograde ejaculation. The large majority of seminal volume (50% to 80%) is due to the alkaline, fructose-rich fluid produced by the seminal vesicles, and the remaining portion is due to the acidic secretions of the prostate directly at the level of the urethra, without communicating with ejaculatory ducts. As a result, low-volume, acidic ejaculate can occur due to ejaculatory duct obstruction. A pH value under 7.2 can be due to a lack of alkaline seminal vesicular fluid or can also occur due to urine contamination (5).

Viscosity

Sperm texture depends on the secretion of seminal vesicles, which are mainly responsible for coagulation, and prostate secretion, which instead contains proteolytic enzymes in charge of liquefaction. Abnormalities is viscosity may indicate presence of infection, defects in prostatic proteolytic enzymes or absence of the seminal vesicles (5).

Concentration and total number

Sperm number is reported as both concentration (millions/mL) and as total sperm count (concentration multiplied by volume in mL). These values are both predictors of conception and are related to time to pregnancy and pregnancy rates (5).

The number of spermatozoa is correlated with testicular volume and function, in the context of an unobstructed tract and a short abstinence time (5).

Oligospermia is a reduced number of spermatozoa in the ejaculate, lower than 15 million spermatozoa/ml or lower than 39 million in total count. Oligospermia is considered severe when there are less than 5 million/ml spermatozoa.

Azoospermia is defined as the absence of spermatozoa in the ejaculate. It must be confirmed by centrifugation. Once verified, azoospermia may reflect either

complete obstruction of the male genital tract or severe impairment of sperm production.
On the other hand, cryptozoospermia is the finding of less than 1 million spermatozoa/ml in the sediment of a centrifuged sample.

Motility

Motility is acquired by sperm through the passage in the epididymis. “Total motility” indicates the percentage of sperm with a motile flagellum. “Progressive motility” instead, refers to the amount of sperm having forward progression. The total number of progressively motile spermatozoa in the ejaculate is of biological significance, meaning that there is a correlation with pregnancy rates (5). Nowadays a four-category classification for staging motility is recommended. Specifically, progressive spermatozoa are further divided into rapidly and slowly progressive. The other two classes include non-progressive spermatozoa and immotile ones (5).
Asthenozoospermia occurs when total motility is lower than 40% or progressive motility is below 32%.

Different conditions can impair this parameter, including infection, anti-sperm antibodies, heat, varicocele or toxins. When persistently abnormal, there can be a higher risk of altered sperm DNA fragmentation.

Vitality

It is estimated by evaluation of cellular membrane integrity. It is particularly important when there are a large number of immotile spermatozoa, in order to understand whether they are dead or alive. Usually, a high number of immotile and dead cells indicates an infection or an epididymal pathology, instead, a high number of live but immotile cells, can be associated to structural defects at the level of the flagellum.

Morphology

The ejaculate contains a wide range of different morphological appearances. It is important to evaluate both the proportion of “normal” spermatozoa, but also to understand the specific morphology of the head, midpiece and tail.

Teratozoospermia indicates the presence of abnormal sperm morphology > 4%.
It must be underlined that there is no correlation between altered morphology and sperm aneuploidy or birth defects in the offspring. On the other hand, though, a normal morphology does not strictly exclude the presence of another pathology within the sperm.

Other cells

In the ejaculate there can be found other cell types including epithelial cells of the genito-urinary tract, leukocytes and immature germ cells (5). Approximately 20% of men have white blood cells in the seminal plasma. Both leukocytes and immature cells are referred as “round cells” and it not simple to discriminate between them.

Semen Parameter	Reference Range	Abnormality	Description
Semen volume	≥1.5 ml		
pH	≥7.2		
Sperm concentration	≥15 million sperm/ml	Azoospermia	Absence of sperm in seminal plasma
		Oligozoospermia	<15 million spermatozoa/ml
		Cryptozoospermia	<1 million spermatozoa/ml
Total sperm count	≥39 million sperm/ ejaculate		
Total sperm motility	≥40% motile sperm	Asthenozoospermia	<40% total motile spermatozoa or <32% progressive motile spermatozoa
Progressive sperm motility	≥32% progressively motile sperm	Asthenozoospermia	<40% total motile spermatozoa or <32% progressive motile spermatozoa
Sperm morphology	≥4% morphologically normal sperm	Teratozoospermia	<4% normal form/morphology
		Oligoasthenoteratozoospermia (OAT syndrome)	Combination of <15 million spermatozoa/ml, <32% progressive motile spermatozoa, and <4% normal form

Figure 5. WHO reference range for semen analysis with examples of main semen parameters abnormalities.

Semen analysis can detect three main types of abnormalities, either quantitative or qualitative sperm defects and semen fluid alterations. When an abnormality is found, further exams are needed in order to reach a clinical conclusion (3).

Ultrasonography

Scrotal ultrasound is the gold standard for testicular investigation by assessing reproductive, inflammatory and neoplasia-related features (20). It is a tool which,

when handled by experienced physicians, allows the assessment of testicular volume, texture and vascularization. Testicular US should be performed as part of routine investigation in all male partners of infertile couples(30).

There is a tight relationship between testicular volume, sperm and hormonal parameters. About 90% of the testis is composed by seminiferous tubules and, as a result, reduced testicular volume (right testis < 12 ml, left testis < 11 ml) is associated with impaired semen parameters, reduced fertility and hypogonadism.

In addition, scrotal US can provide information on abnormalities or agenesis of the epididymis and vas deferens (31). Color Doppler US can detect and grade the presence of varicocele (32), which could have a negative impact on sperm parameters (20).
Furthermore, US can evaluate lesions, suggesting benign or malignant findings, and important feature as infertility is a risk factor for testicular cancer.

On the other hand, transrectal (TRUS) and penile Doppler US should be performed as second level examinations, only in specific cases. The former particularly when suspecting obstructions or abnormalities of the seminal tract, especially in subjects with genital tract infection or inflammation. The latter instead, should be used when facing erectile dysfunction.

Step	
1. Assess the causes and risk factors	• History and physical examination
	• Fertility potential of the female partner
2. Assess the fertility potential	• Semen analysis
3. Proceed to full investigation to define etiology, pathophysiology and to have prognostic and therapeutic information	Consider:
	• Infections
	• Endocrine assessment
	• Scrotal and trans-rectal ultrasound
	• Testicualr cytology/histology
	• Genetic testing
4. Classify in diagnostic categories to define the best therapeutic strategy	• Inflammation and infection of the genital tract
	• Duct obstruction and retrograde ejaculation
	• Primary testicular damage (hypergonadotropic or normogonadotropic)
	• Secondary testicular dysfunction (hypogonadotropic hypogonadism)
	• Idiopathic semen alteration
	• Unexplained infertility
5. Select the treatment strategy	• Etiologic management (remove the cause)
	• Oriented management (act on risk factor)
	• Goal-oriented management (bypass the problem)
	• Empirical management (by acting on testicular function, seminal tract or sperm function)
6. Define the aims of treatment	• If possible, restore natural fertility
	• Allow ART
	• Allow gradual application of ART
	• Improve ART success
	• Preserve fertility
	• Cure the underlying disorder and preserve reproductive health

Figure 6. Schematic flowchart of management of male factor infertility.

3.2 Second-level examinations

Additional tests should be personalized according to the individual clinical picture of the patient. When specific risk factors for male infertility are known or to present or found during initial investigation, second-level exams are suggested (3).

Semen microbiological assessment

When suspecting a genital tract infection or inflammation, standard semen culture and nucleic acid amplification tests should be requested. Infections can be present in the testis, epididymis, prostate and seminal vesicles. Often, the term MAGI (male accessor gland infection) in used referring to them (3).

Suspects can be driven by symptoms like pain during ejaculation and/or seminal alterations like abnormal semen pH or viscosity, positivity of ASA or leukocytospermia.

Results should be considered in the context of other clinical information and especially on imaging studies (3).

HPV search in the semen should be performed, following the exclusion of other pathogens, when there is a known positivity in the female partner, when there is history of repeated abortion or ART failure and in patients with asthenozoospermia.

Imaging

Pituitary MRI can be useful in cases of central, secondary hypogonadism, after the assessment of the endocrine axis, when hypothesizing abnormalities at the level of the hypophysis.

Transrectal ultrasound is very useful in the context of MAGI and seminal tract inflammation, but also allows to adequately assess the presence and eventual obstructions at the level of the vas defers and ejaculatory duct (3).

3.3 Third-level examinations

Testicular cytology and histology

For purely diagnostic processes, fine needle aspiration cytology (FNAC) is the gold standard for the histopathological analysis of the testis. It enables to classify different spermatogenic alterations including Sertoli cell-only syndrome (SCOS), hypospermatogenesis and germ cell maturation arrest. Furthermore, it allows the distinction between obstructive and non-obstructive forms of azoospermia, which is fundamental for the decision of further clinical and therapeutic approach (20). For instance, FSH therapy is more indicated for pictures characterized by hypospermatogenesis rather than maturation arrest.

Biopsy, instead, is suggested when associated with cryopreservation of sperm for future ARTs. It is not recommended as only a step in the diagnostic process, since it is an invasive procedure.

Genetic testing

Genetic contribution to male factor infertility is considerable and appropriate investigations should be performed in a targeted approach in selected patients (3). There is an inverse correlation between sperm count and the risk of genetic anomalies, specifically non-obstructive azoospermic patterns are associated with a very high risk. It is therefore recommended targeted genetic analysis in infertile men to identify the etiology.

Screening includes karyotype analysis, Yq microdeletions, androgen receptor (AR) gene (33)and the cystic fibrosis transmembrane regulator gene (CFTR), especially when there is a documented absence of the vas deferens.

Furthermore, some genetic factors not only have reproductive consequences, but also impact the general health of the patient and might be transmitted to his descendants. Whenever a genetic abnormality is found, genetic counselling is recommended to the couple.

There are several tests allowing the detection of genetic abnormalities, the most used one are sperm aneuploidy test and FISH.

Additional exams

DNA fragmentation

DNA damage within spermatozoa can impact the genetic material either via single or double strand breaks and can be caused by different mechanisms that can be associated with different pathological and environmental conditions (5). It is only partly related to semen quality, indeed it can be present in normozoospermic individuals and it represents an important addition in the investigations of male infertility.

There are several assays differing in method and type of damage detected. Terminal deoxynucleotidyl transferase nick end labelling (TUNEL) directly detects the presence of DNA strand breaks, instead Acridine orange flow

cytometry (AO FCM) detects the chromatic susceptibility to treatment by acid (34). The diagnostic threshold of these methods differs and should be specified.

Urine analysis
When suspecting retrograde ejaculation, post-ejaculation urine analysis should be performed, in order to check for the presence of spermatozoa inside the bladder. This impairment is frequent in diabetic patients due to autonomic neuropathy, in men with spinal cord injury or other neurological problems and in patients who underwent urological procedures like transurethral resection of the prostate (TURP) (3).

Specific blood exams
Depending on clinical data and etiolgy other blood tests can be requested.
A glycometabolic profile, including cholesterol and glycated hemoglobin (HbA1c), should be asked for patients with obesity or diabetes or other CV risk factors.

A window to general health

Infertile men are objectively less healthy and have an increased mortality risk compared to their fertile counterparts of the same age and ethnicity (25). Infertile men have increased risk for metabolic syndrome and osteoporosis but also increased risk of hospitalization and long-term morbidity (35).
Detecting individuals with endocrine or spermatogenic impairment is clinically important for their prognosis and follow-up.

Several studies support evidence showing an association between infertility and several risk factors, including cardiovascular ones. The pathogenetic link remains unclear, hypogonadism is a possible explanation, but associated comorbidities are a frequent event in infertile men with normal testosterone levels and low sperm count (20). Chronic inflammation and early senescence represent possible involved mechanisms.

Therefore, fertility investigations constitute an opportunity to screen and to mitigate long-term risk of non-communicable diseases (NCDs), with relevant real-

life consequences. Testicular function can be considered as a mirror of general health, as it represents an opportunity for medical assessment and disease prevention. The early identification of hypogonadism or other testicular dysfunctions allows for an adequate management, lifestyle modifications and treatment.
Infertility should be viewed as possible symptom of a more constitutional disease, and men should be investigated accordingly (3).

4. CLASSIFICATIONS

4.1 Etiological classification

Traditionally, the most commonly used classification is the etiological one. It includes three macro categories of male factor infertility based on identifiable causes of altered sperm production: pre-testicular, testicular and post-testicular.
Pre-testicular forms involve conditions where the spermatogenic potential of the testes is preserved, but hormonal stimulation targeting the gonads is insufficient, like hypogonadotropic hypogonadism. There is no adequate stimulation by the hypothalamic-pituitary axis.
Testicular causes refer to intrinsic dysfunction resulting in abnormal levels of sperm and hormones production by the gonads. These include genetic abnormalities, infections and inflammations, torsion, trauma, injury or cancer.
Post-testicular category comprises forms where the hypothalamic-pituitary-gonadal axis functions regularly, but there is an impaired exit of sperm, which cannot reach the oocyte. Most common conditions of this group are seminal tract obstruction and ejaculatory or erectile dysfunctions.

4.2 Diagnostic-therapeutic classification

Although the etiologic classification can be useful for the standardization of male infertility diagnosis, it has limited clinical utility as it does not specifically allot individual treatment choices, since each subgroup encompasses heterogeneous etiologies and risk factors, impeding a standardized therapeutic approach. For

example, infections and genetic abnormalities are within the same category, even though they involve very different treatment approaches.
Due to the above explained limitation, a novel classification has been proposed. It consists of new diagnostic categories for male factor infertility based on pathophysiologic mechanisms, accordingly unified to the appropriate therapeutic option: (i) inflammation and infection of the genital tract; (ii) duct obstruction,; (iii) primary testicular damage; (iv) secondary testicular dysfunction; (v) idiopathic infertility with semen alteration; (vi) unexplained infertility, where no semen alterations are found, despite the couple infertility; (vii) varicocele. This last category has been added specifically for cases of clinically impacting varicoceles, only after the exclusion of other potential causes of infertility. Only varicoceles of high or intermediate grade are considered, when associated to verified seminal alterations and/or verified varicocele-induced scrotal injury.
This classification takes into consideration a new approach aimed at seeking the clinical conditions associated with seminal alterations, not merely examining semen analysis.

I. Inflammation and infection of the genital tract

Both these conditions can affect testicular environment, resulting in impaired spermatogenesis. In this case, the purpose of treatment is to resolve the condition with appropriate medical therapy, in order to restore an appropriate environment for sperm production.

II. Duct obstruction

It can either be congenital or acquired and comprehends retrograde ejaculation.
The obstruction can be approached medically or surgically and in case of failure retrieval can be attempted at the level of the testes, epididymis or in the urine.

III. Primary testicular damage

Damage can be of various degrees, and this category is further divided into hypergonadotropic (iiiA) and normogonadotrpic (iiiB), according to the plasma values of FSH, elevated in the first case and unaltered in the latter. This distinction is important since normongonadotropic testicular dysfunction might benefit from empirical therapy with FSH.

IV. Secondary testicular dysfunction

In these forms the hypothalamic-pituitary- gonadal axis is impaired, as in hypogonadotropic hypogonadism, characterized by reduced levels of testosterone, FSH and LH. Homeostatic stimulation can be restored with hormonal replacement therapy or resolution of associated pathologies.

V. Idiopathic infertility with seminal alterations

In this case, potential causes are not detected, but only impaired sperm parameters are found. Empirical therapied are still possible, although associated with variable and unpredictable efficacy.

VI. Unexplained infertility

This condition is characterized by persistent couple infertility, although no alterations were found during investigations. Indeed, semen parameters are normal, as well as hormonal profile and there are no anomalies detected during medical history or physical examination. The absence of identifiable causes is associated to limited therapeutic possibilities and high levels of distress within the couple.

VII. Varicocele

This last extra-category was added following the other six, for patients with clinically impacting varicocele. Particularly, it considers varicocele as an explanation of couple infertility only after the exclusion of other causes and it only includes varicoceles of high grade (IV and V) associated to semen alterations or varicoceles of intermediate grade (III), again associated to semen abnormalities, but also confirmed varicocele-induced scrotal injury verified with contrast-enhanced US.

APPROACH TO TREATMENT

Antibiotic treatment was administered to patients with documented genital tract infections, following sperm culture or urethral swab. It is important to prescribe them only when there is evidence of germs present in a concentration considered pathogenic. Theoretically, the therapy should be based on pathogen identification and on its sensitivity towards antibiotics detected with an antibiogram.

Corticosteroids or non-steroidal anti-inflammatory drugs (NSAIDs) were given to patients with documented genital tract inflammation, like leukocytospermia or other signs of inflammation at imaging, after the exclusion of infections.

HPV clearance occurs naturally by the immune system within 6 months in 60% of cases, but HPV vaccine can be suggested to the couple in order to accelerate the process.

Gonadotropins represent the most evidence-based efficacious treatment for MFI.

FSH treatment acts by stimulating spermatogenesis, especially during the first phases, enhancing it both from a quantitative and a qualitative point of view, in synergism with intratesticular testosterone (11). FSH has traditionally been utilized for cases of hypogonadotropic hypogonadism, with great improvements of seminal parameters and pregnancy outcomes (36). Following these results, it has been proposed also for patients with oligospermic and/ or asthenozoospermic patterns, associated to normal plasma levels of FSH (< 8 UI/L) and hypospermatogenesis at testicular cytology, after the exclusion of seminal tract obstructions (37). Several studies confirmed the efficacy of FSH therapy in this selected population of infertile men. Treatment should be done in a couple-oriented manner and effects on semen parameters can be visible after at least 3-4 months, eventually prolonging the therapy up to 6-12 months when needed.

This type of treatment is not recommended to improve pregnancy rates without a prior appropriate diagnostic work-up.

Selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs) represent an off-label proposal for specific cases of MFI. Although more studies are needed, in clinical practice they are suggested to patients with primary testicular pathologies and FSH concentrations > 8 UI/L. The net effect of these types of therapies is to further increase the levels of FSH, resulting in improved seminal parameters (37). This suggests that a hyperstimulation of testicular function with FSH could also be possible in patients belonging to the 3B category (FSH levels > 8 UI/L).
The choice of the specific drug should be personalized according to the clinical picture of the patient, especially according to the ration between TT and estradiol.

Varicocele correction is recommended only for clinically impacting varicoceles (grades III-IV-V of Sarteschi-Liguori), after the exclusion of other causes of MFI following an accurate diagnostic work-up. Surgical or radiological varicocele repairing is indicated with 1) homolateral testicular hypotrophy; 2) presence of persistent testicular symptoms impacting the patient quality of life; 3) evidence of relevant seminal alterations; 4) progressive worsening of the seminal picture (37). Once again, although varicocele is a common clinical finding among MFI population, it is a rare cause of actual infertility, and it is an exclusion diagnosis after a thorough diagnostic framework.

Antioxidants are suggested after a complete diagnostic pathway to patients with altered seminal parameters and increased oxidative stress signs like DNA sperm fragmentation (37). Particularly, they were proposed to patients with idiopathic oligozoospermia and/or asthenozoospermia. They can be used as a potential aid to idiopathic infertility or can be associated to therapy in cases of abacterial or post-infective inflammation (38). They are not recommended in unselected infertile men populations (20).Their use should be evidence-based, indeed further studies are needed, and should be personalized according to the specific patient findings (37).

Lifestyle changes including weight loss, increased physical activity, smoking cessation and reduced alcohol intake are recommended in men with infertility.

Fertility preservation

Sperm cryopreservation in highly recommended in patients with planned medical or surgical treatment associated to fertility impairment (18). Indicated conditions include cancer and its gonadotoxic treatments, chronic autoimmune and inflammatory diseases, but also urologic disease. These pathologies are associated to a good prognosis and without this prevention, long-surviving patients would face irreversible consequences. These conditions can cause damage by altering spermatogenesis, genome integrity or affect ejaculatory mechanisms.

It is also suggested in patients with active desire of fatherhood and with oligoasthenozoospermia to prevent eventual progressive deterioration of semen quality.

Reproductive cells are maintained in a state of "suspended animation" at cryogenic temperatures (-196°C) reached with either rapid or slow freezing procedures.

The overall rate of patients achieving fatherhood with cryopreserved semen is about 50%, therefore, clinicians are encouraged to discuss post-treatment fertility and advise sperm cryopreservation (20).

EXPERIMENTAL STUDY

Aim

The following monocentric retrospective study aspires to establish the impact of the new pathophysiological classification on the diagnosis and the treatment of male factor infertility in a cohort of infertile male patients. It evaluates the effects of the new proposed categories in a clinical setting, following a precise and comprehensive diagnostic process. Finally, it intends to demonstrate how the idiopathic share can be reduced after an appropriate investigation.

Methods and materials

Sample

Data was retrieved from male patients, with age between 28 and 44 years old, attending the Unit of Andrology and Reproductive Medicine of Padua for investigations on infertility, in a time interval between January 2023 and August 2025.

The main inclusion criterion was couple infertility, either primary, as well as secondary or other forms. Besides, patients with previous ARTs attempts were considered as well.

Male patients of infertile couple with known female factors infertility were excluded from the study, as well as men with semen alterations but no desire of conception. For the present study, only male patients of infertile couples with isolated MFI were considered, where MFI was the major cause of pregnancy absence.

Evaluations

All subjects enrolled in the study underwent a general medical and personal history looking for familiar and individual risk factors towards testicular diseases like testicular cancer and cryptorchidism. They also underwent an andrological physical examination, mainly evaluating testicular volumes, secondary sexual characteristics and eventual presence of abnormalities.

The gonadal function was mainly evaluated through semen analysis, hormonal and testicular US. Sperm evaluation was based on the 6th edition of WHO manual for semen examination with a principal focus on sperm concentration (millions per ml) and total sperm count (total millions). Scrotal US mainly investigated testicular volumes (mL) and testicular features as echogenicity, homogeneity and vascularization, as well as the seminal tract and varicocele, when present.

Further exams, like semen microbiological evaluations, transrectal prostatic US and genetic panels (karyotype, CFTR gene and microdeletions of Y chromosome) were added only when needed.

In particular, general and atypical bacteria, and human papillomavirus (HPV) were investigated in patients with signs and symptoms of semen infections or in

patients with altered semen volume, pH or viscosity and in presence of leukocytes.
TRUS was utilized for the evaluation of prostate volume (cc) and detections of signs of male accessory gland infections or inflammation (MAGI) in patients with specific semen alterations (concentration, total count, motility, morphology, pH, volume, viscosity, leukocytes) as well as in patients with signs and symptoms of chronic prostatitis.

Measures
Anthropometric information (height and weight) was retrieved, together with hemato-chemical exams and semen parameters. Specifically, for gonadal endocrine evaluation, total plasma testosterone (TT nmol/L), sex hormone-binding globulin (SHBG, nmol/L) and albumin (g/dL) were considered for the calculation of free testosterone (cFT, nmol/L) through Vermeulen formula. A threshold of total testosterone below 12 nmol/L or cFT lower than 0,220 nmol/L were considered suggestive for hypogonadism.
Finally, concerning gonadotropins, were considered normal values of FSH (UI/L) between 1.5 and 8 UI/L and between 1 and 9,4 (UI/L) for LH.

Data was collected mainly via the hospital informatic system and sometimes integrated with paper medical records.

Consent
The study protocol followed the standard clinical approach, and the principles outlined in the Declaration of Helsinki.

Following a thorough diagnostic work-up, the sample was divided according to the new diagnostic-therapeutic classification of MFI (see table III).
Moreover, an extra category was added (Category VII) for patients with clinically significant varicocele. Specifically, for high grade varicocele of IV and V grade, associated to semen alterations after the exclusion of other causes, and varicocele

of III grade with semen abnormalities and confirmed varicocele-induced testicular damage detected at contrast-enhanced US.

Category	Sub-Category	Description
I	IA	Infection
	IB	Inflammation
II	n.a.	CBAVD and Acquired Total Obstruction
III	IIIA	Primary Testicular Disease with high FSH
	IIIB	Primary Testicular Disease with normal FSH
IV	n.a.	Hypogonadotropic Hypogonadism
V	n.a.	Idiopathic Semen Impairment
VI	n.a.	Idiopathic Couple Infertility
VII	n.a.	Clinically Significant Varicocele

Table I. New proposal of diagnostic-therapeutic classification of MFI.

I. Evidence of semen infection and/or inflammation or detection of inflammation signs at US, in patients with semen alterations with normal testicular volumes and endocrine profile

II. Congenital absence of the vas deferens, either bilateral (CBAVD) or unilateral (CUAVD), retrograde ejaculation of iatrogenic post-surgical obstruction, in patients with normal testicular volumes and endocrine profile

III. Evidence of signs of primary testicular diseases, with low testicular volume or low TT concentrations with normal or increased FSH and/or LH concentrations, or detected testicular damage at FNAC or presence of a genetic alteration related to primary testicular pathologies

IV. Presence of inappropriately low TT concentrations with inappropriately low FSH and/or LH concentrations, including both congenital hypogonadotropic hypogonadism and acquired or functional hypogonadotropic hypogonadism

V. Presence of idiopathic semen alterations without an identified cause following a thorough and appropriate diagnostic pathway of MFI

VI. Idiopathic couple infertility following a thorough and appropriate diagnostic pathway of both male and female partners

VII. Clinically relevant varicocele responsible of semen alterations, after an accurate exclusion of other possible causes like primary testicular diseases or inflection/inflammation

RESULTS

This study evaluated a total of 505 male patients of infertile couples due to exclusively MFI as a major role of couple infertility, after the exclusion, as previously explained, of male partners of infertile couples with positive female infertility factors.

The table below reports the descriptive analysis of our cohort of patients.

Parameter	**Normal Values**	**Mean +/- SD**
Age of M (years)	n.a.	35.7 +/- 7.8
Age of F (years)	n.a.	35.1 +/- 5.2
Time of Infertility (years)	n.a.	2.9 +/- 2.1
BMI (kg/m2)	19.1-24.9	26.3 +/- 5.4
Sperm Concentration (mil)	>15	11.6 +/- 27.7
Total Sperm Count (mil)	>38	32.4 +/- 80.0
FSH (UI/L)	1.5-8.0	12.1 +/- 11.4
LH (UI/L)	1.0-9.4	6.8 +/- 4.3
TT (nmol/L)	12-30	15.3 +/- 9.1
Right Testis Vol (mL)	12-26	12.0 +/- 5.3
Left Testis Vol (mL)	11-24	11.5 +/- 5.0
Prostate Volume (mL)	15-35	22.4 +/- 7.2

Table II. Descriptive analysis of the cohort of 505 male infertile patients with isolated MFI. Abbreviations: BMI: body mass index; E2: 17-beta-estradiol; F: female partner; FSH: follicle-stimulating hormone; LH: luteinizing hormone; M: male partner; PRL: prolactin; TSH: thyroid stimulating hormone; TT: total testosterone.

In our sample of 505 patients with isolated MFI, it was found that mean age of male partner was 35.7 +/- 7.8 years, whilst mean age of female partner was 35.1 +/- 5.2 years, with a mean time window of couple infertility of 2.9 +/- 2.1 years. In addition, male partners were overall slightly overweight (mean BMI: 26.3 +/- 5.4 kg/m2). Regarding sperm number it was found that mean sperm parameters were 11.6 +/- 27.7 mil/ml of sperm (sperm concentration) and 32.4 +/- 80.0 millions of sperm

(total sperm count). Concerning hormonal parameters, the analysis showed an overall condition of testicular diseases, with a mean FSH concentration of 12.1 +/- 11.4 UI/L, associated with both a mean LH concentration (6.8 +/- 4.3 UI/L) and a mean TT concentration (15.3 +/- 9.1 nmol/L) within the range of normality. Testicular volumes (US) were found to be in the lower limits of normality, with right testis 12.0 +/- 5.3 mL and left testis 11.5 +/- 5.0 mL. Prostate volumes (22.4 +/- 7.2 cc) were found inside the range of normality.

The sample of 505 patients was divided into the seven diagnostic-therapeutic categories proposed, as can be seen in the table below.

Category	**Sub-Category**	**Number of patients**	**Percentage**
I (infection/inflammation)	n.a.	126/505	24.95 %
II (CBVAD/obstruction)	n.a.	16/505	3.17 %
III (primary testicular disease)	IIIA (high FSH)	204/505	40.40 %
	IIIB (normal FSH)	129/505	25.54 %
IV (hypogonadotropic hypogonadism)	n.a.	9/505	1.78 %
V (idiopathic semen alterations)	n.a.	1/505	0.20 %
VI (idiopathic couple infertility)	n.a.	13/505	2.57 %
VII (significant varicocele)	n.a.	7/505	1.39 %

Table III. Diagnostic classification of our cohort of 505 patients with isolated MFI.

In our cohort of 505 patients, we found that the most represented categories were category IIIA and IIIB, followed by category I. In details, category IIIA was found in 40.40 % of patients and category 3B in 25.54%. Category I instead, was found in 24.95% of patients. The conditions labelled as idiopathic infertility, meaning category V and category VI, account for 2.77 % of patients (specifically, 0.20% in V and 2.57% in VI). Low representations were also found in category II with 3.17% of patients and category VII with 1.39% of patients.

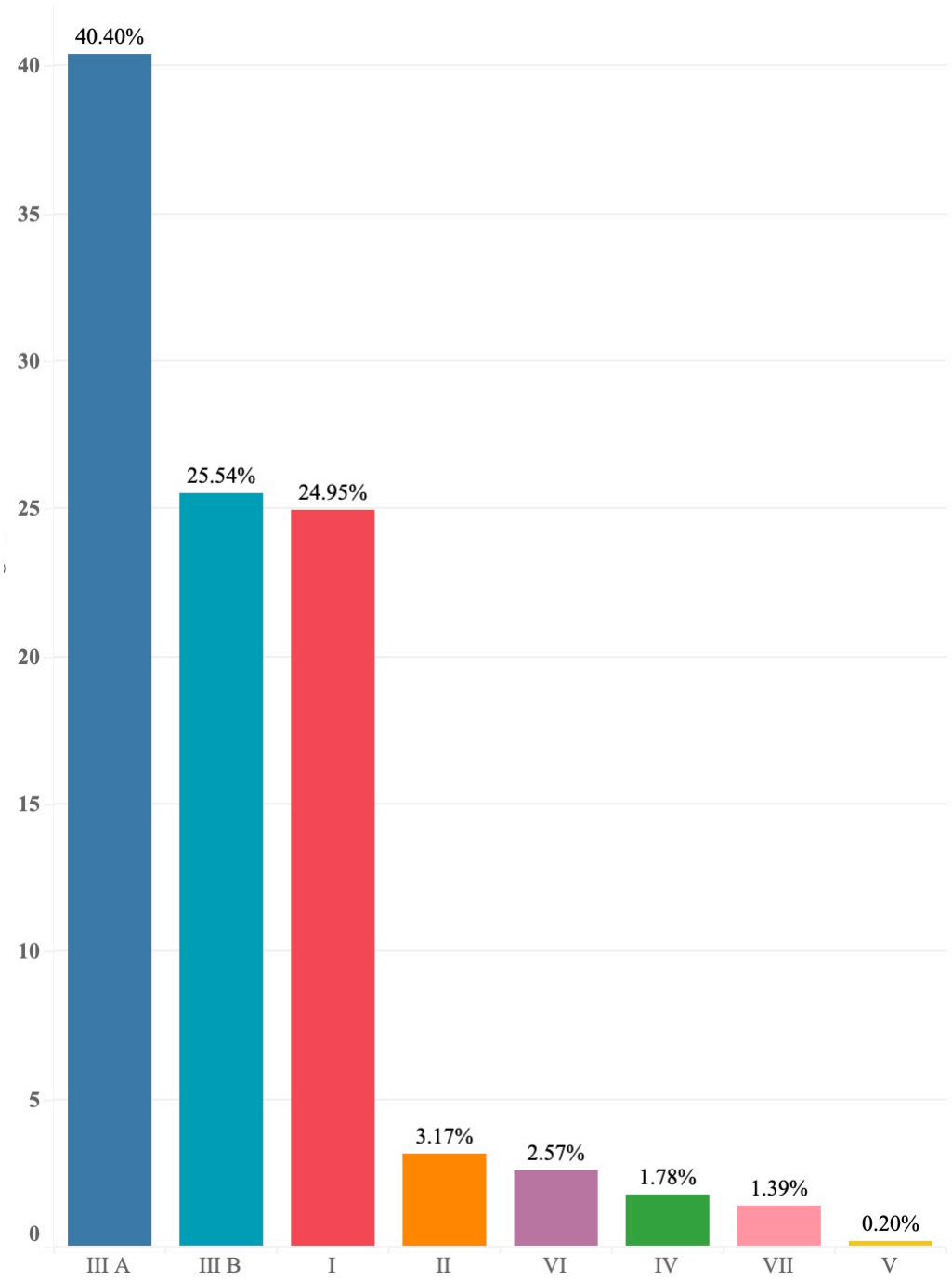


Figure 7. Patient distribution across different categories.

After a pairwise comparison between different categories, no statistically significant differences were found regarding age at first visit, age of female partner, infertility time interval and BMI.

On the other hand, statistically significant differences were found concerning hormonal and seminal parameters, as well as testicular volumes.

In the table below specific data can be seen.

	$\chi 2$	gdl	p
Age at 1° visit	8.15	6	0.227
Infertility time interval	4.79	6	0.571
Age of female partner	8.41	6	0.210
BMI	4.18	6	0.652
FHS	185.11	6	<.001
LH	95.71	6	<.001
Zoi/ml	110.37	6	<.001
Zoi tot	110.37	6	<.001
Dx cc	82.68	6	<.001
Sx cc	87.60	6	<.001
Prostate cc	15.24	5	0.009

Table IV. Principal parameters evaluated in the cohort of patients.

Differences in main parameters according to the category of MFI

We performed a statistical analysis to assess statistical differences across the sample of 505 patients, particularly focusing on the differences among patients with primary testicular disease and those belonging to other groups.

There is no significant difference regarding age of male and female partners at first evaluation, infertility time interval, and BMI of male patients across different categories. On the other hand, when considering hormonal parameters, we found that patients constituting category IIIA, thus the one related to primary testiculopathy, with severe damage to testicular function, we found important differences compared to other categories like IIIB. As we would expect, we found increased levels of primarily FSH (21.5 +/- 12.4 UI/L), but also of LH (9.5 +/- 5.0 UI/L). Contrarily to category III, gonadotropin levels were found to be normal in categories I and II. No relevant difference was found considering TT concentrations in different categories. Concerning semen parameters, both sperm concentrations and total sperm count were found to be reduced in patients of category IIIA (respectively 1.68 +/- 6.84 and 6.17 +/- 29.1 millions of zoa) compared to other categories like I, IIIB and VI. Remarkably, in respect to patients belonging to

category IIIB (respectively 12.9 +/- 35.1 and 104.0 millions of zoa). Finally, patients of category IIIA had both lower bilateral testicular and prostate volumes compared to patients of other groups as IIIB and I.

In the following boxplots a graphical representation of the parameters explained above can be seen.

Figure 8a-k. Graphical representation of the main parameters evaluated in the different subgroups of patients.

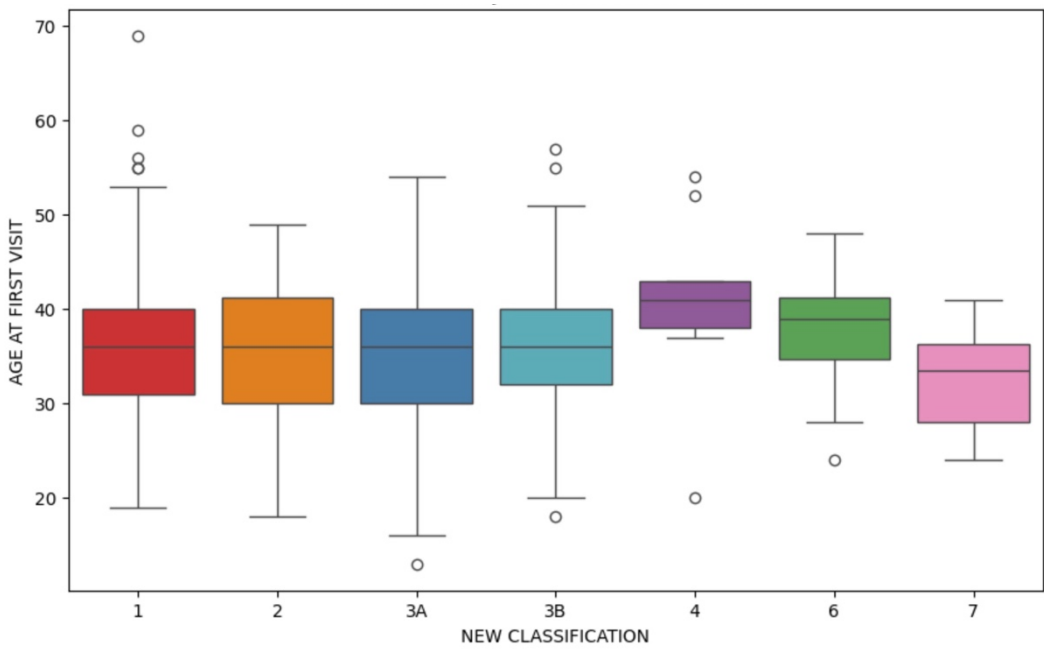


Figure 8a. Age of male partner (years) at first visit in the different groups of patients.

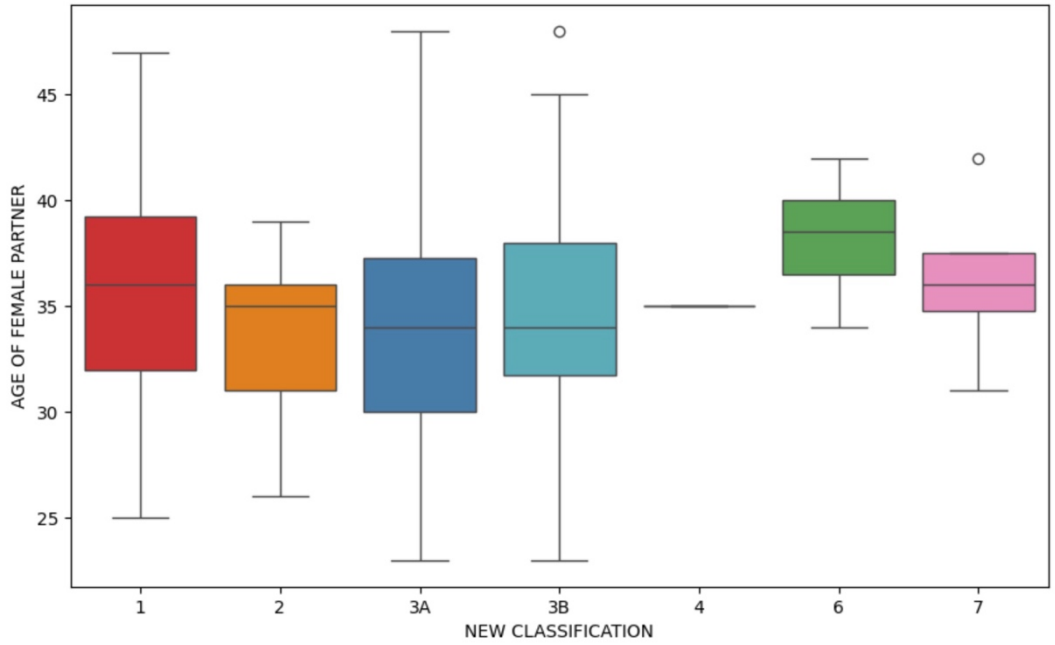


Figure8b. Age of female partner (years) in the different groups of patients.

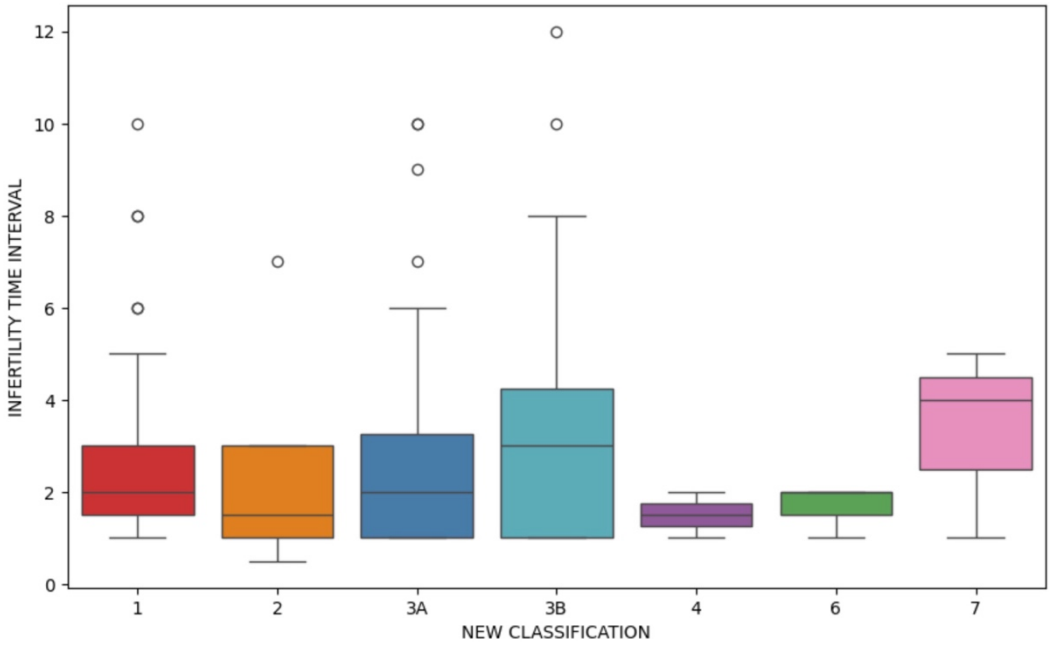


Figure 8c. Infertility time (years) in the different groups of patients.

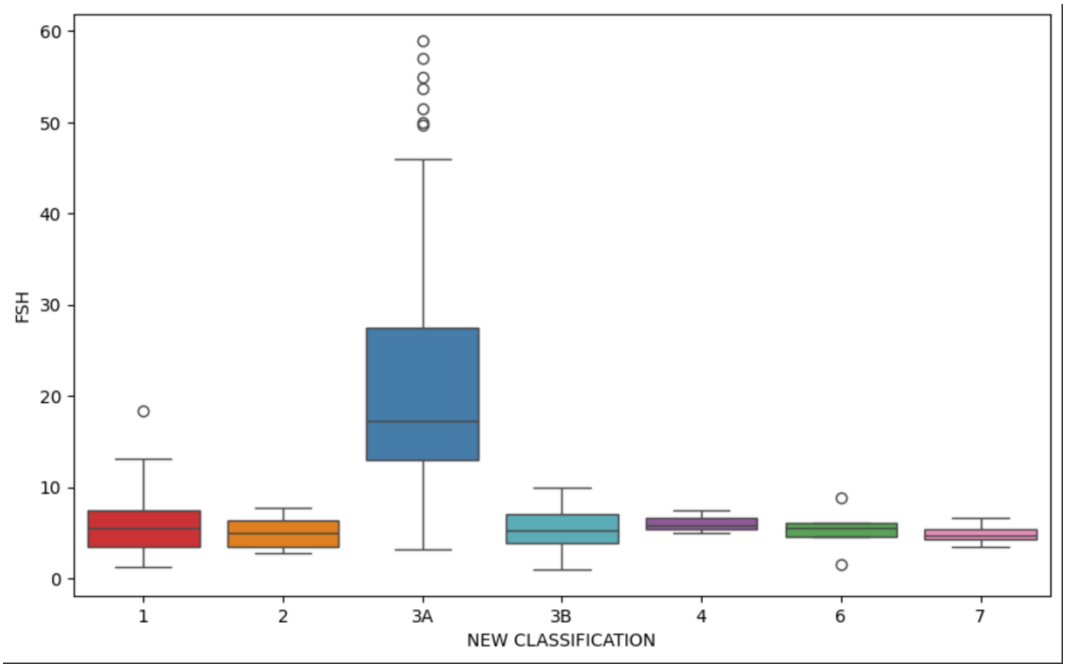


Figure 8d. FSH concentrations (UI/L) in the different groups of patients.

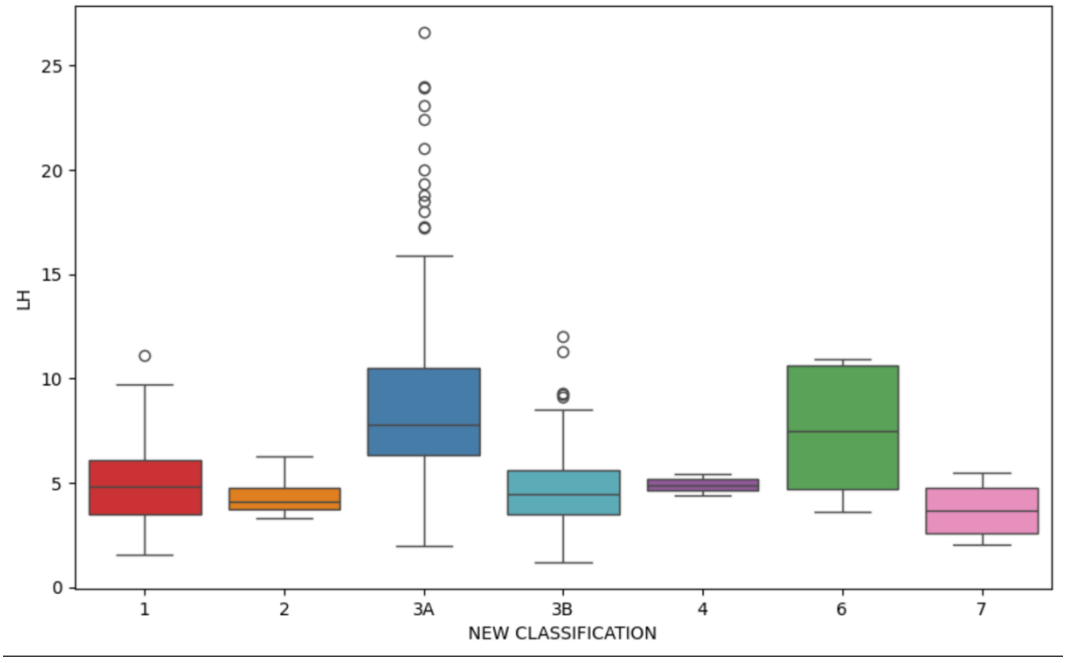


Figure 8e. LH concentrations (UI/L) in the different groups of patients.

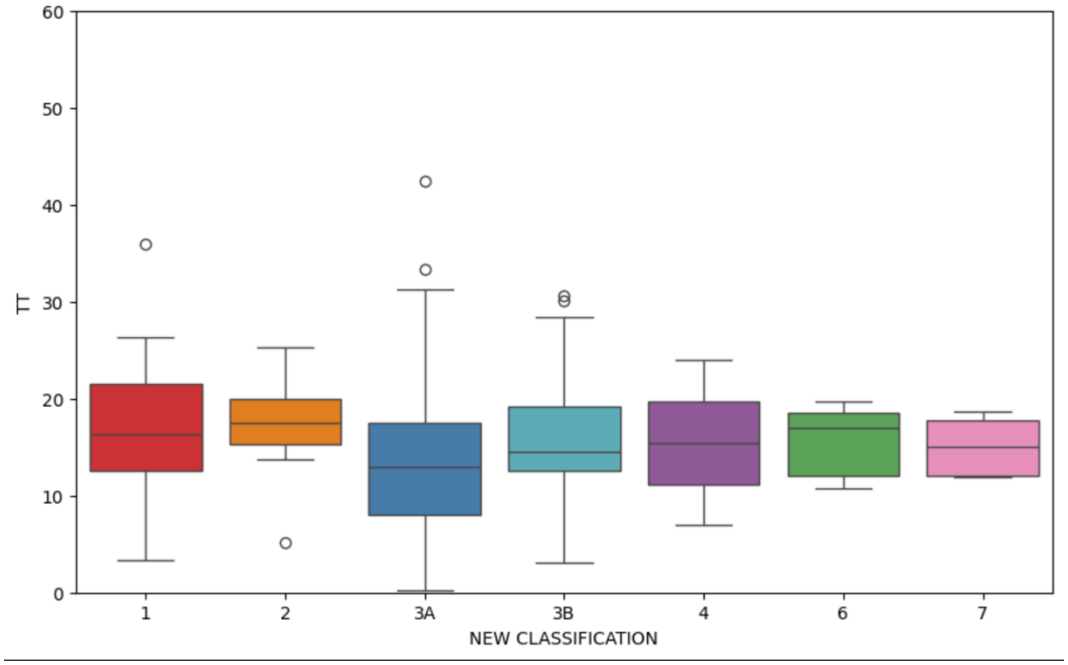


Figure 8f. TT concentrations (nmol/L) in the different groups of patients.

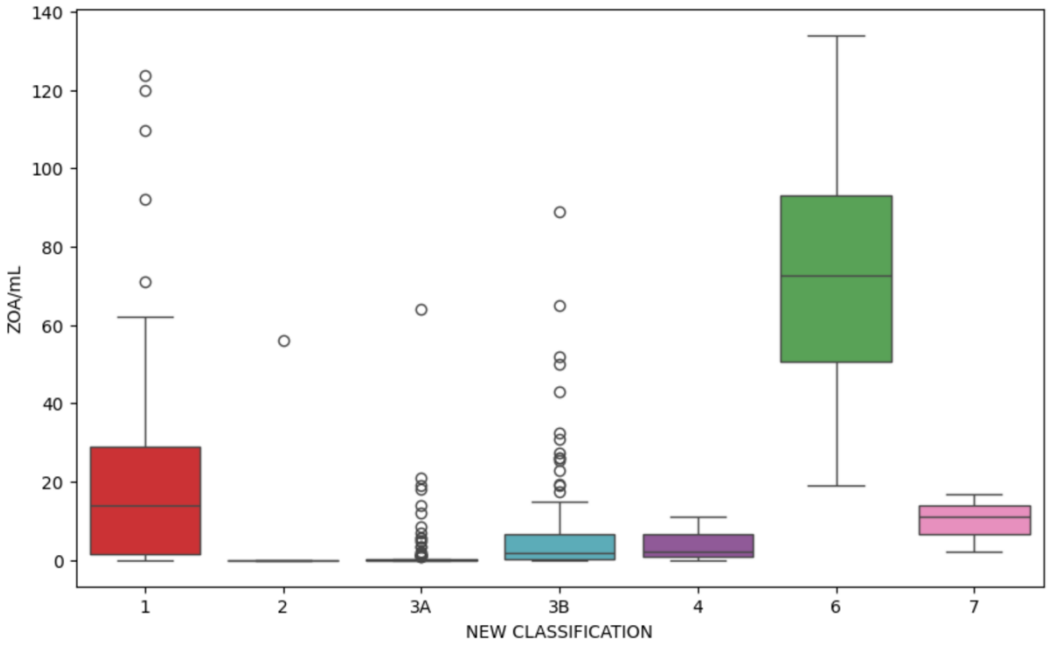


Figure 8g. Sperm concentration (mil/mL) in the different groups of patients.

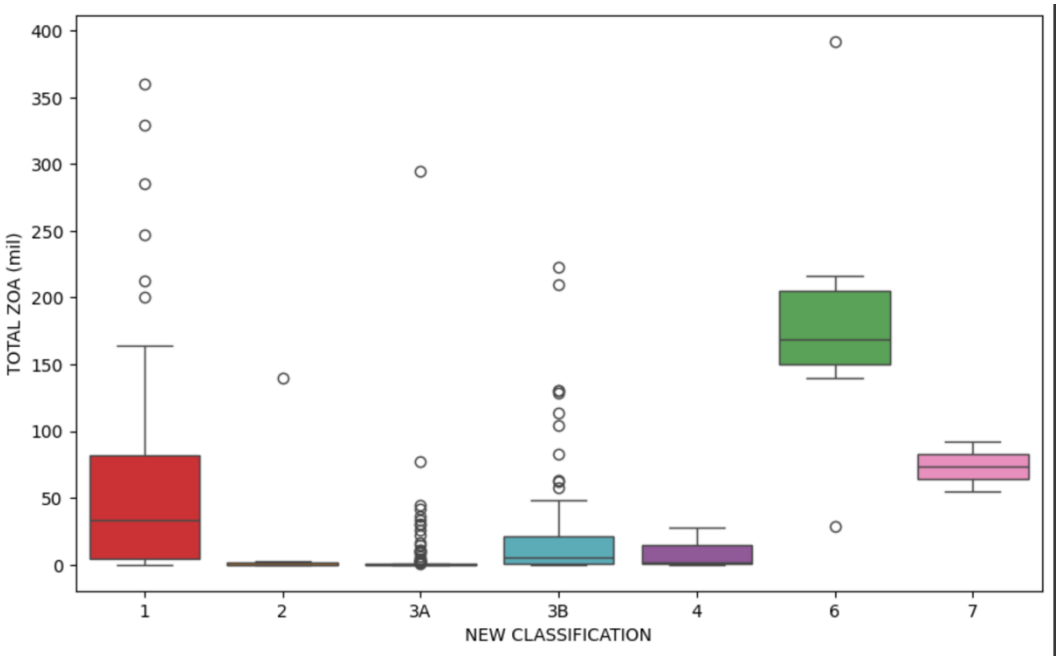


Figure 8h. Total sperm count (millions) in the different groups of patients.

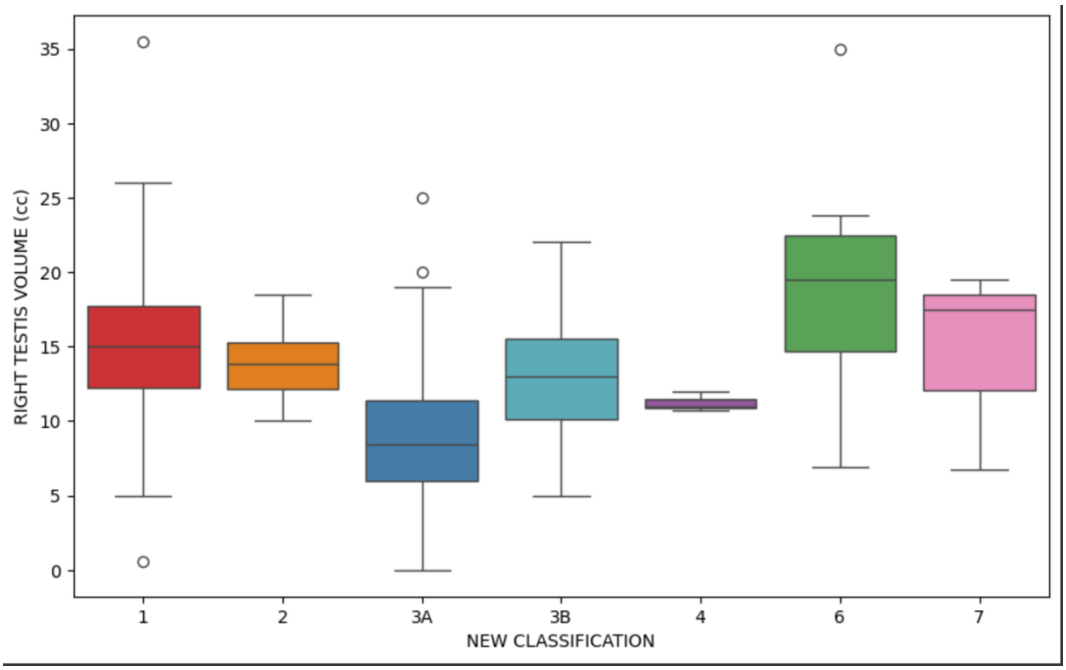


Figure 8i. Right testis volume (mL) in the different groups of patients.

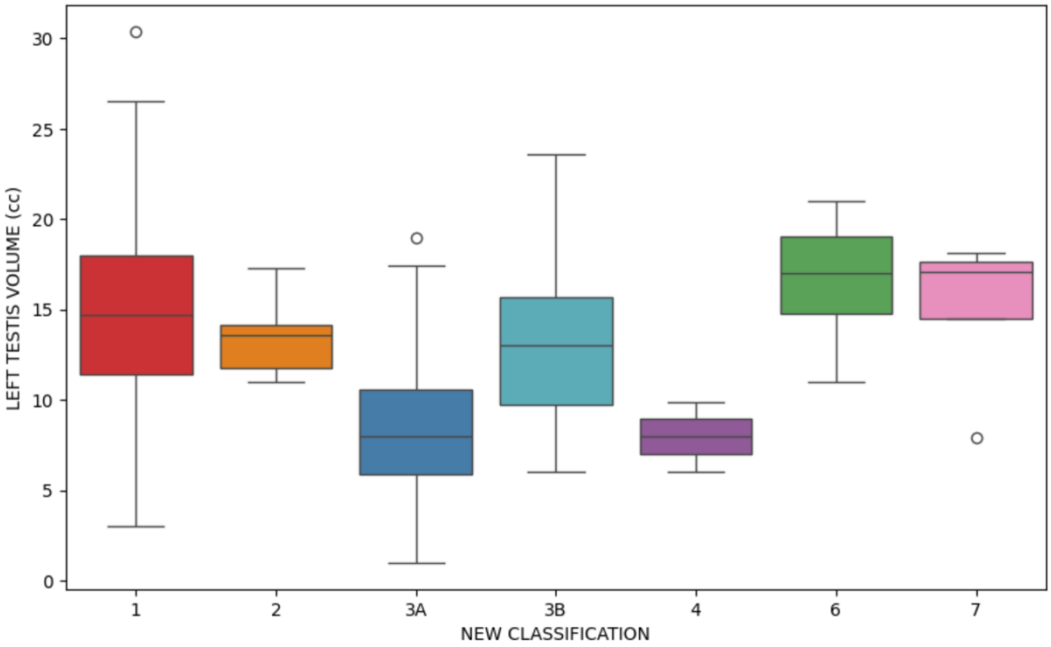


Figure 8j. Left testis volume (mL) in the different groups of patients.

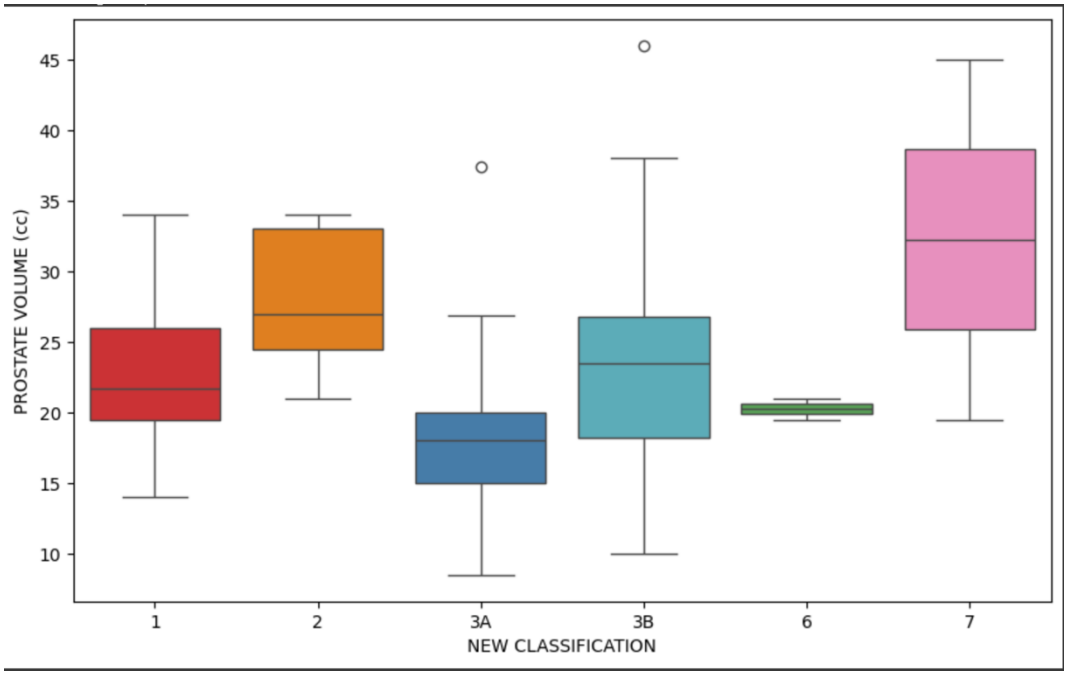


Figure 8k. Prostate volume (mL) in the different groups of patients.

FSH therapy

Furthermore, we conducted a preliminary evaluation of the therapeutic impact of this new classification, focusing on FSH treatment on a subgroup of selected patients with MFI (31 patients, assigned in the category 3B). Inclusion criteria involved the exclusion of obstructions and sub-obstructions via transrectal ultrasound, and the exclusion of infections with semen culture. Finally, only patients with oligozoospermia and/or asthenozoospermia and FSH levels below 8 UI/L, accordingly to the Italian legislation (*nota 74, AIFA*), were considered. FSH therapy has been administered for 3 to 4 months in line with the Italian Health System (fully reimbursed).

Parameter	**n.v.**	**values**
Age of M	n.a.	36.2 ± 6.04
Age of F	n.a.	33.3 ± 5.46
Time of infertility	n.a.	2.95 ± 1.87
FSH (UI/L)	**1.0-8.0**	4.83 ± 1.87
LH (UI/L)	1.0-9.4	4.52 ± 1.63
TT (nmol/L)	12-30	14.2 ± 3.32
Right testis (cc)	12-26	12.3 ± 2.79
Left testis (cc)	11-24	12.4 ± 3.86

Table V. Cut-off values and found values.

We analyzed sperm values, both concentrations and total sperm count, before and after the treatment with FSH. In the boxplots below we can see a graphical representation of the baseline sperm parameters of the selected population.

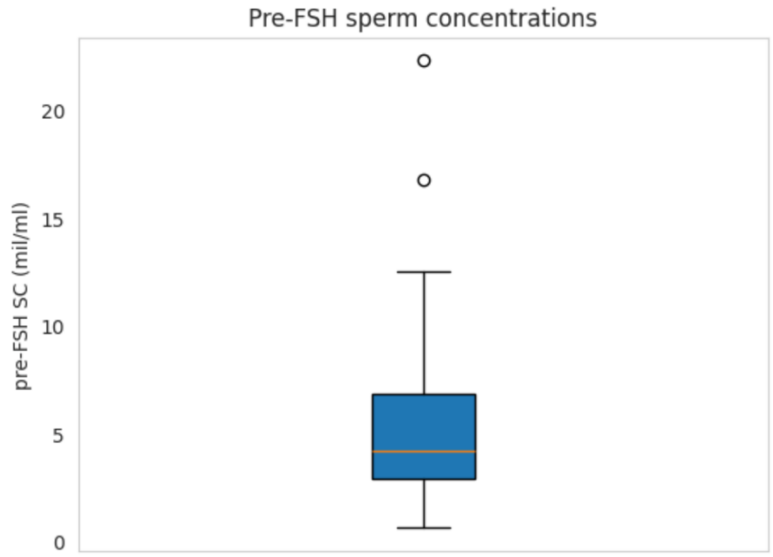


Figure 9. Pre-FSH sperm concentrations (mil/mL).

We can see that the median of sperm concentration of the sample of patient examined is of 4.53 ±4.46 mil/mL (n.v. ≥ 16 mil/mL) before the administration of FSH.

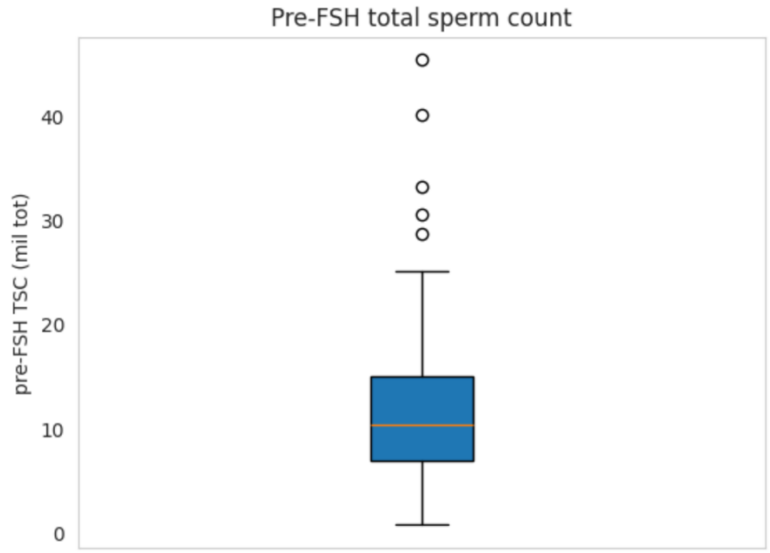


Figure 10. Pre-FSH total sperm count (millions).

We can see that the median of total sperm count of the sample of patient examined is that of 10.20 ±10.70 millions (n.v. ≥ 3.9 millions) before the administration of FSH.

Although in literature an improvement in semen parameters is considered when values increase of about 50%, we took into account a stricter threshold with an increase > 100% in sperm concentration (zoa/mL) or > 100% in total sperm count (total zoa).

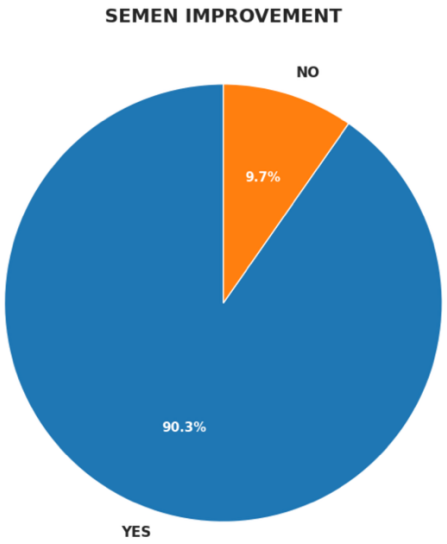
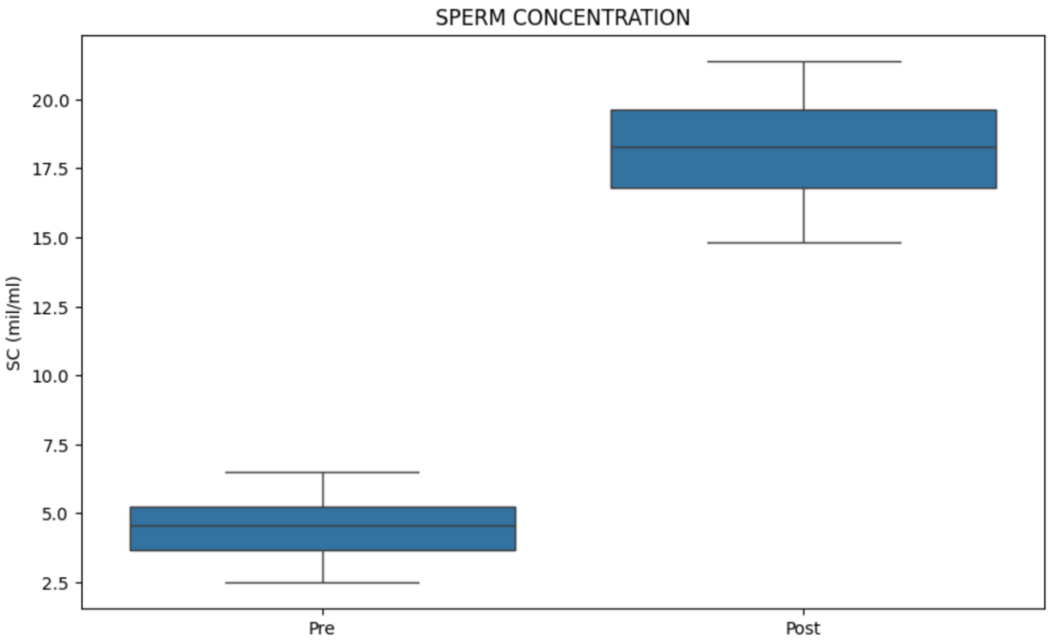
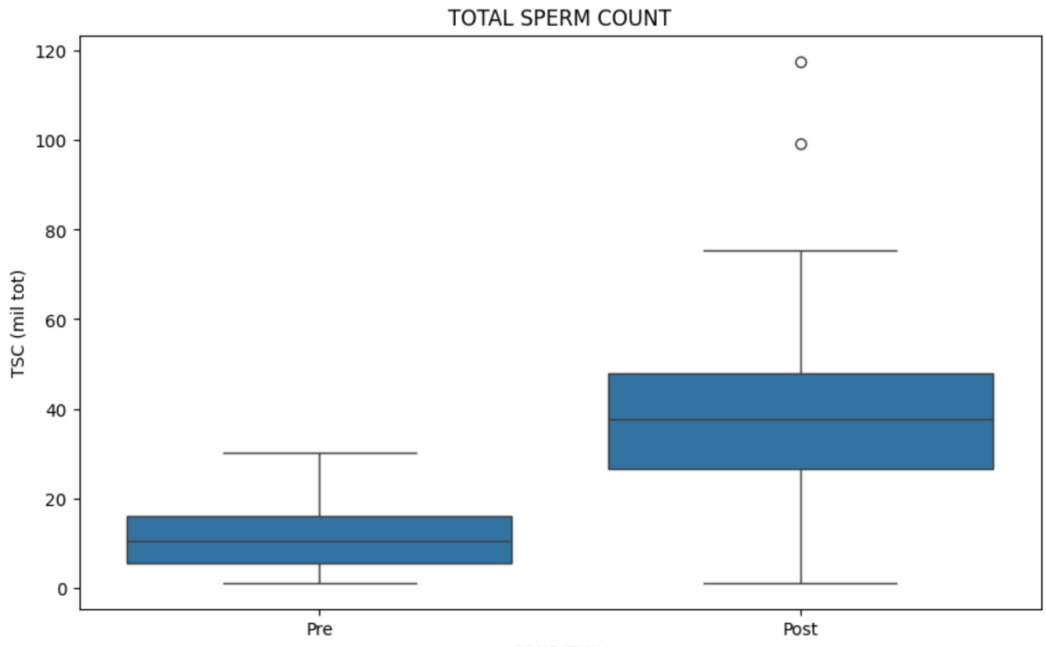


Figure 11. Percentage of semen improvement after FSH therapy in a cohort of 31 patients.



Pre-FSH:4.53 ± 4.46 mil/mL
Post-FSH: 17.5 ± 14.5 mil/mL

Figure 12. Comparison of sperm concentration between pre and post-FSH therapy on a cohort of 31 selected patients.



Pre-FSH: 10.3 ± 10.7 mil
Post-FSH: 38.6 ± 32.1 mil

Figure 13. Comparison of total sperm count between pre and post-FSH therapy in a cohort of 31 selected patients.

We can see how there is a statistically significant increase in sperm number higher than 100%, both in sperm concentration and total sperm count after FSH therapy. In the first case the increase is of ~ 286% on average (from 4.53 ± 4.46 mil/mL to 17.5 ± 14.5 mil/mL) and in the second case there is an increase of ~ 275% on average (from 10.3 ±10.7 millions to 38.6 ± 32.1 millions).

It can be noted that there is a high degree of variability in baseline sperm parameters before FSH administration, but there is also a high degree of variability in sperm parameters after FSH therapy. This occurs as there is a high heterogenicity of individual response and nowadays there are still neither known predictive nor early markers of semen improvement.

DISCUSSION

Couple infertility is a widespread clinical condition affecting 10 to 15% of couples around the world (2) and MFI accounts for at least half of the overall cases, either in combination with female factors or alone (6). However, MFI is often disregarded or only briefly investigated with semen analysis alone and, when alterations in sperm parameters are found, the couple is steadily sent to ARTs (26). Furthermore, a large share of MFI, around 30 to 50%, are considered to be idiopathic and one of the most prevalent etiologies is considered to be varicocele(8).
The conventional anatomical classification divides the causes into pre-testicular, testicular and post-testicular. This classification is useful for research purposes, but it has limited clinical utility in everyday settings, as it does not lead to uniformly standardized treatment options (25). The novel MFI classification proposed, seems to be useful, reliable and simple to use in clinical practice, since it considers simple aspect of male evaluation (medical history, physical examination, testicular volume, semen alterations, hormonal profile and ultrasonographic features). Since patients are categorized according to pathophysiological mechanisms behind infertility, this new classification should better guide treatment options. A comprehensive diagnostic work-up is essential to find an accurate etiological and pathophysiological explanation behind MFI.

It can be noticed that within our study a large proportion of patients belong to categories which are associated with reversible causes of MFI. For instance, 126 patients out of 505 patients, about one fourth of patients (24.95 %), presented a condition of infection/inflammation as a leading cause of MFI, thus fitting in category I of infection and inflammation. These, after an antibiotic or anti-inflammatory treatment, sometimes followed by antioxidant supplements, usually show an improvement of seminal parameters and an increased rate of conception, both spontaneous and through ARTs. Another highly populated category is the third one (IIIa and IIIb), related to primary testicular diseases, with a total of 333 patients out of 505 (> 50%). This occurred also because our unit is a III-level center of referral for complex conditions and often patients arrive with previous visits and investigations.

Contrary to data found in literature, in our study we found only a 2.77% share categorized as idiopathic infertility (category V plus category VI). This shows how, following a thorough diagnostic management, the actual idiopathic share shrinks to about one tenth of the portion found without a proper investigation pathway. This actual percentage of idiopathic infertility confirmed a previous multicentric study recently performed (26), where it was affirmed that the diagnosis of idiopathic infertility should be made only after the evaluation and the exclusion of all known causes of MFI, using a meticulous and complete approach (39).

Another relevant discordant finding of this study is the impact of varicocele on fertility, as we found only 1.39% of patients to have varicocele as a potential cause of MFI, contrarily to a share of 305 found in literature. Also in this case, there should be a complete and appropriate evaluation of all known causes of MFI and varicocele should be considered only when of high grade and after a thorough evaluation with US.

This novel classification is primarily intended for therapeutic management.

As we have specifically seen for FSH treatment, a correct identification of subjects is crucial in order to apply a targeted approach, which can lead to relevant improvement, even when applying strict criteria for both patient selection and for improvement thresholds. In particular, preliminary data of our evaluation showed the importance of the excluding the presence of eventual sub-obstructions, as it can relevantly impact the response to FSH treatment. Indeed, studies which do not foresee the exclusion of sub-obstructions, are associated to an improvement in seminal parameters only of about 50%, compared to an improvement higher than 100% as occurring in our evaluation. It is therefore once more advised to perform an appropriate and complete MFI diagnostic evaluation in order to maximize treatment response and efficacy.

Moreover, progress is being made to identify potential predictive markers of response to FSH therapy, like spermatid count, testicular cytology and polymorphisms in FSHR and FSHB genes, which could lead to the development of new personalized approach for the treatment of MFI (40).

CONCLUSIONS

This study confirms the relevance of the new diagnostic-therapeutic classification proposed for MFI. This classification appears to be reliable, valuable and easily usable in everyday clinical practice.

It is evident that, by using this categorization, the idiopathic share can be narrowed from 50-30% to 3-8% after a thorough diagnostic work-up. An accurate classification of patients with MFI offers better therapeutical chances. This is essential, as by properly categorizing the patients through the understanding of specific pathophysiological mechanisms behind their infertility, a correct therapeutic approach can be addressed. Furthermore, as shown by the study, a large proportion of patients belong to categories which involve reversible causes of MFI, like infection and inflammation. Indeed, after an appropriate treatment, fertility potential is restored and there can spontaneous conception. In addition, finding a precise cause can give an answer to the couple and, at least in part, relieve their distress towards the attempts of conception.

BIBLIOGRAPHY

1. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, De Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, 2017†‡§. Human Reproduction. 2017 Sept 1;32(9):1786–801.

2. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clinical Biochemistry. 2018 Dec;62:2–10.

3. Ferlin A, Foresta C. Infertility: Practical Clinical Issues for Routine Investigation of the Male Partner. JCM. 2020 May 30;9(6):1644.

4. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015 Dec;13(1):37.

5. WHO Laboratory Manual for the Examination and Processing of Human Semen. 6th ed. Geneva: World Health Organization; 2010. 1 p.

6. Sharma A, Minhas S, Dhillo WS, Jayasena CN. Male infertility due to testicular disorders. The Journal of Clinical Endocrinology & Metabolism. 2021 Jan 23;106(2):e442–59.

7. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Human Reproduction. 2007 June 1;22(6):1506–12.

8. Garolla A, Pizzol D, Carosso AR, Borini A, Ubaldi FM, Calogero AE, et al. Practical Clinical and Diagnostic Pathway for the Investigation of the Infertile Couple. Front Endocrinol. 2021 Jan 19;11:591837.

9. Greil AL. Infertility and psychological distress: A critical review of the literature. Social Science & Medicine. 1997 Dec;45(11):1679–704.

10. Eisenberg ML, Esteves SC, Lamb DJ, Hotaling JM, Giwercman A, Hwang K, et al. Male infertility. Nat Rev Dis Primers. 2023 Sept 14;9(1):49.

11. Santi D, Crépieux P, Reiter E, Spaggiari G, Brigante G, Casarini L, et al. Follicle-Stimulating Hormone (FSH) Action on Spermatogenesis: A Focus on Physiological and Therapeutic Roles. JCM. 2020 Apr 3;9(4):1014.

12. Hall JE, Hall JE, Guyton AC. Guyton and Hall textbook of medical physiology: Student consult. Activate at studentconsult.com. Searchable full text online. 12. ed. Philadelphia, Pa: Saunders, Elsevier; 2011. 1091 p.

13. Gardner DG, Shoback D. Greenspan's Basic & Clinical Endocrinology.

14. Foresta C, Moro E, Ferlin A. Y Chromosome Microdeletions and Alterations of Spermatogenesis.

15. De Vries JWA, Repping S, Van Daalen SKM, Korver CM, Leschot NJ, Van Der Veen F. Clinical relevance of partial AZFc deletions. Fertility and Sterility. 2002 Dec;78(6):1209–14.

16. Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, et al. Genital tract infections and infertility. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2008 Sept;140(1):3–11.

17. Gimenes F, Souza RP, Bento JC, Teixeira JJV, Maria-Engler SS, Bonini MG, et al. Male infertility: a public health issue caused by sexually transmitted pathogens. Nat Rev Urol. 2014 Dec;11(12):672–87.

18. Graziani A, Grande G, Martin M, Sorio D, Finocchi F, Corrò S, et al. Fertility Preservation in More than 7000 Male Patients: A Single-Center, Tertiary Care Registry Study over 30 Years. Cancers. 2025 Feb 18;17(4):689.

19. endocrine_disruptors_508.

20. Ferlin A, Calogero AE, Krausz C, Lombardo F, Paoli D, Rago R, et al. Management of male factor infertility: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS): Endorsing Organization: Italian Society of Embryology, Reproduction, and Research (SIERR). J Endocrinol Invest. 2022 May;45(5):1085–113.

21. Thurston L, Abbara A, Dhillo WS. Investigation and management of subfertility. J Clin Pathol. 2019 Sept;72(9):579–87.

22. Choy JT, Eisenberg ML. Male infertility as a window to health. Fertility and Sterility. 2018 Oct;110(5):810–4.

23. Leisegang K, Sengupta P, Agarwal A, Henkel R. Obesity and male infertility: Mechanisms and management. Andrologia [Internet]. 2021 Feb [cited 2025 Oct 7];53(1). Available from: https://onlinelibrary.wiley.com/doi/10.1111/and.13617

24. Baskaran S, Finelli R, Agarwal A, Henkel R. Diagnostic value of routine semen analysis in clinical andrology. Andrologia [Internet]. 2021 Mar [cited 2025 Oct 7];53(2). Available from: https://onlinelibrary.wiley.com/doi/10.1111/and.13614

25. Santi D, Corona G, Salonia A, Ferlin A. Current drawbacks and future perspectives in the diagnosis and treatment of male factor infertility, with a focus on FSH treatment: an expert opinion. J Endocrinol Invest. 2025 Jan 13;48(5):1085–100.

26. Grande G, Garolla A, Graziani A, Astorri AL, Cammarota MV, Merola A, et al. Comprehensive diagnostic and therapeutic approach to male factor infertility aimed at natural fertility: A multicentric retrospective cohort study. Andrology. 2025 Feb 10;andr.70006.

27. Tournaye H, Krausz C, Oates RD. Concepts in diagnosis and therapy for male reproductive impairment. The Lancet Diabetes & Endocrinology. 2017 July;5(7):554–64.

28. Chung E, Atmoko W, Saleh R, Shah R, Agarwal A. Sixth edition of the World Health Organization laboratory manual of semen analysis: Updates and

essential take away for busy clinicians. Arab Journal of Urology. 2024 Apr 2;22(2):71–4.

29. Stahl PJ, Stember DS, Schlegel PN. Interpretation of the Semen Analysis and Initial Male Factor Management. Clinical Obstetrics & Gynecology. 2011 Dec;54(4):656–65.

30. Garolla A, Grande G, Palego P, Canossa A, Caretta N, Di Nisio A, et al. Central role of ultrasound in the evaluation of testicular function and genital tract obstruction in infertile males. Andrology. 2021 Sept;9(5):1490–8.

31. Lotti F, Maggi M. Ultrasound of the male genital tract in relation to male reproductive health. Human Reproduction Update. 2015 Jan 1;21(1):56–83.

32. Bertolotto M, Cantisani V, Drudi FM, Lotti F. Varicocoele. Classification and pitfalls. Andrology. 2021 Sept;9(5):1322–30.

33. Rocca MS, Minervini G, Vinanzi C, Bottacin A, Lia F, Foresta C, et al. Mutational Screening of Androgen Receptor Gene in 8224 Men of Infertile Couples. The Journal of Clinical Endocrinology & Metabolism. 2023 Apr 13;108(5):1181–91.

34. Rex A, Wu C, Aagaard J, Fedder J. Implementation of an in-house flow cytometric analysis of DNA fragmentation in spermatozoa. Asian J Androl. 2020;22(3):246.

35. Ferlin A, Garolla A, Ghezzi M, Selice R, Palego P, Caretta N, et al. Sperm Count and Hypogonadism as Markers of General Male Health. European Urology Focus. 2021 Jan;7(1):205–13.

36. Behre HM. Clinical Use of FSH in Male Infertility. Front Endocrinol. 2019 May 24;10:322.

37. Grande G, Scafa R, Graziani A, Ferlin A. Terapia del fattore maschile di infertilità. L'Endocrinologo. 2025 June;26(3):277–84.

38. Kesari KK, Roychoudhury S, editors. Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility- Volume One [Internet]. Cham: Springer International Publishing; 2022 [cited 2025 Oct 7]. (Advances in Experimental Medicine and Biology; vol. 1358). Available from: https://link.springer.com/10.1007/978-3-030-89340-8

39. Grande G, Graziani A, Ferlin A. Guideline for unexplained couple infertility: misunderstandings on the approach to the male factor. Human Reproduction. 2024 Apr 3;39(4):859–60.

40. Grande G, Graziani A, Scafa R, Garolla A, Santi D, Ferlin A. FSH Therapy in Male Factor Infertility: Evidence and Factors Which Might Predict the Response. Life. 2024 July 31;14(8):969.