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REVIEW

Assisted Reproductive Technology

Severe male factor in *in vitro* fertilization: definition, prevalence, and treatment. An update

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Infertility affects 10%–15% of couples worldwide. Of all infertility cases, 20%–70% are due to male factors. In the past, men with severe male factor (SMF) were considered sterile. Nevertheless, the development of intracytoplasmic sperm injection (ICSI) drastically modified this scenario. The advances in assisted reproductive technology (ART), specifically regarding surgical sperm retrieval procedures, allowed the efficacious treatment of these conditions. Yet, before undergoing ICSI, male factor infertility requires careful evaluation of clinical and lifestyle behavior together with medical treatment. Epidemiologically speaking, women whose male partner is azoospermic tend to be younger and with a better ovarian reserve. These couples, in fact, are proposed ART earlier in their life, and for this reason, their ovarian response after stimulation is generally good. Furthermore, in younger couples, azoospermia can be partially compensated by the efficient ovarian response, resulting in an acceptable fertility rate following *in vitro* fertilization (IVF) techniques. Conversely, when azoospermia is associated with a reduced ovarian reserve and/or advanced maternal age, the treatment becomes more challenging, with a consequent reduction in IVF outcomes. Nonetheless, azoospermia seems to impair neither the euploidy rate at the blastocyst stage nor the implantation of euploid blastocysts. Based on the current knowledge, the assessment of male infertility factors should involve: (1) evaluation – to diagnose and quantify seminologic alterations; (2) potentiality – to determine the real possibilities to improve sperm parameters and/or retrieve spermatozoa; (3) time – to consider the available "treatment window", based on maternal age and ovarian reserve. This review represents an update of the definition, prevalence, causes, and treatment of SMF in a modern ART clinic.

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INTRODUCTION

Male factor is responsible, at least in part, for about 20%–70% of all the causes of infertility.[1,2] A male reproductive impairment could be due to many different factors affecting sperm production, which can ultimately result in oligozoospermia (sperm concentration lower than 15×10^6 ml^{-1}), asthenozoospermia (sperm total motility lower than 40% or progressive motility lower than 32%), teratozoospermia (normal forms lower than 4%), or a combination of these three (oligoasthenoteratozoospermia [OAT]), as indicated by the 2010 World Health Organization reference values.[3] Severe male factor (SMF) infertility involves severe oligozoospermia ($<5 \times 10^6$ sperms per ml of ejaculate), cryptozoospermia (the condition by which spermatozoa cannot be observed in a fresh semen sample, but can be found after centrifugation and microscopic observation of the pellet), or even an absence of spermatozoa in the ejaculate. The latter is defined as azoospermia, a condition affecting 1% of the general male population and up to 10%–15% of the infertile male population.[4,5] Most of the scientific studies investigated the effect of SMF in *in vitro* fertilization (IVF), unfortunately without considering the female factor. As azoospermic couples acquire earlier in their lives an indication of IVF, they tend to be characterized by a younger female counterpart with a good ovarian reserve. Naturally, lower ovarian reserve and response to the ovarian simulation protocol worsen the IVF outcome, defined as the chance to achieve a live birth per intention to treat.[6]

This review aims to focus on: (1) definition, prevalence, and causes of SMF infertility; (2) current and emerging therapeutic approaches in IVF; and (3) the impact of SMF on intracytoplasmic sperm injection (ICSI) outcome, including couples presenting a low ovarian reserve and response.

MALE FACTOR INFERTILITY: DEFINITION AND PREVALENCE

Even if most of the causes of male infertility remain unexplained,[7] the main reasons can be grouped as pretesticular, testicular, and posttesticular forms. For a correct diagnosis of azoospermia, semen analysis should be performed according to the 2010 WHO guidelines,[3] and at least two samples, obtained more than two weeks apart, should be examined. In addition, azoospermia can be divided into two major categories: nonobstructive azoospermia (NOA; about 60% of the cases) due to either inadequate gonadotropin production (pretesticular) or

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intrinsic testicular impairment (testicular) and obstructive azoospermia (OA; about 40% of the cases) due to posttesticular causes.[8,9]

Pretesticular causes

Pretesticular NOA, defined as secondary testicular failure, may arise from endocrinological alterations of the hypothalamic–pituitary axis with a consequent inhibitory effect on spermatogenesis (genetic or not, congenital or acquired hypogonadotropic hypogonadism, with decreased luteinizing hormone [LH] and follicle-stimulating hormone [FSH] and normal or small testes; **Table 1**).

Congenital

In patients with congenital hypogonadotropic hypogonadism (HH), after excluding other secondary forms, gene mutation screening could be performed. Up to date, 35 genes have been identified (*i.e.*, fibroblast growth factor receptor 1 [*FGFR1*], fibroblast growth factor 8 [*FGF8*], SRY-box transcription factor 10 [*SOX10*], Kallmann syndrome-1 [*KAL1*], prokineticin 2 [*PROK2*], kisspeptin [*KISS1*], and kisspeptin 1 receptor [*KISS1R*]).[10] Interestingly, the most frequent form of HH is represented by Kallmann syndrome.

Acquired

Acquired causes of HH include pituitary tumors that can cause local destruction of the anterior pituitary, pituitary trauma, and panhypopituitarism.[11] Other possible causes of hypothalamic–pituitary–gonadal axis dysregulation are anabolic steroid use/abuse[12] and androgen resistance.[13] Androgen resistance is a rare cause of hypogonadism. It occurs in approximately 1:60 000 births, and more than 300 mutations occurring in the androgen receptor (Xq11–q12) have been described. The clinical manifestations depend on the intensity of the defects.[13]

Testicular causes

Testicular NOA involves disorders of spermatogenesis within the testes (genetic or not, congenital or acquired hypogonadism with elevated LH or FSH alongside small testes; **Table 1**).

Congenital

Genetic causes include chromosomal abnormalities, Y chromosome microdeletions, failure of the primordial germ cells to reach the developing gonads, lack of differentiation of the primordial germ cells to spermatogonia, and male germline mutations affecting spermatogenesis.[14–17] Genetic testing is therefore essential to indicate eventual testicular sperm retrieval and avoid unnecessary surgical or medical treatments.[18] Of all the genetic causes, chromosomal abnormalities may account for 20% of the male infertility cases. These alterations can be diagnosed in 15% of azoospermic and 5%–7% of oligozoospermic subjects.[18] Therefore, it is crucial to perform genetic testing before embarking on an IVF treatment. Moreover, KS is considered the most frequent genetic cause of male infertility, and it is due to the presence of an extra X chromosome (karyotype 47,XXY or other variants). Nevertheless, in these patients, the average successful surgical sperm retrieval rate reaches 50%.[19] Y-chromosome microdeletions are also implicated in male fertility since the long and short arms of the Y chromosome (Yq) contain many genes

Table 1: Male factor infertility: classification, main causes, and therapeutic approaches

Classification	*Type*	*Main causes*	*Therapeutic approaches*
Pretesticular	Congenital	Kallmann syndrome and similar	Medical treatment
	Acquired	Trauma, expansive/infiltrative lesions, surgery/RT, and panhypopituitarism	Hormonal treatment Stimulation with gonadotropins Antioestrogens (*i.e.*, clomiphene and tamoxifen citrate)
Testicular	Congenital	Genetic causes Chromosomal abnormalities (*i.e.*, 47,XXY syndrome) Y chromosome microdeletions Undescended testes Cryptorchidism Sertoli cell-only syndrome Androgen insensitivity syndrome	Medical treatment Hormonal treatment Stimulation with gonadotropins (controversial) Antioestrogens (*i.e.*, clomiphene and tamoxifen citrate) Nonhormonal treatment Antioxidants and other nutraceutics Cortisone Antibiotics/anti-inflammatory Surgical treatment Varicocele surgery Sperm retrieval techniques
	Acquired	Orchitis Testicular torsion Trauma Tumors Varicocele Infections Autoimmunity Hormonal disorders Oxidative stress	
	Idiopathic		
Posttesticular	Congenital	Bilateral congenital agenesis of the vas deferens and ejaculatory ducts (*CFTR* gene mutation)	Surgical treatment Sperm retrieval techniques
	Acquired	Previous urethral infections	
Sexual and ejaculatory disorders	Erectile dysfunction, premature ejaculation, retrograde ejaculation, anejaculation		Medical treatment Nonhormonal treatment Dapoxetine, PDE5is Sympathomimetic drugs (*i.e.*, imipranine) Penile vibratory stimulation, electroejaculation Surgical treatment Sperm retrieval techniques

RT: radiotherapy; *CFTR*: cystic fibrosis transmembrane regulator; PDE5is: phosphodiesterase 5 inhibitors

that regulate spermatogenesis and testes' development. Microdeletions on the long arm of the Yq can be detected in approximately 13% of men with NOA and in <5% of men with severe oligozoospermia.[20] Patients with complete azoospermia factor a (AZFa) and b (AZFb) deletions also present having azoospermia with a histological picture of Sertoli cell-only syndrome or spermatogenetic arrest. Conversely, in men carrying AZFc microdeletion, the successful surgical sperm retrieval is about 50%.[18] Therefore, gene mutation screening is considered when a specific disease condition is suspected, *i.e.*, monomorphic forms of teratozoospermia (such as macrozoospermia, globozoospermia, and acephalic spermatozoa), asthenozoospermia (such as primary ciliary dyskinesia), and androgen insensitivity syndrome.[18]

Undescended testis is the most common genital malformation in boys, with a prevalence of 2.7% in male newborns. Cryptorchidism, a condition that is part of the testicular dysgenesis syndrome, may be associated with subfertility, an impaired endocrine axis, and immunologic damage. The prevalence of azoospermia after treating undescended testes is approximately 13% in the unilateral form and 34% in the bilateral one.[21]

Acquired

Among the acquired causes of testicular azoospermia, there are:

1. Testicular torsion,[22] trauma, and orchitis, all with the possible complication of testicular atrophy[23]
2. Drugs and medications that may impair fertility through different mechanisms such as direct gonadotoxic effects, alteration of the hypothalamic–pituitary–gonadal axis, or sexual dysfunction (ejaculation dysfunction and/or reduction in libido).[24] Chemotherapy or radiotherapy, for instance, may induce irreversible damage to spermatogenesis[25–27]
3. Varicocele, which can produce a progressive harmful effect on the testis. Although the broad literature, the exact mechanism by which varicoceles can potentially affect spermatogenesis remains elusive and identifying the individuals that may benefit from surgical treatment of varicocele remains challenging[28,29]
4. Infections[30,31] can be another cause of male infertility. For instance, although available data do not support the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in plasma seminal fluid of infected subjects,[32] in patients who recovered from coronavirus disease 2019 (COVID-19), especially in their reproductive age, andrological consultation and evaluation of gonadal function are always suggested[33]
5. Autoimmunity (presence of antisperm antibodies)[34]
6. Oxidative stress,[35] defined as an excess of reactive oxygen species (ROS) or a deficiency of antioxidant particles, could represent a cause of male infertility. The main antioxidants in semen are enzymatic (superoxide dismutase, catalase, and glutathione peroxidase) and non-enzymatic (vitamins A, E, C, and B, coenzyme Q10, and carnitine); nonetheless, the spermatozoa are susceptible to ROS due to the fatty acids in their plasma membrane.[36,37] A small amount of ROS is required for sperm maturation, acrosome reaction, and capacitation, as well as for sperm–oocyte fusion;[38] on the other hand, their excess induces damage through four mechanisms: (1) loss of membrane integrity, (2) reduced sperm motility, (3) sperm DNA damage, and (4) apoptosis.[36,37] Lastly, the male reproductive hormonal profile might also be altered in case of an excess of ROS[39]
7. Conditions involving incomplete or inadequate gonadotropin production and intrinsic testicular impairment are defined as a mixed form (high FSH and normal volume testes, normal FSH and small testes, or normal FSH and normal testis). However, in about 30% of cases, the cause remains idiopathic.[34]

Posttesticular causes

Finally, posttesticular causes feature normal spermatogenesis and preservation of normal exocrine and endocrine function in association with ductal system alteration or sexual and ejaculatory abnormalities, such as erectile dysfunction or premature ejaculation. In the presence of partial obstruction, severe oligozoospermia or OAT may ensue (**Table 1**).

Congenital

Congenital bilateral absence of vas deferens (CBAVD) is found in 1% of infertile men and in up to 6% of those with obstructive azoospermia. Most commonly, CBAVD is due to a mutation of the cystic fibrosis transmembrane regulator (*CFTR*) gene,[40–42] where the most frequent mutations are F508del, 5T, and R117H.[43] In addition, this disorder may arise from an abnormality in the differentiation of the mesonephric duct.[44] Individuals presenting with CBAVD usually have an average standard testis size and well-preserved spermatogenesis. The caput epididymis is always present, but the corpus and the cauda are only occasionally present. Seminal vesicles are often absent or atrophic. In addition, the semen analysis typically demonstrates a low volume of ejaculate with acidic pH.

Acquired

Acquired causes of OA may include iatrogenic trauma or fibrosis, *i.e.*, postsurgically or postinfectious (orchiepididymitis, prostatitis, or seminal vesiculitis).[45] An infrequent cause of vasal obstruction is inadvertent injuries whilst carrying out a hernia repair. This complication most frequently occurs throughout infancy but can also occur after any inguinal procedure. Ejaculatory duct obstruction is another important condition; these patients present with azoospermia with low volume and dilated seminal vesicles. Typically, patients affected by ejaculatory duct obstruction present normal secondary sex characteristics, testis size, and hormonal profiles. Notably, the medical history, physical examination,[46,47] and hormonal assessment[48,49] are all required parameters to evaluate azoospermic patients comprehensively.[50] The most common cause of iatrogenic obstruction of the vas deferens is bilateral vasectomy performed for elective sterilization.

DIAGNOSIS

The individual diagnosis of the underlying causes of infertility is pivotal to plan and implement appropriate and coherent treatment strategies (**Table 1**). Men with one or more abnormal semen parameters or presumed male infertility should be evaluated by a male reproductive expert (andrologist as well as urologist).[51] A complete medical evaluation is always necessary and should include clinical and surgical history, childhood illnesses, genital trauma, medications and allergies, past infections, prior radiation therapy, and/or chemotherapy. Furthermore, a physical examination is essential for the correct evaluation of an azoospermic patient. It is important to screen the male hypothalamic–pituitary–gonadal axis by measuring serum testosterone, FSH, and LH levels and essential to detect the majority of clinically significant endocrinopathies. A more comprehensive evaluation includes the dosage of prolactin, thyroid-stimulating hormone (TSH), and estradiol levels; in case of low total testosterone, free testosterone may be evaluated (through a calculation using sex hormone-binding globulin [SHBG] and albumin).[49] The information obtained from a complete endocrine profile may help to elucidate the etiology.

Furthermore, ultrasound is a useful tool in detecting abnormalities related to male infertility, such as testicular volume, vascularization, and structure. In-depth, transrectal ultrasound plays a key role in assessing OA.[52–54]

Karyotype and Y-chromosome microdeletion analysis should be performed in men with primary infertility and azoospermia or severe oligozoospermia with elevated FSH. Furthermore, *CFTR* mutation carrier testing should be carried out in men with vasal agenesis or idiopathic obstructive azoospermia.

Additionally, patients with increased round cells on semen ($>1 \times 10^6$ ml^{-1}) and pyospermia should be evaluated for a potential infection.[51]

Finally, in addition to standard semen analysis, sperm DNA fragmentation (SDF) might also be helpful to diagnose male infertility. The tests most commonly performed are the comet assay (single-cell gel electrophoresis), the terminal deoxyuridine nick and labeling assay (TUNEL) test, the sperm chromatin structure assay (SCSA), and the sperm chromatin dispersion (SCD) test.[55,56]

THERAPEUTIC APPROACHES

Lifestyle changes can be useful in the management of OAT. Indeed, smoking and alcohol consumption, as well as overweight/obesity, are associated with worse semen parameters;[50] hence, clinicians may assess these risk factors.[51] In addition, infertile men should be soon diagnosed and followed, as they could show an increased chance of morbidity and mortality.[57]

Medical treatment

Medical treatment comprises hormonal and nonhormonal management.

Hormonal management

Spermatogenesis requires adequate endocrine stimulation. Hormonal treatment using FSH (75–150 IU, three times a week) in association with human chorionic gonadotropin (HCG; 2000 IU one or two times a week) is effective in azoospermic patients with HH.[58,59] It is controversial to use gonadotropins to increase the function of the hypothalamic–pituitary axis in unexplained male infertility with normal FSH values.

However, a recent position statement[60] and guidelines[61] suggested using purified or recombinant FSH in normogonadotropic males with idiopathic male factor infertility to improve spontaneous pregnancy rate and ART success in couples with male infertility and to ameliorate sperm chromatin integrity and spermiogenesis. In these cases, the analysis of polymorphisms on the follicle-stimulating hormone receptor (*FSHR*) and follicle-stimulating hormone subunit beta (*FSHB*) genes could be performed to predict the clinical response to FSH treatment; however, it is currently indicated only for research purposes.[60] In case of hypothalamic defects, pulsatile administration of GnRH is possible, but, up to date, it is not recommended. Macroprolactinoma, another treatable form of acquired HH, should be treated with dopamine agonists. Androgens have no role in treating male reproductive dysfunction, and testosterone should not be prescribed,[62] while antiestrogens (*i.e.*, clomiphene and tamoxifen citrate), which increase endogenous FSH and LH secretion, represent another possible therapeutic strategy.[63]

Nonhormonal management

The processes of differentiation and maturation of spermatozoa are highly influenced by external factors, such as ROS, temperature, and lifestyle. Therefore, both nonhormonal medical treatments and food supplements are prevalent in treating male infertility. However, sufficient data confirming the beneficial effects of these drugs have yet to come.[9,64] A position statement suggested the use of antioxidants in patients with idiopathic infertility in the presence of documented abnormal sperm parameters and altered sperm DNA fragmentation.[65,66] Other clinical conditions that could benefit from medical treatment are infections with antibiotics/anti-inflammatory,[30,31] autoimmunity with cortisone therapy,[34] and sexual and/or ejaculatory dysfunction (*i.e.*, dapoxetine for premature ejaculation, phosphodiesterase type 5 inhibitors [PDE5is] for erectile dysfunction, and sympathomimetic drugs for retrograde ejaculation).[65,67]

Surgical treatment

Varicocele surgery

Repairing varicocele and the benefits it may offer in improving pregnancy and live birth rates remain uncertain. In fact, even though a significant improvement in sperm concentration, total count, and total motility were observed in several studies,[68,69] its procedural effect on infertile men undergoing IVF is still debated. Some studies reported no benefit from varicocele repair, while other studies based on microsurgical varicocelectomy showed improved pregnancy and live birth rates after IVF.[70–72] American Urological Association and American Society for Reproductive Medicine (AUA/ASRM) guidelines suggested surgical varicocelectomy in infertile men with palpable varicocele and abnormal semen parameters; however, azoospermic men are an exception to this statement; these patients should be informed of the absence of definitive evidence supporting varicocele repair improvements on the condition before ART.[73]

Sperm retrieval techniques

Sperm retrieval techniques are surgical methods that are necessary to obtain spermatozoa from the epididymis and testicles of azoospermic men. Subsequently to these techniques, the retrieved sperm can either be directly used for ICSI or can be cryopreserved.[9,17,73,74]

The method of choice for sperm retrieval is based on the type of azoospermia and the surgeon's experience. The techniques performed are (1) percutaneous epididymal sperm aspiration (PESA); (2) testicular fine needle aspiration (FNA); (3) microsurgical epidydimal sperm aspiration (MESA); (4) testicular sperm extraction (TESE); (5) microdissection testicular sperm extraction (micro-TESE). PESA and FNA are indicated in case of suspected OA, while MESA and TESE/micro-TESE are indicated for both OA and NOA. Infertility secondary to retrograde ejaculation should be treated with sympathomimetics (as described above) and alkalinization of urine, as well as induced ejaculation or surgical sperm retrieval.[62] Finally, after vasectomy, either surgical reconstruction or surgical sperm retrieval, or both, are possible options.[62]

Predictive factors of sperm retrieval in patients with OA and NOA

Spermatozoa can be retrieved either from the epididymis or from the testicles in almost all cases of OA, regardless of the cause of obstruction and the technique used (both aspiration and biopsy). Microsurgical ductal reconstruction has also been proposed as a cost-effective treatment in selected cases of OA (*i.e.*, postvasectomy). Nevertheless, recanalization may not be an available option for some couples, as in cases of congenital obstructions and postinfectious obstruction or failed vasectomy reversals. Unlike men with obstructions, in men with NOA, TESE is the technique of choice. In such cases, spermatogenesis may be focal, and spermatozoa can be found and used for ICSI in approximately 30%–60% of the cases.[75] The main goals of sperm retrieval are (1) the acquisition of an adequate number of sperm for both immediate use and cryopreservation; (2) the retrieval of the highest quality of sperm;

and (3) the minimization of damage to the testis, to preserve testicular function (*i.e.*, testosterone production). Predictive factors for successful TESE were previously defined; nevertheless, only contradictory data have been published until now.[76–79] Precisely, the preoperative factors considered are (a) serum FSH; (b) testicular volume: a recent meta-analysis showed that a testis volume higher than 12.5 ml predicted sperm retrieval rate (SRR) >60% with an accuracy of 86.2%;[80] (c) serum inhibin B; (d) genetics: Y chromosome microdeletions may help predict the success of micro-TESE, *i.e.*, men with AZFc microdeletions have high probabilities of a successful micro-TESE, whereas men with AZFa or AZFb should refrain from undergoing TESE;[81,82] men affected from KS show successful micro-TESE rates similar to men affected from NOA;[83,84] (e) age; (f) cryptorchidism; (g) histopathology: it has been reported that men with just Sertoli cell-only syndrome show a lower rate of spermatozoa recovery compared to men with predominantly hypospermatogenesis and maturation arrest. However, the requirement for a separate surgical procedure for the diagnosis is very limited. At present, no clinical or biochemical marker can surely predict the presence of active focal spermatogenesis within the testis. Finally, the use of testicular spermatozoa for ICSI has been proposed for patients with OAT and high DNA fragmentation or with cryptozoospermia. However, the couple should be informed that this approach is based on low-quality evidence.[85]

ICSI FOR SMF INFERTILITY

In 1986, Baker *et al.*[86] adopted an evidence-based approach to identify a group of men with an untreatable condition of sterility for the first time, accounting for 12% of the cases of male infertility. The advent of IVF/ICSI opened to new investigations of male infertility. However, even if several etiologies of male infertility are known, its treatment approach in ART remained almost unchanged.[87]

Differently from female infertility,[88,89] only a limited number of men affected by a primary male factor infertility may be treated.[9] Although this technology has significantly evolved over time, conventional IVF failed to solve problems associated with SMF infertility, as it is correlated to poor fertilization and pregnancy rates.[90] Azoospermia associated with primary testicular failure was considered untreatable before 1985. These azoospermic patients (15%–20% of the infertile male population) were diagnosed as sterile, and sperm donation was their only possible strategy for conception.[91] The only treatable exceptions were the pretesticular forms due to a dysregulation of hypothalamic–pituitary–gonadal axis, which could be treated with FSH together with hCG, or the posttesticular obstructive cases that could have undergone reconstructive surgery. The introduction of MESA[91] led to breakthrough discoveries with important clinical implications in patients affected by CBAVD, failed vasovasostomy or vasoepididymostomy, and any other irreparable obstructions unsolvable with surgery.[92] The main advance in IVF, especially regarding the treatment of male infertility, is dated back to 1992: the ICSI.[93] Its advent revolutionized IVF and was quickly adopted worldwide. The fertilization and pregnancy rates drastically improved, especially for patients undergoing MESA[94] or TESE because of OA and NOA.[95–98] Notably, some authors demonstrated better ICSI outcomes (in terms of embryo developmental potential) when using spermatozoa from OA patients compared to NOA patients,[99,100] possibly because part of the sperm maturation occurs in the epididymis. Furthermore, ICSI is also required in case of preimplantation diagnosis, *in vitro* maturation, and cryopreserved oocytes usage.[101–105] However, in cases of specific rare disorders associated with male reproductive failure (globozoospermia and absolute sperm immobility) secondary to ultrastructural deficiencies within the sperm, ICSI is often not resolutive.[106] Over the past two decades, the use of ICSI in patients with borderline or even normal semen characteristics has increased without no clear evidence of its benefits compared to conventional IVF. In fact, the Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology concluded that there is insufficient evidence to support the routine use of ICSI in patients without male factor infertility. Therefore, the choice to use either IVF or ICSI is yet unclear and will depend on future prospective studies comparing the outcomes of the two techniques.[107–110]

Hereafter, the indications to various ART treatments are defined according to the clinical characteristics of the male partner. Intrauterine insemination (IUI) might be considered when:[111,112] (1) inseminating motile count (IMC) after washing is 0.8–5 million; (2) sperm morphology is 5% (normal); (3) total motile sperm count in the native sperm sample is 5–10 million; (4) total motility in the native sperm sample is 30%. IVF might be considered when:[113,114] (1) the minimum motile count in the native semen sample is at least 0.2–1 million spermatozoa; (2) sperm morphology is 5% (normal). The following are instead unequivocal indications to ICSI: (1) surgically retrieved testicular and epididymal sperm; (2) immotile spermatozoa; (3) round-headed spermatozoa (globozoospermia).[114]

Finally, physiological intracytoplasmic sperm injection (PICSI) and intracytoplasmic morphologically-selected sperm injection (IMSI) should be considered alternatives to ICSI;[115] however, the indication to these techniques is still a matter of debate, and a recent meta-analysis does not support their clinical utility.[116]

Impact of SMF on ICSI outcome

To date, only a few data have been published regarding the impact of the severity of male factor infertility on ICSI outcomes, and no specific guidelines are available. European Academy of Andrology guideline[85] suggested assisted reproduction as a symptomatic therapy after excluding other therapeutical options.

Data collected and analyzed include male age, blastocyst development, euploidy rate, and reproductive competence of the tested embryos. The influence of advanced paternal age on sperm quality and the reproductive outcome has been addressed in several studies.[117,118] Male age has been correlated with fertilization rate and embryo cleavage at 48 h and 72 h, respectively; nevertheless, the first results showed neither significant association[119] nor adverse effects on ICSI outcomes.[120,121] Despite the fact that advanced paternal age could induce deleterious effects on semen quality, it does not seem to compromise reproductive outcomes when the female partner is not of advanced age.[119] Still, the impact of extremely advanced paternal age on reproductive outcome needs to be clarified. Bartolacci and colleagues suggested that advanced male age negatively impacts fertilization and blastulation rates while neither interfering with developing good-quality blastocyst, nor establishing pregnancy after ICSI.[122] Cioppi and colleagues used the term "paternal age effect (PAE)" to define the greater risk of congenital disorders, such as monogenic diseases, in children conceived by fathers of advanced age.[123] Various authors[124,125] claimed that SMF might result in a higher prevalence of aneuploid embryos after IVF. However, their conclusions are mainly based on older FISH analyses of a limited number of chromosomes in a limited number of cleavage-stage embryos. In this regard, our group published an observational study based on 1219 cycles performed in 1090 couples which were clustered according to male factor in normozoospermic, moderate male factor, OAT, OA, and NOA. Interestingly, it was observed that the poorer the semen characteristics, the higher the risk

of fertilization failure and developmental arrest before the blastocyst stage. However, the euploidy rate and the reproductive competence of the euploid blastocysts were independent of the male factor[126] (**Figure 1**). Importantly, these results were reproduced through the quantitative polymerase chain reaction (qPCR)-based analysis of trophectoderm biopsies[127,128] and mostly were euploid single blastocyst transfer. The outcomes were confirmed after being corrected for all the putative confounders, including maternal age that is namely the most important cause of increasing aneuploidy rates, mainly due to meiotic impairments beyond the age of 35 years.[129–134] Perinatal and obstetrical outcomes were also similar across the different couples clustered according to the male factor. In a recent study, Coates and colleagues investigated the euploidy outcomes in embryos obtained from SMF patients versus normozoospermic patients. They reported an increase of sex chromosome abnormalities.[135] Therefore, more recently, we assessed the prevalence of sex chromosome aneuploidies among 7549 blastocysts biopsied during qPCR-based preimplantation genetic testing for aneuploidies (PGT-A) cycles. However, in contrast to Coates and colleagues, the univariate and multivariate logistic regression analyses conducted from our dataset showed that only maternal age and blastocyst morphology are correlated with the prevalence of vital sex chromosome aneuploidies in the embryos (47,XXY; 47,XXX; 45,X).[136]

SMF and low ovarian reserve and response

Commonly, couples having an azoospermic man tend to be characterized by younger female partners with an associated good ovarian reserve. Moreover, as these women are being proposed IVF treatment earlier in their lives, their response to the controlled ovarian stimulation tends to be better than older women. Hence, this positive outcome in the female partners often compensates for the moderate or reduced male fertility. In general, IVF aims to overcome the natural biological barriers to successful fertilization to obtain a healthy live birth. In addition to SMF, a low ovarian reserve and response worsen the probability of achieving such an outcome. Mahesan and colleagues evaluated the influence of maternal age on the clinical outcomes after ICSI using surgically recovered sperm.[137] Their report concludes that older women had significantly fewer oocytes retrieved and a lower probability of having a blastocyst transferred. An increased quantity of oocyte retrievals is required to collect enough MII oocytes to produce at least one chromosomally normal blastocyst. In general, fully exploiting the ovarian reserve to maximize the number of MII oocytes collected is pivotal, especially considering poor responder patients. Recently, the increasing knowledge of human ovarian follicular waves[138–142] opened new horizons regarding controlled ovarian stimulation, intending to improve the efficiency and efficacy of IVF.[143] This enhancement is achievable by collecting a higher number of MII oocytes in the shortest time frame possible. The classic theory stating that a single cohort of antral follicles grows only during the follicular phase of a menstrual cycle has been overtaken by the evidence that follicles may be recruited at any time throughout the ovarian cycle.[144] Therefore, a novel ovarian stimulation protocol has been hypothesized, validated, and implemented: double ovarian stimulation in the follicular and luteal phases of a single ovarian cycle (DuoStim). Such innovative protocol has been recently demonstrated by our group as an effective strategy to increase the chance of finding at least one euploid blastocyst per ovarian cycle, in poor prognosis patients characterized by a limited reproductive time window.[145] We also highlighted that luteal phase stimulation (LPS)-derived cohorts oocytes are larger (1 more oocyte on average collected) and equally competent as follicular phase stimulation (FPS)-derived ones.[146] In the future, this ovarian stimulation strategy could also improve the conditions of couples suffering from SMF infertility (severe OAT, cryptozoospermia, and azoospermia), maximizing the number of oocytes retrieved per ovarian cycle to be then used for ICSI, thereby increasing their reduced probabilities of obtaining euploid blastocyst(s).

FUTURE PERSPECTIVES

The use of ICSI with ejaculated or epididymal and testicular spermatozoa is of fundamental importance in treating male factor infertility. The drawback of this success is that nowadays, only limited research is ongoing to find other solutions to treat severe male infertility.[147] Yet, in the future, new evidence may arise thanks to the broader use of ICSI with testicular spermatozoa, as well as from the implementation of novel stimulation protocols aimed at increasing the ovarian response (and the chance to identify at least one competent blastocyst) in a single ovarian cycle, thereby, counterbalancing the negative impact of SMF on the fertility outcome. Furthermore, some innovative approaches have been proposed, and these include the differentiation of embryonic stem cells into either male or female gametes,[148] the *in-vitro* culture of spermatogonial stem cell,[149] the clonal expansion of spermatogonia,[150] and the creation of artificial gametes.[151] Certainly, several studies are required before any of these avant-gardes may be clinically applied, and the development of whole-genome-scaled techniques might generate a better identification of disease-related abnormalities in known or novel genes.

At last, studying epigenetics could very well increase knowledge in this field. In fact, azoospermia is a complex disorder, and its etiology results from genetic and epigenetic changes. Specifically, recent studies highlighted an association between genomic DNA methylation in testicular cells and azoospermia,[152] as well as the dynamic changes in chromatin organization/re-packaging and transcriptomes during human spermatogenesis.[153] New findings will increase our knowledge regarding the diagnosis, counseling, and management of infertile patients, ultimately changing the current therapeutic strategies.

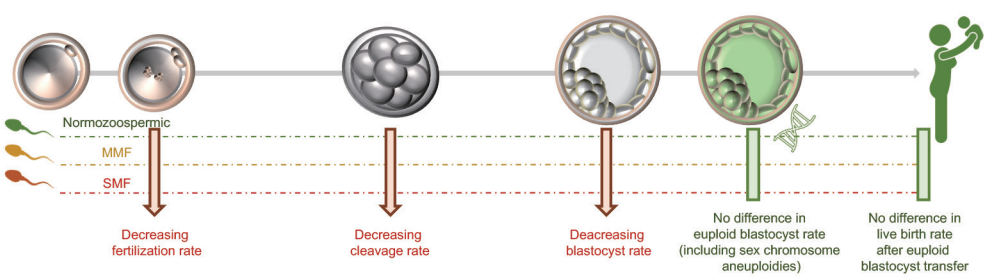

Figure 1: Impact of severe male factor on ICSI outcome. MMF: moderate male factor; SMF: severe male factor; ICSI: intracytoplasmic sperm injection.

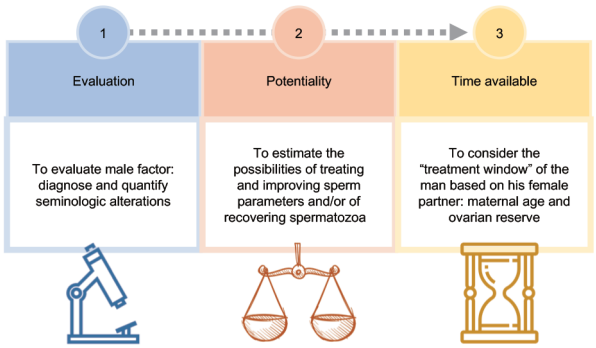

Figure 2: Workflow for the assessment of severe male factor in *in vitro* fertilization.

CONCLUSIONS

At present, male infertility is a key issue; hence, individualized research and diagnosis of the underlying causes of infertility are necessary. When applicable, IVF is considered feasible in overcoming SMF in order to reach a previously improbable live birth. ICSI was a breakthrough in IVF, allowing the treatment of OA and NOA patients, and improving the outcomes of OAT patients. Thus far, no solid and useful predictive factors for successful testicular sperm retrieval in azoospermic patients have been outlined. Moreover, SMF impairs early embryonic competence in terms of fertilization rate and developmental potential to the blastocyst stage. Nevertheless, once the blastocyst is obtained, its euploidy rate (including the prevalence of sex chromosome aneuploidies) and implantation potential are independent of the sperm quality.

In conclusion, the assessment of the male factor should involve (**Figure 2**):

1. Evaluation: to diagnose and quantify the seminologic alteration
2. Potentiality: to determine if there are real possibilities to treat and improve sperm parameters and/or to recover spermatozoa
3. Time available: to consider the "treatment window" of the male partner (the production of a mature spermatozoon from a testicular stem cell takes around 74 days, and the timing of the treatment can vary, depending on the etiology) relative to the characteristics of the female partner in terms of maternal age and ovarian reserve
4. Research efforts to improve our current limited understanding of male reproductive biology.

AUTHOR CONTRIBUTIONS

RM and FMU conceived the review. RM, AV, DC, and LD drafted the manuscript, NU, SF, LR, FL, AL, and HT contributed to the final version of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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