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·*Review*·

Male idiopathic oligoasthenoteratozoospermia

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Abstract

Idiopathic oligoasthenoteratozoospermia (iOAT) affects approximately 30% of all infertile men. This mini-review discussed recent data in this field. Age, non-inflammatory functional alterations in post-testicular organs, infective agents (*Chlamydia trachomatis*, herpes virus and adeno-associated viruses), alterations in gamete genome, mitochondrial alterations, environmental pollutants and "subtle" hormonal alterations are all considered possible causes of iOAT. Increase of reactive oxygen species in tubules and in seminal plasma and of apoptosis are reputed to affect sperm concentration, motility and morphology. iOAT is commonly diagnosed by exclusion, nevertheless spectral traces of the main testicular artery may be used as a diagnostic tool for iOAT. The following can be considered therapies for iOAT: 1) tamoxifen citrate (20 mg/d) + testosterone undecanoate (120 mg/d) (pregnancy rate per couple/month [prcm]: 3.8%); 2) folic acid (66 mg/d) + zinc sulfate (5 mg/d); 3) *L*-carnitine (2 g/d) alone or in combination with acetyl-*L*-carnitine (1 g/d) (prcm: 2.3%); and 4) both carnitines + one 30 mg cinnoxicam suppository every 4 days (prcm: 8.5%). Alpha-blocking drugs improved sperm concentration but not morphology, motility or pregnancy rate. Tranilast (300 mg/d) increased sperm parameters and pregnancy rates in an initial uncontrolled study. Its efficacy on sperm concentration (but not on sperm motility, morphology or prcm) was confirmed in subsequent published reports. The efficacy of tamoxifen + testosterone undecanoate, tamoxifen alone, and recombinant follicle-stimulating hormone is still a matter for discussion. *(Asian J Androl 2006 Mar; 8: 143–157)*

Keywords: idiopathic oligoasthenoteratozoospermia; male infertility; diagnosis; pathogenesis

1 Introduction

Idiopathic oligoasthenoteratozoospermia (iOAT) is defined as defective spermatogenesis of unknown etiology and is regarded as undetectable by the common laboratory methods [1]. This mini-review presented recent data in the field.

Databases that were used to find relevant published reports were: Medline database, EMBase and Cochrane reports. Prospective studies were considered [2]. The efficacy of drugs was evaluated using specific parameters (see the section "Therapy"). A prospective/longitudinal research plan could not always be used for iOAT study (see subsections "Infective agents" and "Environmental pollutants" under the section "Etiology"), in these cases it was specified in the text. Reviews and meta-analyses were chosen within high-level journals (impact factor higher than 1.060).

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2 Definition

Infertility means that a couple has failed to achieve a pregnancy within one year of regular (at least three times per month) unprotected intercourse. One objection to the general use of the term "infertility" is that the condition which is actually meant may instead be that of subfertility, as the ability to father or to conceive may exist with a different partner. However, here too it is difficult to delineate clearly between definitions. Infertility pertains to approximately 15% of sexually active couples [3]. A male factor is present in approximately 40% of infertility cases. It is considered a male factor when an alteration in sperm concentration and/or motility and/or morphology could be found in at least one sample of two sperm analyses which comply with World Health Organization (WHO) 1999 guidelines collected between 1–4 weeks apart. "Idiopathic" means that no etiological factor could be found with the common clinical, instrumental or laboratory methods. Approximately 30% of OAT infertile men are diagnosed as "idiopathic". iOAT is classified as: isolated astheno ± teratospermia (no alteration in sperm con-centration); moderate (sperm concentration $< 20 \times 10^6$/mL and $> 5 \times 10^6$/mL); or severe (sperm concentration $< 5 \times 10^6$/ mL) [4].

3 Etiology

Descriptions of reputed etiologies of iOAT have at least two biases: 1) two patterns have been described whose alterations are linked to male infertility with normal sperm parameters [5, 6]; 2) the sum of the percentages of patients with different etiologies of iOAT gave a result much higher than 100%. This means that etiologies overlap and/or that the primary cause (if any) of iOAT is still unknown and/or that more than one cause is needed to affect sperm patterns.

Nevertheless, the following factors are accepted etiologies of iOAT, therefore the concept that iOAT is composed of an assortment of infertilities is confirmed [7].

3.1 Age

Semen volume and sperm motility, but not sperm concentration, continuously decrease between the ages of 22 years and 80 years, with no evidence of a threshold [8].

3.2 Non-inflammatory functional alterations in post-testicular organs

Low seminal concentration of prostate-specific antigen, zinc, fructose [9] and prostatic acid phosphatase [10], and low seminal activity of neutral alpha-glucosidase are linked to isolated asthenospermia as well as to increased viscoelasticity [11] and osmolarity of seminal plasma [12].

Three spermatogenesis-specific genes were found to be unmethylated in adult spermatogenetic cells in the testis, but were then found to be remethylated in mature spermatozoa in the vas deferens. A role of post-testicular organs (epididymis?) on DNA male gamete methylation might be suspected [13]. DNA methylation is involved in imprinted gene expression in animal cells [14]. The methylation occurs at cytosine-guanine (CpG) dinucleotides at which methyl groups become covalently bound to cytosine residues [15]. Methylation mediates transcriptional repression by recruiting histone deacetylase [16]. Most tissue-specific genes are fully methylated in sperm and in almost all somatic cells of the adult organism. In tissue expression the same genes undergo a striking demethylation that is necessary for gene transcription. Specific genes that are expressed in somatic cell types have distinctive patterns of DNA methylation that have been correlated to the expression profile [17], even though more recent studies suggest that methylation is not necessary for transcription repression in many organs [18]. Even some germ cell-specific genes are regulated by (de)methylation [19] at CpG dinucleotides. Changes in CpG methylation are involved in senescence (even at the epydidymal level [20]) and tumoringenesis [21].

3.3 Infective agents

Longitudinal observational studies to determine the role, if any, of *Chlamydia trachomatis* (CT) asymptomatic infection in the aetiology of iOAT look extremely difficult. The alternative is to contrast and compare between fertile and infertile patients with regards to the detection and occurrence of CT, CT products, and/or of an immune response towards the CT products.

Some researchers estimate the prevalence of asymptomatic CT infection at approximately 20% in men with iOAT. As this percentage is higher than in the control population, asymptomatic CT infection has been regarded by some as a cause of iOAT [22, 23]. On the contrary, other researchers have maintained that CT prevalence in

iOAT and in fertile subjects is similar (approximately 5%) [24]. Different detection techniques have been regarded as an explanation for this difference [25]. Furthermore, CT antibodies were not significantly related to the outcome of seminal analyses, including an anti-sperm antibodies analysis and an analysis of several parameters of sperm function [26]. Therefore, proof of the role asymptomatic CT infection has in infertility is considered inconclusive.

Herpes virus and adeno-associated viruses have been linked to OAT without significant leukospermia [27, 28].

3.4 Gamete genome

Y chromosome microdeletion [29–31], androgen receptor gene defects, and/or somatic karyotype aberration [30] are rarely found in iOAT patients (1%–3%).

Of the 11 955 rat loci investigated, 1 268 were identified as having been specifically transcribed in germ cells during the course of gametogenesis; 200 of these genes are important for the male germ cell development [32]. Approximately 4% of the mouse genome is dedicated to the expression of post-meiotic male germ cells. Targeted disruption of 19 of these genes has displayed their critical roles in fertility [33]. In order to be considered a key for iOAT, a gene must display three characteristics: 1) it should be specifically expressed in the germ cell line; 2) it should have an essential role in spermatogenesis; and 3) its altered expression should be associated with iOAT [34].

For example, cyclic adenosine monophosphate-responsive element modulator (CREM) is a transcription factor which is highly expressed in testicular post-meiotic germ cells and which regulates the expression of several germ cell-specific genes by controlling the imbalance between apoptosis and development. Its activation depends on follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [35]. In histological patterns of the testicle, CREM expression was found significantly reduced in severe OAT patients who had round spermatid maturation arrest or mixed atrophy in combination with normal blood levels of testosterone, FSH and LH (i.e., iOAT patients) [36, 37]. However, azoospermic patients lacking spermatid elongation but who had spermatid maturation arrest were not found to necessarily have defective CREM expression [38].

The *BOULE* gene (a member of the deleted in azoospermia [*DAZ*] gene family) encodes a key factor (a cdc 25 phosphatase protein) which promotes progression through meiosis. A major group of azoospermic infertile men with meiotic arrest completely lack the *BOULE* gene and its target (cdc 25 phosphatase) expression [39]. Furthermore, several patients with a defective expression of BOULE protein and of cdc 25 phosphatase could be correctly classified as iOAT because their testicle displayed a histological picture of mixed atrophy, their sperm analyses displayed OAT and their serum displayed normal levels of testosterone, FSH and LH [39].

Pyridoxal-kinase mRNA splice variant (*PKH-T*) gene is expressed in the adult testes and in spermatozoa, but it has no expression in the testis of men with Sertoli cell-only syndrome. *PKH-T* was found to be expressed in three out of four patients with spermatogenetic arrest, and in one out of two patients with spermatid arrest. It was unexpressed in a minority of OAT patients with normal serum levels of testosterone, FSH and LH [40].

3.5 Mitochondrial alterations

In asthenospermia, both mitochondrial membrane potential [41] and DNA mitochondrial [42] content are impaired. Mitochondrial DNA oxidative damage has been observed in asthenospermic infertile men [43] and has been confirmed in experimental models [44]. These alterations are very similar to the peculiar mitochondrial modifications found in apoptotic sperm cells [45–49].

3.6 Environmental pollutants

The possible effect of environmental pollutants on sperm quality is a matter of discussion.

The hypothesis that environmental hormonally-active compounds of industrial origin (endocrine disrupters) may affect semen quality was first synthesized in 1992 [50].

Several laboratories published a decline in sperm concentration, motility, and morphology using archival data [51–56], yet there was no detectable deterioration in sperm analyses in areas that are similar in terms of human pollutant exposure [57–62]. Therefore, on one hand, it has been maintained that the current data are insufficient to support a causal relationship between human exposure to endocrine disruptors and the decline of sperm count and sperm quality [63], on the other hand, endocrine disruptors are regarded as developmental and reproductive toxicants which can increase seminal reactive oxygen species (ROS) concentration [64]. In addition, an association among urinary phthalate

concentration, increased FSH and lowered inhibin B concentration has been found [65].

3.7 "Subtle" hormonal abnormalities

A decreased LH pulse frequency has been found to occur in iOAT men whose amplitude parallels the severity of the disorder [77].

The Gly102Ser variant of LH might be implicated in iOAT [78].

iOAT displays a shift toward lower testosterone (T) serum levels, lower calculated free T index, and lower T/LH ratio, and a shift toward higher serum LH levels, higher estradiol (E2) levels, and higher E2/T levels [79]. Also, significantly lower inhibin (I) levels and significantly higher FSH levels have been found in i(O)ATs when compared to the levels in fertile men [82].

All of these etiologies can be correctly classified as etiologies of iOAT. The causal link between these pathologies and iOAT is not always evident, nevertheless, the common final result is the impairment of spermatogenesis.

Despite this range of possible etiologies, male infertility also appears to have a familial occurrence especially among brothers and maternal uncles, who often have normal blood levels of FSH and LH [56]. Observations were also consistent with an autosomal recessive mode of inheritance in over half of the cases [57]. These published reports fit with a genetic etiology of iOAT which of late has received great consensus. Such a consensus is lacking, however, in the case of pollution and infection. On one hand, genetic etiology is consistent with iOAT being composed of an assortment of infertilities; on the other hand, it provides a holistic approach to the disease. Therefore, even though in the future genetic etiology will not prove to be the most probable cause of iOAT, it is currently the most convenient to accept.

4 Pathogenesis

The above etiologies are regarded to affect spermatogenetic process. Impaired spermatogenesis leads to increased ROS concentration and to unbalanced germ cell apoptosis. The result of these processes is an affected sperm concentration, motility and morphology.

4.1 Increased ROS

Increased ROS concentration and reduced total antioxidant capacity (TAC) were found in the tubula and the seminal plasma of the majority of iOAT. ROS originates from cellular physiological metabolism of O_2 in aerobic conditions. Seminal cells include mature and immature gametes, leukocytes, epithelial cells, and red blood cells. ROS are mainly produced by leukocytes and immature gametes, therefore an increase in one or both cell types increases ROS and reduces TAC [72]. Leukocytes and/or immature gametes can be found in several types of OAT (hormonal alterations [73], inflammation [74], and varicocele [75]), therefore increased ROS and reduced TAC are not characteristic of iOAT. Physiological ROS concentration has positive effects. ROS are metabolic intermediates in metabolism of prostanoid, in the regulation of vasotonous, in gene regulation, and in facilitating sperm capacitation and acrosome reaction, but at a higher concentration they exert negative effects [74]. WHO [4] defines a 10^6 leukocytes/mL threshold above which leukocytes and/or their product(s)/ROS impair(s) fertility. This threshold is commonly accepted today [4] but is still under discussion [76–79]. Therefore it may be assumed that the main source of ROS in iOAT is immature gametes.

ROS are short-lived chemical intermediates which contain one or more electrons with unpaired spin. In order to overcome this state of unpaired electrons, they are highly and unspecifically reactive molecules able to interact with lipids, amino acids and nucleic acids [74]. Seminal plasma is the human fluid with the highest concentration of anti-oxidants to protect gametes from ROS. Seminal plasma TAC is the sum of the potential anti-ROS activities: 1) of enzymes (superoxide dismutase, catalase, glutathione peroxidase); 2) of low molecular weight substances (α-tocopherols, β-carotene, ascorbate, urate); and 3) of the transition metal chelators (transferring, lactoferrin, ceruloplasmin) [80].

ROS exert their activity on all cellular structures during the course of tubular spermatogenesis and sperm maturation during the migration through the seminiferous tubules and epididymis [74].

ROS attack nuclear and mitochondrial DNA, inducing fragmentation, aberrant recombination, and/or defective packaging [81]. 2-Deoxyguanosine, a normal DNA base, is oxidized to 8-OH-deoxyguanosine. Whereas the former nucleotide binds to cytosine, the latter will form a base pair with thymine during DNA replication. Thymine will bind to adenosine so that a point mutation is introduced in the DNA [82]. The extent of DNA damage depends on the degree of oxidative stress [83].

ROS irreversibly damages cell membranes. Mam-

malian sperm cell membranes have a highly specific lipid composition: a high content of polyunsaturated fatty acids (PUFA), plasmalogens and sphingomyelins. The unusual structure of their membrane is responsible for the flexibility and the functional ability of spermatozoa. ROS-dependent peroxidation of PUFA is an autocatalytic self-propagating reaction. The first step (initiation) is the extraction of a hydrogen atom from an unsaturated fatty acid. The second step (propagation) is the formation of a lipid-alkyl radical followed by its rapid reaction with oxygen to form a lipid-peroxyl radical. The peroxyl radical can extract a hydrogen atom from a PUFA with the concomitant formation of a lipid radical and lipid hydroperoxide. As the peroxyl and alkyl radicals are regenerated, the cycle of propagation could continue indefinitely or until one of the substrates is consumed or terminated in the radical-radical reaction [84–86].

An increase in ROS changes the tertiary structure and expression of proteins, protein membrane receptors, and membrane transport proteins, which in turn results in disturbances in the ionic balance. The function of enzymes with thiolic (SH) groups may also be altered by ROS [87].

Sperm epididymal maturation involves extensive protamination and chromatin condensation [88, 89]. Increased ROS concentration has been linked to DNA denaturation in infertile men [90]. DNA denaturation is negatively correlated with semen parameters [91] but is positively correlated with cytoplasmic residues [92], that is, with the morphological markers of immature gametes.

Death of gametes (necrosis and/or apoptosis, i.e., oligo-/terato-spermia), reduction in the percentage of sperm typical forms, and impairment of sperm motility (i.e., asthenospermia) are resulting patterns. Asthenospermia is caused by axonemal damage mostly as a result of adenosine triphosphate depletion [93, 94].

Despite the progress in our knowledge of ROS activity, the causal link between these alterations and iOAT is not always evident.

4.2 Modified apoptosis

Apoptosis or programmed cell death (PCD) is critical for mammalian tissue morphogenesis because it eliminates unwanted or defective cells through an orderly process of cellular disintegration without inflammation, thus ensuring the production of intact functional spermatozoa [95]. Therefore, as a general rule, PCD is involved in the removal of arrested germ cells from the testicles of patients with spermatogenetic failures [96]. PCD is an active process; it involves specific gene expression and requires mRNA and protein synthesis. Apoptosis of type A_2, A_3 and A_4 spermatogonia reduces the number of germ cells produced to approximately 25% of that expected if all A_1 spermatogonial progeny were to survive. It has been proposed that individual spermatogenesis is the result of a framework involving the imbalance between pro-apoptotic and pro-survival factors [45]. In fact, the BCL-2 protein family can either support cell survival (BCL-2, BCL-XL, BCL-W, MCL-1, A1/BFL-1) or promote cell death (BAX, BAK, BCL-XS, BAD, BID, BIK, BOK, DIVA, HRK). BCL-2 family members interact competitively, thereby regulating activation of the proteases (cysteinyl aspartate-specific proteinases [caspases]) which dismantle the germ cell [97]. Furthermore, the C-kit stem cell factor system, bone morphogenetic protein 8B [45], lactate [98], and sphingosine-1-phosphate [99] inhibit male germ cell apoptosis, whereas the fibroblast-associated death receptor (Fas)/Fas-ligand (Fas-L) system is regarded as a pro-apoptotic factor [45].

Interestingly, toxicants and increased concentration of ROS from activated leukocytes or from immature spermatozoa trigger PCD [45–47]. These substances may [100] or may not [101] interact with the Fas/Fas-L system to activate caspase [48, 101], which leads to cellular morphological and biochemical alterations and finally to death [45–49].

The evidence of ethnic differences in the susceptibility of germ cells to PCD [102] may indicate that alterations of PCD may occur in the absence of an exogenous triggering. The degree of decreased expression of survivin (a PCD inhibitor) parallels the severity of iOAT [102], just as the overexpression of the *Fas* gene has been associated with altered meiotic and post-meiotic sperm cell maturation [103].

Ejaculated spermatozoa from infertile men display a number of apoptosis markers: various degrees of plasma membrane translocation of phosphatidylserine; DNA fragmentation; and active capsase-3 (the main executor of apoptosis) with an apparent exclusive cellular location in the mid-piece [104]. The apoptotic process is probably set in motion before ejaculation [105] because healthy human ejaculated spermatozoa cannot initiate apoptosis, at least not under *in vitro* conditions [106].

Apoptosis has been inversely linked with sperm motility [107], morphology, vitality and concentration [108].

Increased apoptosis was found in a variety of infer-

tilities, such as hormonal infertilities [109], anti-sperm auto-antibodies (ASA)-associated infertility, varicocele, testicle torsion [110] and inflammation [111]. Therefore, increased PCD is not atypical of iOAT.

In conclusion, the pathogenetic mechanisms of iOAT are not peculiar to this kind of disease and can be found in several types of OAT.

5 Diagnosis

iOAT is commonly diagnosed by exclusion; the differential diagnosis is presented in Table 1.

Impaired spermatogenesis of iOAT was assayed with testicular arteries duplex examination and inhibin B serum levels.

Table 1. Differential diagnosis of male infertility [112].

Reproductive failure mechanism		Methods of diagnosis
Chromosomal [113-116]	X chromosome disorders Y chromosome disorders Autosomal disorders	Objective examination, Y microdeletion detection, karyotype, screening of cystic fibrosis, hormonal profiles, androgen receptor detection, semen analysis.
Developmental	Hypospadias Ductal obstruction Didymal-epididymal interruption	Clinical history, objective examination, scrotal echography (vasography; fructose, *L*-carnitine and alpha-glycosidase seminal plasma determination), semen analysis.
Testicular pathology	Cryptorchidism Ectopic testicle Retarded descent (Floating testicle) Testicular tumors Bilateral atrophy Testicular torsion Trauma	Clinical history, objective examination, scrotal echography, semen analysis.
Genital tract inflammation [117]	Urethritis Prostatitis Epididymitis Orchitis	Clinical history, objective examination, scrotal echography, seminal leukocyte concentration/mL, urethral swab, urine analyses, sperm and urine cultural analysis.
Varicocele	Objective examination, scrotal bilateral echo-color Doppler examination, semen analysis.	
Endocrine [118, 119]	Pituitary disorders Hypothalamic disorders Testicle disorders Thyroid disorders Adrenal gland(s) disorders	Hormonal profiles, semen analysis.
Iatrogenic	Surgery Drug or radiation administration	Clinical history, objective examination, semen analysis.
Sexual related aetiologies	Erectile deficiency Disturbed ejaculation	Clinical history, semen analysis.
General diseases	Renal diseases Liver diseases Neurological diseases Gastrointestinal diseases Haematologic diseases Autoimmune diseases Infectious diseases (AIDS) Psoriasis, sarcoidosis Diabetes	Specific tests, clinical history, semen analysis.
Sperm auto-antibodies	Agglutination, microagglutination, immunofluorescence [120], semen analysis.	
Idiopathic oligo-astheno-teratospermia		Semen analysis, exclusion criteria.

iOAT displayed peak systolic velocity (PSV) levels significantly lower than those of normospermic fertile men, inflammatory OAT patients, and varicocele OAT patients [121]. An echo-colour Doppler semi-quantitative score has been used to distinguish obstructive azoospermic patients from non-obstructive azoospermic patients affected by primary testicular pathology [122]. The intratesticular arterial blood flow and maximum blood flow velocity were significantly lower in patients with germ cell hypoplasia or maturation arrest [123]. The pulsatility index of the testicular transmediastinal artery was significantly higher in patients with obstructive azoospermia than in those with non-obstructive azoospermia [124]. Arterial impedence of undescended testes in adults may have a predictive value and provide more accurate information about histology than testicular volume [125]. These data link blood arterial supply to spermatogenesis [121]. Currently there is no direct explanation for this phenomenon. Testicular arteries are target organs for androgens [126] and in infertile men testicular arteries have a narrow lumen caused by enlarged endothelial cells, a thickened subendothelial layer, and an abundant adventitia rich in connective fibres and ground substance [127].

The clinical usefulness of a blood level measurement of inhibin B is considered rather low [128]; inhibin B serum levels are regarded as markers of Sertoli cell function, but the prediction of the quality of spermatogenesis is not higher than FSH [129]. iOAT men who had been exposed to phthalate displayed increased levels of inhibin B, lowered levels of FSH, and phthalate urinary excretion [65].

There are two pathologies that may confound the practitioner: sperm auto-immunity and male accessory gland inflammation (MAGI).

Disruption of the epithelial (i.e., Sertoli cell) blood barrier elicits ASA. Some reputed causes of ASA are vasectomy, vas obstruction, testicular trauma, torsion, malignancy and infection of the urogenital tract [130]. ASA are found in 26%–55% of infertile couples, in up to 19% of fertile men, and in 43% of fertile women, which means that not all ASA cause infertility [131].

In order to be defined as an "autoimmune disease", three criteria must be met: direct proof, indirect evidence and circumstantial evidence [132]. The direct proof is easy to obtain because ASA can be transferred to the sperm of healthy persons and an impairment of functions can be demonstrated in the receiving cells [132]. Indirect proof (i.e., animal models) is difficult to obtain. Spermatozoa are not amenable to conventional molecular biology techniques because of the lack of much functional mRNA and the lack of many post-translational modifications of germ cells during maturation [132, 133]. Circumstantial evidence (i.e., the favourable response to immuno-suppression) was not demonstrated because it is impossible to treat infertile patients with cytotoxic drugs [132].

ASA are made of numerous antibodies interacting with multiple sperm components [134]. These findings support the hypothesis there might be a relatively non-specific binding of antibodies to the surface of spermatozoa [135] probably through crystalizable fragment (FC) or disulphide bonds [131].

On the other hand, ASA are reputed to affect the following: cervical mucus penetration [135], acrosome reaction [132], zona binding [136], zona penetration [137], oolemma binding [138] and pronucleus formation [139]. The effects of ASA on motility [140, 141] and on *in vitro* fertilization [139, 142, 143] are questionable. The WHO recommends direct testing for ASA on the surface of spermatozoa [4], nevertheless only some trials on iOAT therapy look for sperm autoimmunity [144–146], therefore underestimating the role of ASA in infertility. Our opinion is that ASA detection may be a prudent technique for interpreting results in infertility management.

Seminal leukocyte concentration greater than 10^6/mL is considered the lowest threshold of inflammation. Controversial results have been published about the efficacy of antimicrobial therapy on sperm quality and pregnancy rate in MAGI, even though seminal leukocyte concentration was found to fall below 10^6/mL after therapy [147, 148].

Some reasons for discrepancy may be: poor patient selection [147], inadequate drug administration [148], persistence of leukocytes [149] and/or of their products (ROS) [150], elastase [151], interleukin (IL)-8 and IL-6 [151].

Regardless, the vast majority of trials for idiopathic oligoasthenoteratozoospermia consider 10^6/mL leukocytes to be the threshold for the diagnosis of MAGI.

6 Indications for therapy

If sperm concentration exceeds 20×10^6/mL, the probability of spontaneous conception depends only to a minor extent on sperm motility, viability, and morphology.

The lack of prognostic significance contrasts with the fact that these characteristics discriminate well between the semen of fertile and subfertile men. Men whose sperm concentration is over 20×10^6/mL but who have abnormal motility or morphology of idiopathic origin have a 40% higher probability of achieving spontaneous conception than men in whom the sperm alterations are related to a demonstrable cause [130]. Therefore it is difficult to justify the therapy of an iOAT patient with a sperm concentration of 40×10^6/mL spermatozoa and a class A WHO motility of 24%.

7 Therapy

Evidence-based medicine indicates that studies without a placebo-treated group should not be taken into consideration. "Placebo treatment" differs from "no treatment" because a placebo can improve sperm parameters due to a psychological mechanism [144, 145]. It is mandatory to establish whether an improvement in sperm count and quality is statistically or clinically significant, because the latter involves the former, but not vice versa. Spontaneous pregnancy rate when the partner is free from any detectable factor of infertility is an accepted method, even though 11%–17% of infertile couples do not display any cause of infertility as detected by the common techniques [130]. Therefore to define a drug as active, it should improve sperm patterns and pregnancy rates in at least one blind, prospective, placebo-controlled trial; more of these trials from independent groups are necessary in order to define a drug as unquestionably active [2].

Despite these instruments, difficult-to-interpret data still materialize. Two typical examples are recombinant FSH (rFSH) and tamoxifen citrate (TC). rFSH was reputed effective when compared to a "no treatment" group [152, 153], or ineffective when compared to a "placebo-treated" group [154]. A recent meta-analysis showed that rFSH given to men with iOAT increases the clinical pregnancy rate, the fertilization rate, and the rate of doubling sperm count significantly [155].

OAT decreases the monthly chance of conception in a dose-dependent manner, adding its effect to the duration of infertility [156]; an increased sperm concentration in OAT infertile males has been associated with disproportionately higher fecundability [157]. Further pregnancies can be expected on the basis of hyperbolic regression between sperm concentration and fecundability [157]. Therefore, treatments which double sperm concentration will have a stronger effect on the probability of conception when the initial sperm count is low, rather than when the initial sperm count is high [158]. The TC data were principally reviewed by Vanderkerckove *et al*. [159] and Comhaire [158]. Vanderkerckove maintained that there is not enough evidence to use TC to increase the fertility of iOATs [159]. Comhaire showed that, because a lower sperm concentration is related with a lower fecundability, the pregnancy rate with TC treatment is stronger in trials containing lower treatment-independent pregnancy rates [158]. In addition, Comhaire [158] also found an increased sperm concentration in trials that were discharged by Vanderkerckove [159].

The effects on seminal parameters of TC (10 mg × 2/d) combined with testosterone undecanoate (TU) (40 mg × 3/d) were studied in men with iOAT. The results were that TC + TU improved total sperm count, motility and functional sperm fraction after 3 and 6 months [160]. A further report showed a 33.9% (pregnancy rate per couple per month [prcm] = 3.8%) rate of incidence of spontaneous pregnancy in the TC + TU treatment group at 9 months, and a 10.3% (prcm = 1.1%) rate of spontaneous pregnancy in the placebo group at 9 months [161]. TC was introduced as an empirical treatment for iOAT [162] because of its stimulatory action on gonadotropin secretion [163], its direct effect on Leydig cell function [126], and because of its production of 5a-dihydrotestosterone in tubules and epididymis [164]. To overcome the putative inferior androgenic environment in the reproductive tract of men with iOAT, androgen-dependent epididymal and accessory gland function [165] were boosted by androgen administration [160].

The effects of folic acid and zinc sulfate treatment on semen variables in fertile and subfertile men were studied. One hundred and eight fertile and 103 subfertile men were randomly assigned to receive one of four treatments for 26 weeks: folic acid and placebo; zinc sulfate and placebo; zinc sulfate and folic acid; and two placebos. Folic acid was given at a daily dose of 5 mg, and zinc sulfate was given at a daily dose of 66 mg. Subfertile men demonstrated a significant 74% increase in total normal sperm count and a minor increase of 4% in abnormal spermatozoa. A similar trend was observed in fertile men. The beneficial effect on fertility, if any, remains to be established. Despite a putative role of zinc on post-testicular organs, a direct anti-oxidant effect of both drugs on tubules was sustained [166].

Although there is no clear pathophysiological hypoth-

esis for the use of α-blocking agents in the treatment of iOAT, studies have been performed using these substances [167]. Two placebo-controlled studies claimed an improvement in sperm concentration but not of ejaculated volume, morphology, motility or pregnancy rate when compared to placebo [168, 169].

In bioptic testicle specimens, mast cell counts were higher in idiopathic infertile men than in normospermic men [170]. Tranilast (a mast cell blocker) was first used in an uncontrolled study and demonstrated a clinical benefit on semen parameters and conception rates (prcm = 9.5%) [171]. A further double-blind controlled trial showed that tranilast significantly increased sperm concentration, but not motility, morphology or pregnancy rate when compared to placebo [172]. It has been concluded that tranilast demonstrates a certain clinical benefit in terms of improvement in semen parameters involving severe iOAT, but it does not appear to afford clinical benefit in the long term [173]. A second mast cell blocker (fexofenadine) was found ineffective [174].

Acetyl-*L*-carnitine (ALC) 500 × 2 g/d + *L*-carnitine (LC) 1 × 2 g/d have been shown to increase sperm count, sperm quality and pregnancy rate in patients with an ultrasound picture of genital inflammation and leukocyte sperm concentration < 10^6/mL [175]. LC alone 2 g/d was shown to increase sperm concentration and motility [144] in iOATs, although a higher activity on iOAT was found using LC 2 g/d + ALC 1 g/d [136]. Another therapy studied was intermittent administration of a non-steroidal anti-inflammatory drug (NSAID). Cinnoxicam (C) (one 30 mg suppository every 4 days) was found to increase sperm concentration and quality in low grade varicoceles [176]. Therefore one 30 mg C suppository every 4 days was used in combination with LC 2 g/d + ALC 1 g/d. This three-drug association significantly increased sperm concentration, motility, morphology, and pregnancy rates when compared to placebo and to the two carnitines alone [146]. Although more effective than placebo, a recent analysis of literature indicates that the spontaneous prcm following carnitine therapy is 2.3% both in placebo-controlled and open studies [177]. The use of LC + ALC + C gave a prcm of 8.5% in iOAT patients (51% at 6 months) [146].

Carnitines have several activities which might be useful for the male gamete: free-radical production is reduced by depleting fatty acid peroxidation; carnitines restore the phospholipid composition of mitochondrial membranes; carnitines enhance cellular energetics in mitochondria which increases cytoplasmic acetyl-coenzyme A concentration through the higher availability of acetyl groups, which in turn results in an increase in mitochondrial respiration and monoamine-oxidase activity and thus increasing the metabolism of histamine; carnitines stabilize cell membrane fluidity by regulating phospholipid levels and reducing ceramide production and insulin-like growth factor 1. Carnitines prevent cellular death and apoptosis. Most of their activities take place in the tubular microenvironment rather than in epididymal functions [144–146]. No biochemical study was performed regarding the role of C suppositories in increasing the efficacy of carnitines. The effect of oral and injected NSAIDs on sperm analyses was lower than that of suppositories. A more direct effect of suppositories on seminal plasma may be presumed because of the rectal-prostatic lymphatic pathways. Hydrophilic NSAIDs were less active than C, whose lipophilia facilitate absorption [176]. An animal model showed that chronic treatment with NSAIDs at low doses improved sperm quality and fertility [178]. Carnitine administration was found to increase E2 prostaglandin concentration [179], which affects sperm count [180, 181]. Finally, polyzoospermia has been associated with lowered prostaglandin content of tubuloseminal plasma and OAT has been associated with increased prostaglandin content of tubuloseminal plasma [180, 181]. NSAIDs stabilize lysosomal membranes, thereby partially preventing apoptosis [176].

The efficacy of conventional anti-ROS drugs (vitamin E, vitamin C and essential fatty acids) is controversial. An uncontrolled study found that N-acetyl-cysteine or the combination of vitamins A + E with essential fatty acids improved sperm concentration, and that acrosome reaction reduced ROS and sperm oxidized DNA [182], but no significant difference was found in terms of sperm motility, morphology, or round cells. A double-blind placebo-controlled study maintained that vitamin E + selenium was effective because they combination improved sperm motility and lipid peroxidation markers. The number of patients who dropped out (n = 34) is much higher than the actual group studied (n = 20 patients), therefore its conclusions might be reputed as unreliable [183]. Vitamin E + C was ineffective in a prospective double-blind controlled trial [184].

Finally, T + TC and (C+)ALC + LC may be regarded as active drugs, whereas TC and FSH are considered to most likely be active drugs. As yet, no drug can be defined as unquestionably effective. Actually C + ALC

proved significantly more active than placebo in two blind prospective controlled trials [145, 146], therefore it is likely that these drugs are going to be regarded as unquestionably effective.

8 Conclusion

The seasonal [185], regional [186], and racial [187] differences in sperm count and quality make it difficult to consider iOAT data as absolutely valid, therefore further multicentric studies should be performed in this field.

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