



# Genetic evaluation of patients with non-syndromic male infertility

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## Abstract

**Purpose** This review provides an update on the genetics of male infertility with emphasis on the current state of research, the genetic disorders that can lead to non-syndromic male infertility, and the genetic tests available for patients.

**Methods** A comprehensive review of the scientific literature referenced in PubMed was conducted using keywords related to male infertility and genetics. The search included articles with English abstracts appearing online after 2000.

**Results** Mutations in 31 distinct genes have been identified as a cause of non-syndromic human male infertility, and the number is increasing constantly. Screening gene panels by high-throughput sequencing can be offered to patients in order to identify genes involved in various forms of human non-syndromic infertility. We propose a workflow for genetic tests which takes into account semen alterations.

**Conclusions** The identification and characterization of the genetic basis of male infertility have broad implications not only for understanding the cause of infertility but also in determining the prognosis, selection of treatment options, and management of couples. Genetic diagnosis is essential for the success of ART techniques and for preserving future fertility as well as the prognosis for testicular sperm extraction (TESE) and adopted therapeutics.

**Keywords** Male infertility · Non-syndromic · Gene panel · Whole exome sequencing · Genetics

## Introduction

The World Health Organization (WHO) defines infertility as a disease of the reproductive system and describes it as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1].

Infertility is a global health problem and is considered a pathology of itself. Approximately 10–15% of couples confront to infertility, and it is estimated that approximately 50% of such cases have a female origin and 20–30% a male one. The remaining 20–30% of infertility cases is due to a combination of male and female factors [2]. The ability to reproduce depends on the proper functioning of the male and female reproductive systems. Different causes can be responsible for infertility; endocrine dysfunction, inflammatory diseases, genital tract obstruction, female or male gametogenesis dysfunctions, implantation failures, erection or ejaculation problems [3], and infertility can even have psychological or psychiatric origins. In addition, studies have shown that environment and lifestyle can also affect fertility [4].

The complexity of the process explains why the etiology of only some cases of infertility is diagnosed. Actually, for nearly 40% of cases, no diagnosis is established and such cases remain classified as “idiopathic” [5]. It is believed that about 50% of the idiopathic cases could be explained by a genetic defect [6].

Since the 70s, we know that genetic anomalies can affect human fertility [7]. Genetic anomalies can be categorized into two kinds: (i) karyotype anomalies comprising numerical or structural anomalies and (ii) genetic anomalies affecting one

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specific gene. In instances of genetic anomalies, one can distinguish syndromic affections, which are associated with pathological syndromes as well as infertility. This group is defined as syndromic infertility and usually infertility is not, in general, of primary concern. On the other hand, in non-syndromic infertility, gene mutations cause absent or abnormal spermatogenesis without any other symptoms. This review focuses on genes related to non-syndromic male infertility. Recent advances in molecular biology and medical genetics have enabled the discovery of the genetic etiologies of male infertility. For the past 10 years, we have witnessed the emergence of a new field of research, the “genetics of infertility.” New genetic tools that implement a whole-genome approach have led to the identification of an increasing number of gene mutations as the cause of specific infertility phenotypes. Early successes in the field have also attracted a large number of new players. Our personal prediction is that the list of “infertility” genes will grow exponentially in the next 10 years, and as a consequence, the more diagnostic tests will be offered. We can thus hope that the number of idiopathic infertility will be reduced as diagnostic tests available for more couples will increase.

New results in the genetics of infertility will lead us to modify the management of couples and integrate genetics into the daily practice of the artificial reproductive techniques (ART) [8]. Undeniably, there is a very close link between these two practices, ART and genetics. One contributes to the birth of children and thus to the transmission of genetic heritages from one generation to the next, and the other ensures that this heritage is not affected by deleterious anomalies [9].

A “rough draft” of the human genome was finished in 2000 (announced jointly by U.S. President Bill Clinton and the British Prime Minister Tony Blair on June 26, 2000). It was a historical turning point in the field of human genetics, and therefore in this review, we limit our literature search after the year 2000. Our targeted readers are clinicians in reproductive medicine, and our focus is non-syndromic infertility caused by single gene mutations. We first describe the initial management of males presenting alterations revealed by semen analysis or spermocytogram then briefly describe the strategies allowing the identification of infertility genes. We then introduce the genetic tests available for different kinds of male infertility and how they can help the management of couples. Genetic examinations are usually carried out for etiological purposes, but they also aid in providing adequate care for patients and, possibly, for members of their families.

### Initial management of a couple where the man has an anomaly detected after semen analysis or spermocytogram

For men, a semen analysis is a systematic examination and it is essential. It must be done without waiting for other results

either from the man or even less so, from his spouse. In the WHO laboratory manual for the examination and processing of human semen (5th ed., 2010), semen analysis is defined as a quantitative analysis of sperm which is fundamental in the evaluation of male fertility [10].

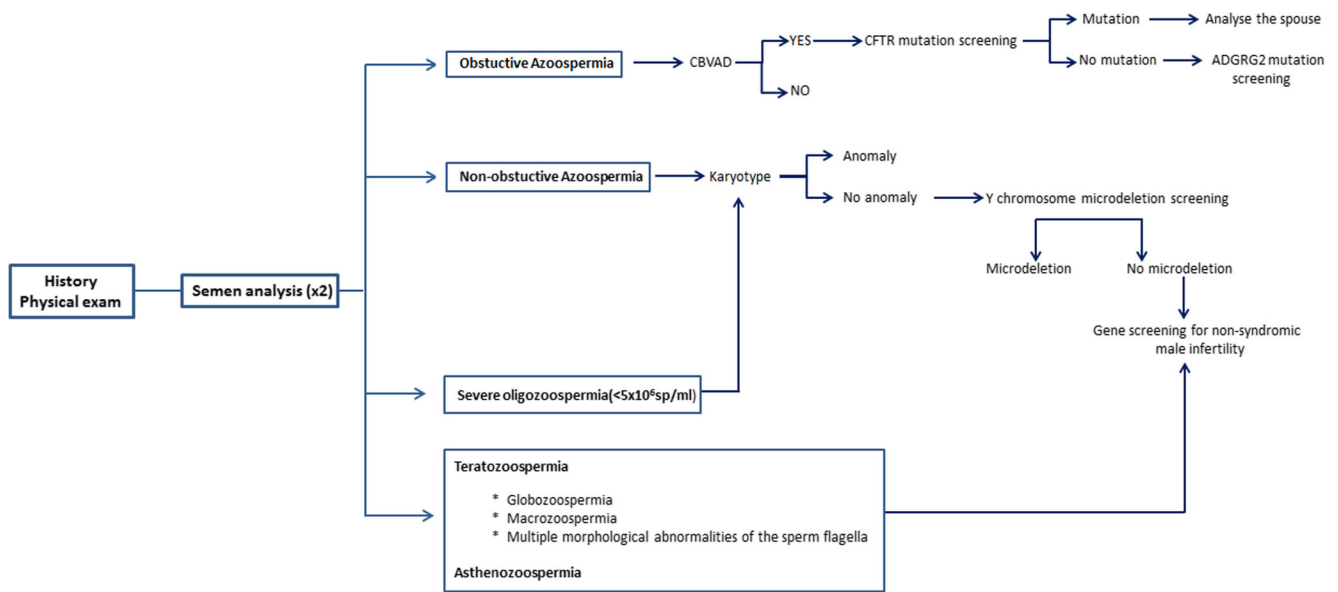
Gene-expression analysis studies from Schultz et al. estimate that more than 2300 genes in the mouse genome are expressed predominantly in the male germ line [11]. We extrapolate that it would be similar in man, and theoretically, a mutation in any of these genes may be responsible for a spermatogenic defect, and therefore, it is likely that many “idiopathic” forms may have a genetic origin.

The proposed workflow after the diagnosis of a male infertility phenotype is shown in Fig. 1. The results of semen analysis may prompt an examination of the karyotype and/or search for Y chromosome microdeletions. A search for mutations in the cystic fibrosis transmembrane conductance regulator (CFTR, OMIM no. 602421) gene will be recommended in case of obstructive azoospermia associated with a congenital bilateral absence of vas deferens (CBAVD) detected at the clinical examination. In 80–90% of CBAVD cases, the etiology is mutations in the *CFTR* gene [12, 13]. Recently, the adhesion of G protein-coupled receptor G2 (ADGRG2, OMIM no. 300985) gene, a novel pathogenic gene for CBAVD, was identified [14]; however, the incidence of ADGRG2 gene mutations is still unknown.

Karyotype anomalies have emerged as one of the major causes of male infertility. They involve loss or gain of a chromosome, part of a chromosome, or abnormal rearrangement at the chromosomal level without loss of genetic material. About 15% of azoospermic men and 2% of oligozoospermic men carry chromosomal anomalies [15], rates that are much higher than in the general population which is approximately 0.6% [16]. Some chromosomal aberrations are inherited, while others arise de novo. They can be subdivided further into sex chromosome aneuploidies, structural chromosome aberrations, and copy number variations.

The most common genetic cause of azoospermia is Klinefelter syndrome (KS) with a 47,XXY karyotype (Fig. 2a), which accounts for 14% of azoospermia cases [19, 20]. Among all cases of KS and irrespective of the age of the patient, a testicular biopsy (TESE) associated with an intracytoplasmic sperm injection (ICSI) allows 50% of patients to father [21]. So far, studies suggest that the risk of chromosome anomaly in the offspring of 47,XXY patients is low; hence, reproductive genetic counseling for them can be reassuring, and management of the pregnancy can proceed with caution [22, 23]. However, until conclusive information is available, such couples should be offered extensive genetic counseling.

Translocations, inversions, Y chromosome microdeletions, autosomal deletions, and duplications are considered as structural chromosome aberrations. Rearrangement of parts



**Fig. 1** The workflow for genetic tests in male infertility. According to semen analysis, karyotype, Y chromosome microdeletion testing or specific gene mutation screening can be suggested to patients

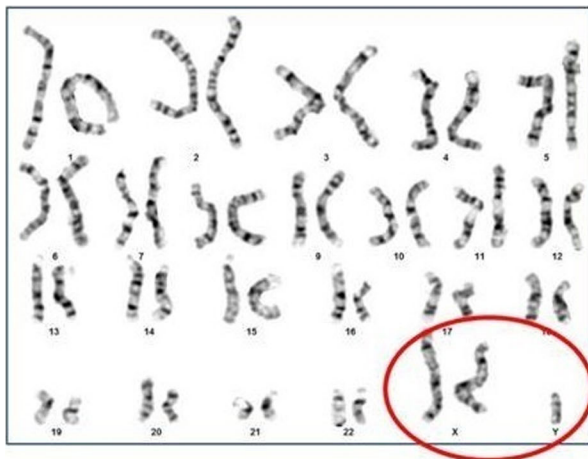
between non-homologous chromosomes are defined as translocations, which can be either exchange of material between non-homologous chromosomes (reciprocal) or fusion of two acrocentric chromosomes (Robertsonian). Robertsonian translocations (Fig. 2c) are much more frequent in oligozoospermic males than in azoospermic males. Although the prevalence of Robertsonian translocations is only 0.8% in infertile males (1.6 and 0.09% in oligozoospermic and azoospermic men, respectively), this is nine times higher than in the general population [24]. On the other hand, reciprocal translocations (Fig. 2b) are more common in azoospermic than in oligozoospermic males. Inversions (Fig. 2d) are rarely seen in infertile men. Apart from infertility, patients who display a chromosomal structural aberration have most often a normal phenotype.

Reciprocal translocations involving the X chromosome lead to an azoospermia in the vast majority of cases [25]. Translocations involving autosomes, whether reciprocal or Robertsonian, have very variable effects on spermatogenesis. This can range from azoospermia to almost normal semen parameters. In general, Robertsonian translocations induce moderate but variable oligozoospermia. Sperm concentration less than 15 million spermatozoa per milliliter of semen is defined as oligozoospermia according to the World Health Organization’s (WHO) 2010 criteria [10]. Normal spermatogenic parameters are found in 30% of the Robertsonian translocation carriers. The study of the chromosomal content of the spermatozoa from these patients by FISH shows an aneuploidy rate that varies little, in the range of 12% [26]. The situation with reciprocal translocations is much more complex. Indeed, the rate of aneuploidy observed in the spermatozoa of patients carrying a reciprocal translocation is extremely variable

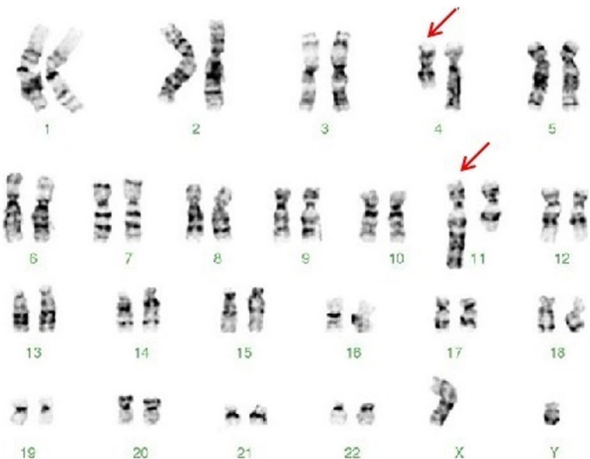
ranging from 40 to 80% [27, 28]. As reciprocal translocations occur rarely at the same breakpoints and therefore each patient harbors a specific translocation, it is impossible to predict the rate of aneuploidy. The risks associated with aneuploid spermatozoa are early developmental arrest leading to an absence of implantation, miscarriage, or even the birth of children with chromosomal defects entailing generally severe phenotypes. For couples where one partner has a translocation, it is possible to propose either a prenatal diagnosis (PND) or a pre-implantation genetic diagnosis (PGD).

In instances of non-obstructive azoospermia or severe oligozoospermia (according to WHO recommendations [10], sperm count less than 5 million spermatozoa/ml in the ejaculate); the absence of a karyotype anomaly should prompt the attending physician to request a search for Y chromosome microdeletions [29]. A significant role for the Y chromosome in spermatogenesis was established in the seventies when large terminal deletions of the long arm of the Y chromosome (Yq) in six men with azoospermia were detected upon karyotype analysis [7]. They named the defined region azoospermia factor (AZF). Later on, three AZF regions were detected: AZFa, AZFb, and AZFc (azoospermia factors a, b, and c) (Fig. 3a). Microdeletions of Y chromosomes are found in about 5–10% of men with oligozoospermia and in up to 15% of azoospermic patients [30].

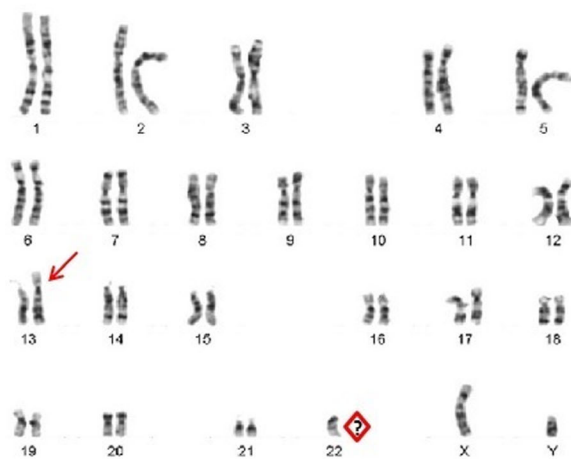
The current guidelines by the European Academy of Andrology (EAA) and European Molecular Genetics Quality Network (EMQN) for the detection of Yq microdeletions recommend the use of two markers in each AZF region in two multiplex polymerase chain reactions (PCR). Each PCR also has to include a marker for sex-determining region Y (SRY), located on the short arm of the



(a) 47,XXY



(b) 46,XY,t(4;11)(q21;q23)



(c) 45,XY,der(13;22)(q10;q10)



(d) 46,XY,inv9(p11q13)

**Fig. 2** Examples of karyotype anomalies. **a** Klinefelter syndrome—47,XXY (adopted from [www.qfg.com.au](http://www.qfg.com.au)). **b** Reciprocal translocation involving chromosome 4 and 11 (adopted from <http://what-when-how.com/genetics>). **c** Karyotype in patient with Robertsonian translocation

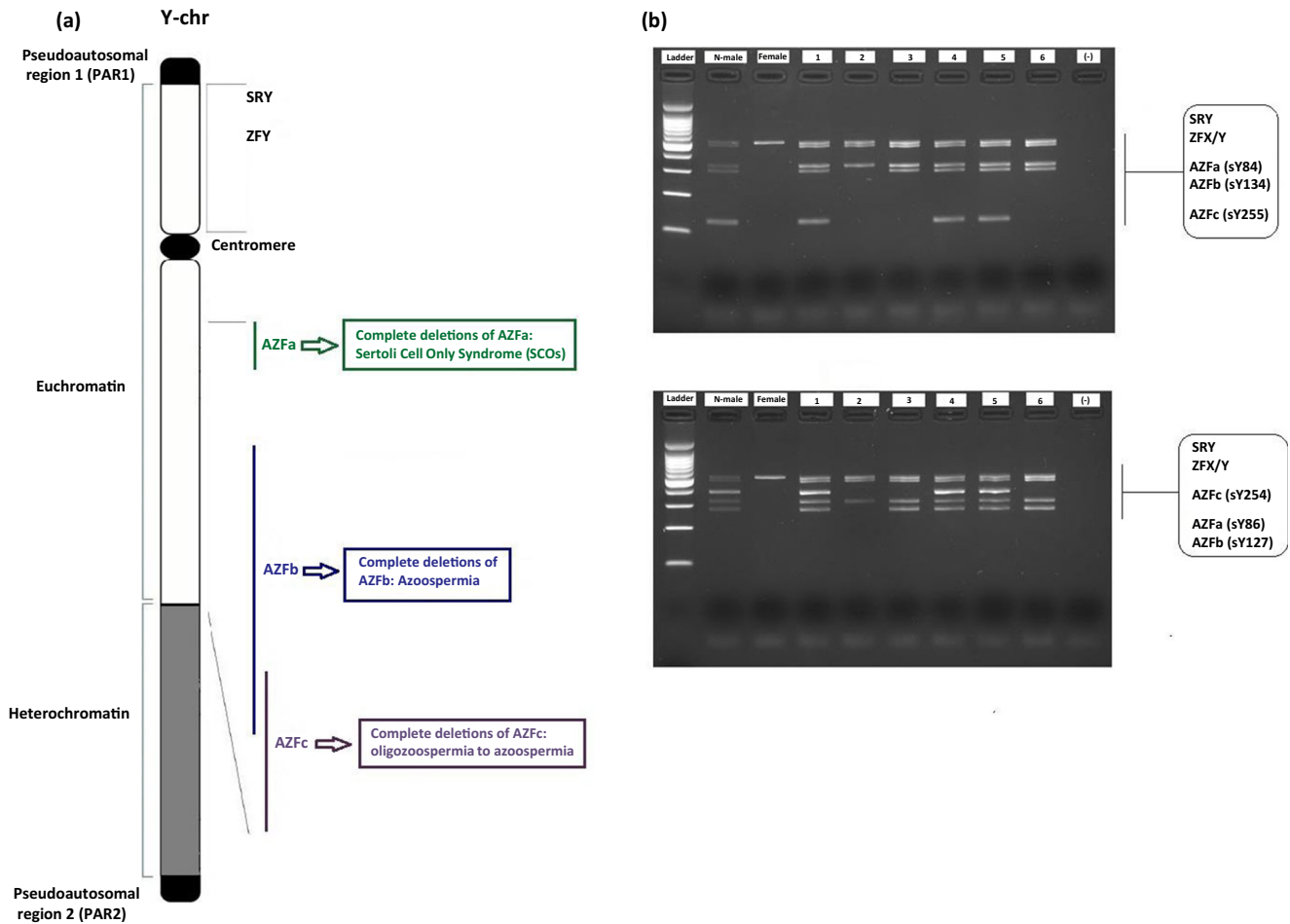
involving chromosome 13 and 14 (adopted from Răchışan et al. 2017 [17]). **d** Karyotype of a patient with inversion 9 (adopted from Jeong et al. 2010 [18])

Y chromosome, and a marker for *ZFX/ZFY*, a gene located on the X and Y chromosomes [29]. Examples of both multiplex PCRs are shown in Fig. 3b. A DNA sample from a fertile male, a woman, and a blank (water) control should be run in parallel with the set of primers.

Deletions encompassing the entire AZFa region usually produce the severe phenotype designated Sertoli-cell-only syndrome (SCOs), and complete deletions of the AZFb region induce maturation arrest of germ cells [29] which results in azoospermia. In a few cases, sperm retrieval has been reported in patients with complete AZFb and AZFbc deletions but, as yet, no clinical or chemical pregnancy after ICSI in cases with complete AZFb/b-c microdeletions has been reported [31–33]. Testicular sperm extraction (TESE)/ICSI programs should be suggested after careful genetic counseling.

On the contrary, AZFc deletions result in a variable infertility phenotype, ranging from severe or mild oligozoospermia to azoospermia. Absence of spermatozoa in the ejaculate is not predictive of testicular biopsy results; it is therefore justified. In 50% of patients with the AZFc deletion, testicular biopsy is positive. There is still a debate as to whether oligozoospermic patients with an AZFc deletion become azoospermic over time [34]. Hence, it is important to propose sperm cryopreservation to patients with AZFc microdeletion.

The most frequent deletion subtypes comprise the AZFc region (~80%) followed by AZFa (0.5–4%) and AZFb (1–5%) regions [29]. In addition to these deletions, it is possible to observe complete deletions of the AZFabc or AZFbc region [29]; in all cases, this is accompanied by complete azoospermia, for which it is useless to propose a testicular biopsy.



**Fig. 3** Y chromosome microdeletion testing. **a** Schematic diagram of human Y chromosome indicating the male-specific region of Y chromosome. AZFa-c: azoospermia factor a-c; PAR1 and PAR2: the pseudoautosomal regions. Genotype/phenotype relations of complete AZF deletions is given for each region. **b** Examples of multiplex PCRs

recommended by European Academy of Andrology (EAA) and European Molecular Genetics Quality Network (EMQN) for the detection of Yq microdeletions. Two STS loci in each AZF region are analyzed and PCR products run on 3% agarose gel

Other forms of semen analysis anomalies, namely, teratozoospermia and asthenospermia, are not indications to search for microdeletions of the Y chromosome. Alternatively, as we will see, particular gene screening may be proposed.

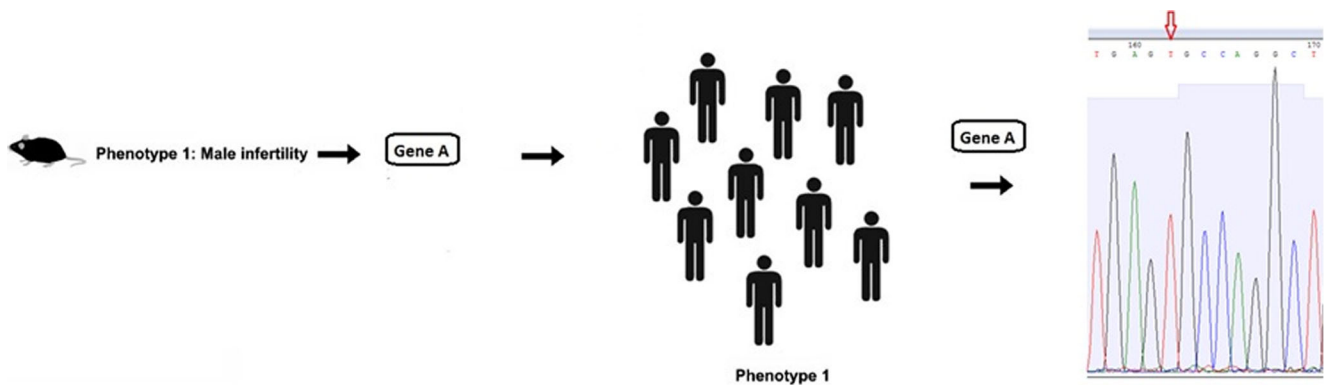
### Identification of “infertility” genes

Before introducing cases of patients who may benefit from a genetic diagnosis, we would like to briefly describe the methods used to identify infertility genes. Obviously, clinicians play an important role in this process and their collaboration is fundamental since they are the only ones who can recruit suitable patients to be examined, allowing the identification of infertility genes. So, it is important that they know how this should be done and how to contribute to this research.

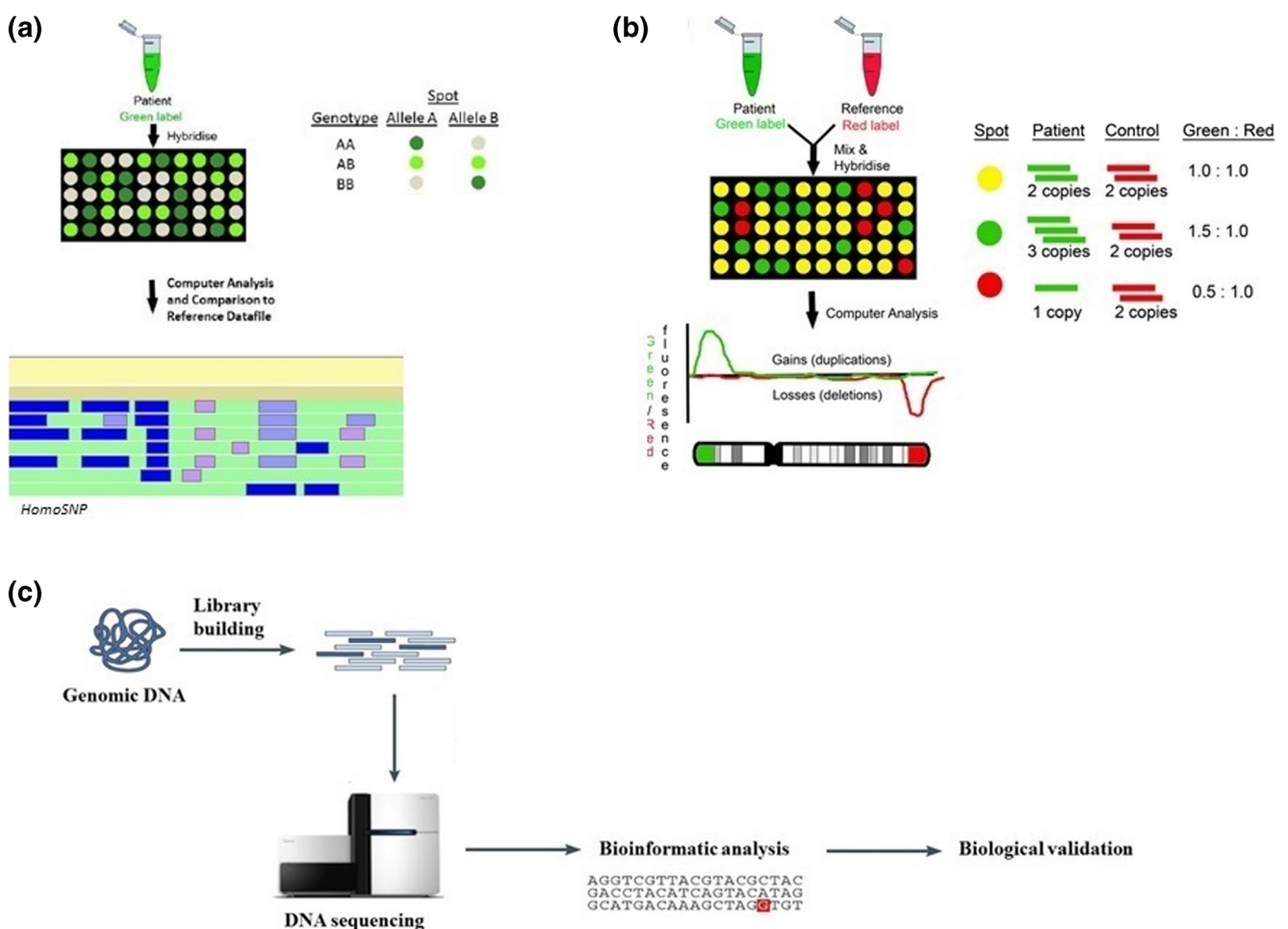
To identify mutations, geneticists have different strategies at their disposal. One of the oldest, but not the most fruitful, therefore less frequently used, is called the candidate gene

approach (Fig. 4). The candidate gene approach depends on the assumption that the gene function is conserved throughout evolution. Thus, an infertility gene in an animal model should, if mutated, cause a similar phenotype in humans as that observed in the animal model, usually the mouse. The method involves identifying a cohort of patients with the same phenotype as in the model animal and sequencing the human homolog of the candidate gene in these patients. Prior knowledge of the function and expression of the candidate gene is necessary to predict the biological plausibility of its role in the phenotype studied. This method is time consuming for a highly unpredictable result; so far, only one gene has been identified with certainty by this strategy, *TEX11* [35]. This gene has also been identified with comparative genomic hybridization (CGH) array technique by another group, and the publication appeared a few months before the candidate gene study [36].

Whole-genome approaches are much more effective and affordable (Fig. 5). Such strategies involve the analysis of single nucleotide polymorphism (SNP) arrays (Fig. 5a),



**Fig. 4** Candidate gene approach. The method involves identifying a cohort of patients with the same phenotype as in the model animal and sequencing the human homolog of the candidate gene in these patients



**Fig. 5** Whole-genome approaches. **a** Single nucleotide polymorphism (SNP) arrays. SNP array technology is based on the discrimination between the two possible SNP alleles (A or B) for a specific position in the genome. The hypothesis is the whole region surrounding the mutation is identical in the group of patients with same phenotype; therefore, all the polymorphic markers, SNPs, of the area are homozygous and can be readily detected. Regions of homozygosity can be visualized by specific programs such as HomoSNP (adopted from Karampetsou et al. 2014 [37]). **b** Comparative genomic hybridization (CGH) arrays. The patient DNA and a normal control DNA, used as reference, are differentially

labeled using fluorescent dyes. The two DNAs are then mixed together and hybridized on a microarray slide on which each spot represents a specific locus in the genome. The relative intensity between the two fluorescent dyes is calculated for each probe (adopted from Karampetsou et al. 2014 [37]). **c** High-throughput sequencing. High-throughput sequencing technologies enable researchers to perform massively parallel sequencing. In practice, it can be used for whole-genome (WGS) or whole-exome sequencing (WES); both create an enormous amount of raw data requiring complex bioinformatic analyses to extract useful information

GCH arrays (Fig. 5b), or high throughput sequencing technologies (Fig. 5c). The latter is the preferred method due to its effectiveness and the considerable reduction in cost.

High-throughput sequencing can be performed on an entire genome, about 3 billion base pairs, or only the coding parts, called the “exome” comprising 2% of the genome, around 60 million base pairs. With the knowledge that 80 to 85% of disease-causing mutations are found in the exome [38], and that the complexity of the bioinformatics analysis increases with the size of the genome, most studies are carried out by sequencing the exome. Indeed, regardless of the genetic diseases studied, there is a significant increase of articles using this method for the identification of causal mutations.

### Patient recruitment for the identification of infertility genes

Like all research in human genetics, searching for infertility genes is based on the study of patients, more precisely infertile patients and of course the quality of diagnosis is critical.

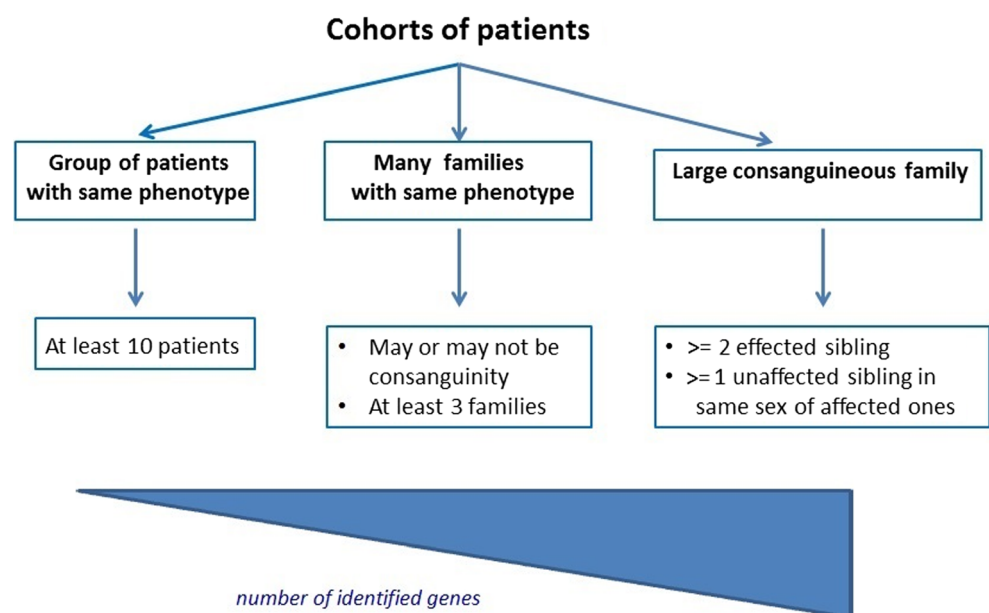
Different cohorts of patients can be examined (Fig. 6). In the case of a cohort of genetically unrelated patients presenting an identical phenotype, a frequent mutation inherited from a common ancestor, known as a founder mutation, is searched for. This is how the c.144delC (p. L49Wfs22) mutation in *AURKC*, which is found in 50% of North African patients with macrocephaly, was identified [39]. The phenotype studied could also be due to the specific architecture of a locus, which displays instability. Deletion of the entire *DPY19L2* gene in globozoospermia is an example of such a phenomenon. This deletion is found in more than 60% of globozoospermia cases because of the presence of two highly identical repeats, namely

low copy repeat (LCR) sequences surrounding the gene and that are capable of recombining between each other and therefore provoking the deletion of *DPY19L2* [40–42].

On the other hand, studying cohorts of patients can be problematic because of the multiple possible causes of infertility, genetic or not, and the low level of discernment of diagnostic tests. Phenotypic homogeneity remains a major paradigm of sporadic cases. There are various causes of azoospermia, and it is very difficult, if not impossible, to classify them. Currently, about 17 genes have been identified in man which when mutated lead to severe non-syndromic oligozoospermia and/or azoospermia without overlapping with female infertility [36, 43–55]. On the other hand, such an approach makes it possible to demonstrate transmission modes of any kind, recessive, dominant, or X-linked.

The hereditary nature is generally confirmed by the existence of familial cases of the pathology investigated. The analysis of large families of well-documented male infertility with some degree of consanguinity provides an alternative approach to identify genes involved in infertility. The genetic etiology of a phenotype within a family has a high probability of being identical which allows a straightforward analysis. Therefore, consanguineous family-based studies have largely proven successful. As a result, geneticists have a natural preference to study families. Thus, clinicians or reproductive biologists are solicited for the recruitment of families, consanguineous or not, with one or more cases in the same family. Again, the limitations stem from the uncertainty of the hypothesis that these families present defects in the same gene or the uncertainty of the diagnosis. In this strategy, the recessive transmission mode is primarily assumed but dominant or X-linked transmissions are not completely excluded.

**Fig. 6** Cohorts of patients that can be involved in genetic studies for the quest of infertility genes. In the case of a cohort of genetically unrelated patients presenting an identical phenotype, a frequent mutation inherited from a common ancestor, known as a founder mutation, is searched for. Family-based studies provide an alternative approach to identify genes involved in infertility. The genetic etiology of a phenotype within a family has a high probability of being identical which allows a straightforward analysis



Following the identification of a variant within a gene, whether through the analysis of a cohort of patients or family cases, it is essential to demonstrate that it is really a mutation that is the cause of the observed pathology. Indeed, the genome is interspersed with variants (most of them corresponding, as far as science can tell, to polymorphisms having no phenotypic consequences) and in such genetic studies, many variants remain of unknown significance. If a variant does not fulfill criteria using either of these sets (pathogenic or benign), or the evidence for benign and pathogenic is conflicting, the variant is classified as of uncertain significance (*variant of uncertain (or unknown) significance*, VUS) [56]. There are different non-exclusive methods to determine whether a variant is causal or not. The first one is to check the frequency of a candidate variant within the human population. This can be achieved by analyzing available databases such as the gnomAD database which includes 123,136 exome sequences and 15,496 whole-genome sequences from unrelated individuals. If the variant is frequently found, it will not be considered causal. Actually, this would mean that a large part of the population would be affected by the pathology. In general, a variant may be considered as interesting if it is found, in the examined databases, at a frequency below 1% [57]. Other criteria include the pattern of expression, which must be compatible with the phenotype studied, here infertility. Once a variant is selected, functional research can be carried out. This can be done in many ways; the strategy mainly depends on the gene identified and the available knowledge of its function. A lack of suitable human germ cell models limits functional studies in the field of infertility. The emergence of techniques allowing the differentiation of pluripotent stem cells able to go through meiosis may facilitate these functional analyses. The most elegant way to demonstrate the role of a protein is to create, if it does not exist already, a mutant mouse model and see if the mouse phenotype recapitulates the human one.

In addition, a convincing way to prove that a variant is a mutation is to find the same or other mutations in the same gene among a group of patients displaying the same phenotype. This will also enable an estimation of the frequency at which the identified gene can be involved.

The set of infertility genes identified so far, which we believe the data are firm enough to consider as responsible for non-syndromic male infertility phenotype when mutated, are shown in Table 1. For PubMed search “male infertility, non-syndromic, genetic, gene mutation” were used as keywords. The Genome Aggregation Database (*gnomAD*)—[gnomad.broadinstitute.org/](http://gnomad.broadinstitute.org/)—and The Alamut® Software Suite were used for validation of the mutations.

## Misters: Mr. Brown, Mr. Pink, Mr. White, Mr. Blonde, Mr. Blue and Mr. Orange

(Reference to the film by Quentin Tarantino, “Reservoir Dogs” 1992)

### Obstructive azoospermia

Mr. Brown after a first consultation had undergone a semen analysis. The result indicates an obstructive azoospermia. Examination of the patient reveals a congenital bilateral absence of vas deferens (CBAVD). A search for a mutation in the *CFTR* gene should be offered to the patient. Indeed, CBAVD is the most severe form of cystic fibrosis (CF) and mutations in the *CFTR* gene are responsible for CBAVD in 80–90% of cases entailing obstructive azoospermia [12, 69]. If Mr. Brown bears mutations, then screening for a mutation in the same gene should be offered to his spouse in order to eliminate the risk of transmitting CF to the future child. If a mutation is found in the wife, a pre-implantation genetic diagnosis (PGD) can be offered. If Mr. Brown carries no mutation in the *CFTR* gene, then ADGRG2 gene mutation testing may be offered. Karyotype and microdeletion searches of the Y chromosome are not recommended to patients with an obstructive azoospermia.

### Non-obstructive azoospermia

Mr. and Mrs. Pink return in consultation following the first investigations. Mr. Pink suffers from a non-obstructive azoospermia without any karyotype anomaly or Y chromosome microdeletion. In this case, he may be offered a mutation screen using a panel of genes that have recently been identified (see Table 1). This test has several advantages: (i) it allows a precise diagnosis and identifies the cause, which is important for the psyche of the patient; (ii) it helps to choose the best treatment and counsel not only for him but also for his spouse and relatives. Among approximately 17 genes identified for azoospermia, there is no predominant gene, so all of them need to be tested. So far, the research on these genes has not been able, because of the scarcity of patients with mutations, to determine if any of them could be used to predict the presence or absence of spermatozoa after a TESE, as for AZFa or AZFb deletions. However, there is no doubt that this information will be available in the future. The identification of such genes is the starting point of a long-term research effort.



**Table 1** The set of genes identified so far, for non-syndromic male infertility. *NOA* non-obstructive azoospermia, *OAT* oligoasthenoteratozoospermia

Phenotype		Genes (OMIM no.)	Reference	
Teratozoospermia	Globozoospermia	<i>SPATA16</i> (609856)	Dam et al. [58]	
		<i>DPY19L2</i> (613893)	Koscinski et al. [42]	
	Macrocephaly	<i>AURKC</i> (603495)	Dieterich et al. [39]	
	Sperm flagellar problem (MMAF)	<i>DNAH1</i> (603332)	Ben Khelifa et al. [59]	
		<i>SEPT12</i> (611562)	Kuo et al. [60]	
		<i>CFAP43</i> (617558)	Tang et al. [61]	
		<i>CFAP44</i> (617559)	Tang et al. [61]	
	Acephalic spermatozoa	<i>BRDT</i> (602144)	Li et al. [62]	
		<i>SUN5</i> (613942)	Zhu et al. [63]	
	Asthenozoospermia		<i>CATSPER1</i> (606389)	Avenarius et al. [64]
		<i>GALNTL5</i> (615133)	Takasaki et al. [65]	
		<i>SLC26A8</i> (608480)	Dirami et al. [66]	
		<i>SPAG17</i> (616554)	Xu et al. [67]	
Total fertilization failure (ff)—ICSI		<i>PLCZ1</i> (608075)	Escoffier et al. [68]	
Spermatogenic Failure	Azoospermia and/or oligozoospermia	<i>DAX1</i> (300473)	Mou et al. [43]	
		<i>TEX11</i> (300311)	Yatsenko et al. [36]	
		<i>TEX15</i> (605795)	Okutman et al. [44]	
		<i>MAGEB4</i> (300153)	Okutman et al. [45]	
		<i>TAF4B</i> (601689)	Ayhan et al. [46]	
		<i>ZMYND15</i> (614312)	Ayhan et al. [46]	
		<i>HSF2</i> (140581)	Mou et al. [47]	
		<i>KLHL10</i> (608778)	Yatseenko et al. [48]	
		<i>MEIOB</i> (617670)	Gershoni et al. [49]	
		<i>TEX14</i> (605792)	Gershoni et al. [49]	
		<i>DNAH6</i> (603336)	Gershoni et al. [49]	
		<i>HIWI</i> (605571)	Gou et al. [50]	
		<i>SPINK2</i> (605753)	Kherraf et al. [51]	
		NOA	<i>SOHLH1</i> (610224)	Choi et al. [52]
			<i>NPAS2</i> (603347)	Ramasamy et al. [53]
			<i>TDRD9</i> (617963)	Arafat et al. [54]
		NOA / OAT	<i>NANOS1</i> (608226)	Kusz-Zamelczyk et al. [55]

References [36, 39, 42–55, 58–68]

## Severe oligozoospermia

Mr. White suffers from severe oligozoospermia (< 5 million sperm/mL of ejaculate). Karyotype analysis reveals no anomaly and he has no Y chromosome microdeletion. Similarly to Mr. Pink, he may also be offered a screen for mutations using a panel of genes that have recently been identified as provoking oligozoospermia when mutated (Table 1). Furthermore, the mutation of certain genes can lead to a progressive oligozoospermia, evolving towards a complete azoospermia. In such a situation, it is wise to offer Mr. White a sperm cryopreservation, as soon as possible. Severity may be variable within the same family. It is therefore important to inform the patient about the consequences of such mutation; if the patient agrees to inform his brothers, it will then be possible

to offer a genetic test and sperm cryopreservation to those affected. So far, only the *TEX15* gene falls into this category so further research is needed to determine whether other genes behave similarly and which mutations are critical since they can affect the function of the encoded protein to a variable extent.

## Macrocephalia

The Blonde couple returns to consultation; the spermocytogram detects a macrocephaly with more than 70% of affected spermatozoa, associated with multiple flagella. In this situation, it is interesting to test the *AURKC* gene which is mutated in 83.7% of the cases with one of two recurrent mutations [70]. The first

one, c.144delC (p. L49Wfs22), is found in North African populations and the other, c.744C > G (p.Y248\*), is found in European populations [39, 71]. Finding a mutation leads to an unfortunate but necessary interruption of any ART procedure. Indeed, it has been clearly demonstrated that in such a situation, all spermatozoa display karyotype anomalies, the majority of spermatozoa being tetraploid. Most probably, meiosis continues without cytokinesis. Alternative solutions should be offered, ranging from sperm donation, adoption, or abandoning a parental project.

## Globozoospermia

Mr. Blue's spermocytogram shows total globozoospermia, meaning that more than 95% of the spermatozoa have no acrosome. Several genes have been described for globozoospermia; among them, one predominates since it was found mutated in almost 75% of cases [41]. The gene, known as *DPY19L2*, was identified for the first time by the study of a Jordanian consanguineous family [42]. The multiple studies following the first one revealed *DPY19L2* mutations as a major cause of globozoospermia. In more than 50% of the cases, deletion of the entire *DPY19L2* gene occurs due to a genomic recombination event between two almost identical LCR sequences situated on either side of the gene. Besides this recurrent mutation, point mutations can be found without any recurrence in the population [40–42]. So far, among the other genes identified in globozoospermia, only mutations of *SPATA16* have been firmly identified as causative [58, 72]. Again, genetic analysis allows a precise diagnosis, providing valuable information for the rest of the family and help in choosing the appropriate treatment. Kuentz et al. studied a cohort of patients suffering from globozoospermia and designed a new clinical approach to improve the results of ART with ICSI. We showed that an ICSI associated with artificial oocyte activation restores fertilization and the resulting pregnancy rate to the same level as obtained during routine ICSI cases [73]. This is explained by the absence of an acrosome and thus of the oocyte meiosis activating factor, namely, the phospholipase C zeta (PLCz) factor, provided by the acrosome.

## Multiple morphological abnormalities of the sperm flagella (MMAF)

Following a semen analysis that demonstrates a teratozoospermia with multiple anomalies of the flagella, the Orange couple returns in consultation. To date, four genes have been identified for sperm flagella problems, namely, *DNAHI*, *SEPT12*, *CFAP43*, and *CFAP44* [59–61]. In patients with a mutation in *DNAHI*, an ICSI can be proposed leading to fertilization and pregnancy rates similar to those of the routine ICSI cases. The

value of a genetic search for mutations in *DNAHI*, in addition to those mentioned above and even if the 20 identified patients declared no other problems, lies in a risk of other problems linked to ciliary dyskinesia. Indeed, *DNAHI* is involved in the formation of cilia, and the importance of cilia in the development of certain organs and their function has been clearly established. The multiple anomalies of the flagella can thus be considered as ciliopathies. It remains to be established whether the effects of a mutation in *DNAHI* are limited to spermatozoa or may also involve other organs where the gene is expressed. Again, it cannot be excluded that different mutations may have different effects with varying severities, involving only spermatozoa or additionally affecting other organs.

A patient could have combined phenotypes, meaning that semen analysis could reveal a combination of count, morphology, and/or motility problems together such as oligoasthenoteratospermia. In these cases, there is so far no clear option and therefore it is difficult to give advice.

## Discussion and future perspectives

The emergence of new molecular biology technologies allowing the analysis of the complete genome has revolutionized the genetics of infertility. In just 10 years, an increasing number of genes has been identified as responsible, when mutated, for non-syndromic male infertility (Table 1). The identification of such genes is the starting point of long-term research projects. Recently, this new area of research has begun to have clinical consequences. Indeed, several genetic diagnostic laboratories are now offering the analysis of a panel of infertility genes involved in a variety of male and female infertilities.

The high-throughput sequencing of a list of genes, known as a “gene panel,” is the most recent test available in genetics of infertility. The panel will inevitably evolve as new genes are identified. Today, there are commercial panels available in the USA and in Europe. The choice of genes may vary from one laboratory to another. The shortcomings are that it is limited to genes in the panel, translation from research to diagnosis is so recent, and the cost-benefit relationship is not yet well-defined. Indeed, the data available to establish the causality of certain variants described in the literature may be insufficient. The choice of genes remains up to the best judgment of the geneticist elaborating the panel.

For almost all the genes recently described in infertility genetics, the frequency of the identified mutations will have to be determined. Without excluding some exceptional cases, as illustrated with globozoospermia and macrocephalia, it is probable that this frequency will remain low, rarely more than 1 to 2%, since gametogenesis is a complex process with concerted actions of many genes. Additionally, the correlation

between genotype and phenotype as well as the possible variation of inter-individual severity still remains to be defined for almost all of the genes identified so far. Not only is more knowledge of previously identified genes required, but a significant number of genes remains to be identified.

The search for infertility genes has led to the emergence of new knowledge about the human reproductive process, which will ultimately improve the management of couples.

## This is a call for collaboration!

Certainly, one of the major difficulties in the realization of this kind of research is the recruitment of patients. Therefore, any clinician who would like to participate in this work should be brought closer to research teams working on the subject. They will be welcomed with open arms.

With this in mind, our team is looking for collaboration with clinicians capable of recruiting patients with infertility of unknown origin. We work preferentially on large consanguineous families, but also with several small families having consanguinity or not, and cohorts of individuals from the same geographical region with the same phenotype.

We focus on men with non-obstructive azoospermia including maturation arrest (MA) and Sertoli cell only syndrome (SCOs), severe oligozoospermia, and multiple flagellar anomalies. Of course, if a clinician encounters a family which is interesting, we will consider with the clinician about the possibility of analyzing it.

To conclude, let us note that the study of the genetics of infertility is paradoxical. Genetics studies the transmission of such defects from one generation to the next, while ART seeks to ensure their transmission to the next generation which would be impossible without medical means.

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