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Genetic testing in couples undergoing assisted reproduction technique protocols

Liborio Stuppia†, Valentina Gatta, Ivana Antonucci, Rossella Giuliani, Anna Paola Scioletti & Giandomenico Palka

†*G. d'Annunzio University, Department of Biomedical Sciences, Via dei Vestini 35 66013, Chieti-Pescara, Italy*

Background: An increasing number of couples undergo assisted reproduction techniques (ART) to generate a child, with the risk of the transmission to the offspring of a genetic defect underlying the condition of infertility. *Objective*: To review the most common genetic causes of infertility and identify the appropriate genetic testing to be carried out to reduce the risk of genetic defect in the offspring. *Method*: Review of the literature in the field. *Results/conclusion*: Cytogenetic investigation and screening of the *CFTR* gene are the only genetics testing suggested in all the couples undergoing ART; other tests should be performed only in selected cases.

Keywords: assisted reproduction techniques, *CFTR* gene mutations, chromosome aberrations, genetic counselling, infertility of the couple, Yq microdeletions

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1. Introduction

Infertility represents a major health problem in western countries, as about one in six couples seek medical assistance owing to the incapacity to generate a child [1]. A dramatic increase in the prevalence of infertility has been evidenced in the last few years, probably due to factors inducing a damage in the gametes' quality, such as increased maternal age, lifestyle habits and environmental agents [1]. Despite the large number of available tools for the study of the pathogenesis of male and female infertility, in many cases the etiology of the disease has not been identified, and no specific therapy can be provided. As a consequence, increasing numbers of couples undergo assisted reproduction techniques (ART) in order to generate a child. Since the birth of the first baby conceived using *in vitro* fertilization [2], about one million babies have been born worldwide as the result of ART protocols. Nevertheless, the possible health effects of these protocols are still debated, as a slightly elevated risk of birth defects in children born following ART has been reported [1]. In particular, as > 100 different genetic diseases are related to male and female infertility, there is concern for the risk of transmission of genetic defects to the offspring, in particular for cases submitted to the intracytoplasmic sperm injection (ICSI) technique, based on the use of a single sperm for egg fertilization [1,3]. This technique allows men with very few (if any) sperms in the ejaculate to father a child using a single spermatozoon retrieved from the epididymis or the testis, bypassing the natural sperm selection preventing the formation of zygotes with major abnormalities [4-6]. Thus, couples enrolled in an ART protocol should be submitted to an appropriate genetic counselling and genetic testing to disclose the presence of hidden genetic alterations that can be transmitted to the offspring. On the other hand, in these couples, screening of genetic diseases not related with infertility but showing a high prevalence of healthy carriers in the general population should be considered. In both cases, the aim of the genetic testing is to provide

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the couple with accurate information about the reproductive risk, and to offer prenatal diagnosis when appropriate. This latter topic involves also the application of preimplantation genetic diagnosis (PGD), representing an alternative approach to amniocentesis in countries where this technique is legally permitted [7]. As the aim of this review is to analyze the application of genetic testing in couples before fertilization, PGD and other forms of prenatal diagnosis are not considered.

In this study, the most common genetic anomalies associated with male and female infertility and the appropriate genetic testing to perform in order to prevent the birth of a child affected by a genetic disease are described. Moreover, the application in couples undergoing ART of specific genetic testing to rule out the condition of a healthy carrier of genetic diseases with high prevalence in the population is discussed.

2. Genetic causes of male and female infertility

2.1 Chromosome abnormalities

Several studies have investigated the presence of chromosome abnormalities in couples undergoing ART, reporting a prevalence ranging from 1.3 to 13.1% (**Table 1**) [5,8-19]. Despite the variability among different series, probably related to the different compositions of the populations examined, these data demonstrate an increase of abnormal karyotypes in infertile couples, the expected prevalence of chromosome abnormalities in the general population being ~t 0.85% [20]. In males, the prevalence of chromosome abnormalities appears to be inversely related to the sperm count, the highest incidence being detected in azoospermic patients [5-6,18,19]. In women, genotype–phenotype correlation is less evident, a gametogenesis defect being more difficult to assess than in men; however, it has been reported that women with a history of at least one spontaneous pregnancy loss show the highest incidence of chromosomal abnormalities [5].

Chromosome aberrations can be detected in infertile couples in the form of numerical alterations of sex chromosomes and of structural rearrangements of both sex chromosomes and autosomes (**Table 2**). The most common chromosome aberration is represented by the 47,XXY karyotype (Klinefelter syndrome [KS]), showing a prevalence of 1 in 500 – 1000 males and detected in up to 10% of azoospermic patients [5,6,21]. The diagnosis of KS is commonly made during adolescence or adulthood in males showing small testes, hypergonadotropic hypogonadism and gynecomastia [22]. The spermatogenesis failure in KS patients results in azoospermia or, less frequently, severe oligozoospermia at sperm count. High stature is a constant feature of KS, owing to the presence of three copies of the *SHOX* gene, mapped within the pseudoautosomal region 1 (PAR1) of the X and Y chromosomes and involved in the regulation of stature [23]. Although further clinical findings (such as feminized body habitus) can be present in a portion of KS patients, in most cases infertility is the only relevant clinical feature, and this syndrome must be suspected in all cases of azoospermia or severe oligozoospermia. This latter condition is generally associated with the presence of a 47,XXY/46,XY mosaic (detectable in ~ 15% of KS cases), allowing a residual spermatogenesis in some seminiferous tubules. After the introduction of ART, oligozoospermic KS patients can father a child by ICSI with testicular or ejaculated spermatozoa. Most children fathered by KS patients using ART are chromosomally normal, probably because seminiferous tubules with residual spermatogenesis have a normal 46,XY karyotype. However, it has been reported that KS patients submitted to ICSI have an increased risk of fathering a 47,XXY or 47,XXX child [18,24,25]. The production of aneuploid spermatozoa probably derives from the meiosis of a few 47,XXY spermatocytes or from meiotic abnormalities occurring in 46,XY germ cells in a compromised testicular environment [18]. Thus, the presence of a 47,XXY karyotype should be assessed in all infertile males with non-obstructive azoospermia or severe oligozoospermia, and appropriate genetic counselling should be provided to KS patients with residual spermatogenesis undergoing ICSI protocols.

In addition to the 47,XXY karyotype, it has been reported that men with a 47,XYY karyotype, although generally fertile, are more frequently represented in infertile populations [19]. XYY fertile males do not transmit the extra Y chromosome to their progeny [26,27], probably because the extra Y chromosome is lost before meiosis [28]. However, it has been shown that severe oligozoospermia in 47,XYY males is often associated with the persistence of the extra Y chromosome in 30 – 100% of spermatocytes I at pachytene stage [29-31].

Another chromosomal cause of male infertility is represented by the 46,XX males. This condition has a frequency of ~ 1:20,000 in the general populations [32], and is characterized by azoospermia owing to the complete absence of germ cells as the only clinical feature. In 80% of cases 46,XX males derive from an abnormal exchange between the X and Y chromosomes during the paternal meiosis involving the *SRY* gene [33], mapped close to the PAR1 boundary on the Y chromosome. The deriving 23,X sperms can fertilize a 23,X egg producing a 46,XX zygote carrier of the *SRY* gene, which is able to induce a testicular differentiation of the primary gonad, with androgen secretion and the development of a full male phenotype. However, owing to the absence of all the genes mapped within the Y chromosome long arm (Yq), these patients are not able to produce any sperm; 46,XX males generally are not enrolled in ART protocols, and there is no risk of transmission of their condition.

In females, the most frequent chromosome abnormality associated with infertility is the 45,X karyotype (Turner syndrome). Owing to the presence of very typical clinical features (amenorrhea, short stature, lack of development of internal and external genitalia), the diagnosis of this condition is usually made during adolescence. Thus, the typical Turner

Table 1. Prevalence of chromosomal aberrations in infertile patients in different literature reports.

Authors	Ref.	No. of patients	Prevalence of chromosomal abnormalities (%)
Hens *et al.*, 1988	[8]	1000	1.3
Lange *et al.*, 1993	[9]	144	9
Mau *et al.*, 1997	[10]	300	9
Scholtes *et al.*, 1998	[11]	2280	7.2
Van der Ven *et al.*, 1998	[12]	610	3.3
Meschede *et al.*, 1998	[13]	894	3.8
Peschka *et al.*, 1999	[14]	1562	13.1
Gekas *et al.*, 2001	[15]	3208	5.7
Sonntag *et al.*, 2001	[16]	1622	3.3
Clementini *et al.*, 2005	[5]	4156	1.9
Riccaboni *et al.*, 2008	[17]	5266	1.3

syndrome is not usually observed in the female partners of couples undergoing ART. However, milder phenotypes with secondary amenorrhea or premature ovarian failure (POF) can be caused by structural abnormalities of the X chromosome or mosaicisms of sex chromosomes [34]. This latter abnormality represents a large proportion of all chromosomal alterations detected in infertile women [5,11,13,15]. The role played by mosaicisms of sex chromosomes on female fertility and ICSI outcome is still controversial [11,15,16], although it has been suggested that mosaicisms > 8% have potential effects on the female reproductive axis [5].

Besides numerical chromosome aberrations, structural rearrangements can also be detected in infertile couples. Among these, Robertsonian translocations, involving acrocentric chromosomes, are the most frequent in humans, with a prevalence of about 1 in 1000 newborns in the general population [35]. Robertsonian translocations are generally associated to normal phenotype, but can induce impaired gametogenesis and/or production of gametes with an unbalanced combination of the parental rearrangement, affecting fertility and/or pregnancy outcome. The prevalence of Robertsonian translocations in infertile males is nine time higher than in the normal population [18]. Although it has been demonstrated that the actual frequency of unbalanced sperm in men with Robertsonian translocation is lower than theoretically expected, leading to 1 – 2% unbalanced zygotes, couples should be informed about the possible consequences of undergoing ART in the presence of a Robertsonian translocation in one of the two partners [19]. When the Robertsonian translocation involves the same chromosome pair, the couple must be informed that only trisomic or monosomic embryos will be produced, with no possibility of a normal offspring.

Reciprocal translocations, consisting of the exchange of chromosome material between any chromosomes, represent another common structural aberration in humans, being detected in 0.9/1000 newborns and showing a 7 times higher prevalence in infertile men [18]. A higher frequency of sperm carriers of unbalanced chromosomes than in Robertsonian translocations has been observed for these rearrangements [19]. In women, balanced chromosome translocations do not usually cause disruption of ovarian function, unless the X chromosome is involved, but infertility may be caused by recurrent spontaneous abortions because of unbalanced forms [36]. In couples undergoing ART, the possible consequences for the offspring of the presence of a reciprocal translocation are related to the chromosomes involved, and the breakpoint position. Many of these imbalances are not compatible with survival, whereas others cause serious mental and physical handicaps. Several fetuses with unbalanced segregations of reciprocal translocations have been reported after ICSI [13,37].

Another class of chromosome structural aberrations potentially related to infertility is represented by chromosomal inversions, that is, the presence of two chromosome breaks occurring in the same chromosome followed by a break healing in an inverted order. Chromosomal inversions can be divided into paracentric (both breakpoints are in one chromosome arm) and pericentric (breaks occur in both chromosome arms and include the centromere in the inversion). These aberrations can perturb spermatogenesis and lead to the production of unbalanced gametes through the formation of an inversion loop, with an overall 10 – 15% risk for the offspring [19].

A particular class of chromosome structural aberrations related to male infertility is represented by the rearrangements of the long arm of the Y chromosome (Yq). In fact, deletions or other rearrangements involving the band Yq11 are associated to azoospermia or severe oligozoospermia. However, cytogenetically detectable rearrangements represent only a small portion of all the Yq alterations associated with male infertility, the largest part being represented by the Yq microdeletions, which are described in the next subsection.

2.2 Yq microdeletions

The most frequent molecular cause of severe infertility in men is represented by the microdeletions of the Y chromosome long arm (Yq), detectable in ~ 10% of patients with unexplained azoospermia or severe oligozoospermia [38-43]. Yq microdeletions cannot be classified together with other structural chromosomal rearragements associated with infertility, as in most cases they are not detectable by cytogenetic investigation. On the other hand, they are not 'single gene' disorders, usually being characterized by the loss of function of several genes. Yq microdeletions can thus be considered as 'genomic' rather than 'chromosomal' or 'genetic' disorders. A large amount of data has been collected about the prevalence and the consequences at the genomic level of Yq microdeletions from the first cytogenetic indication, suggesting the presence of an azoospermia factor (AZF) on Yq [44]. These microdeletions

Table 2. Frequency and associated phenotypes of the most common chromosome abnormalities detected in male infertility.

Chromosome abnormality	Associated phenotype	Prevalence in general population	Prevalence in infertile patients (%)
47,XXY	Azoospermia (85%) Severe oligozoospermia (15%)	1:500 – 1:1000	Azoospermia 5 – 10 Oligozoospermia 2 – 5
47,XYY	Normal, occasionally infertility	1:500	NR
46,XX	Azoospermia	1:20,000	NR
Robertsonian translocations	From normospermia to azoospermia	1:1000	0.5 – 1
Reciprocal translocations	From normospermia to azoospermia	0.9:1000	0.5 – 1

involve three different loci named AZFa, AZFb and AZFc [45], and remove genes that are probably involved in male germ cell development and maintenance [46-50]. The AZFa region is ~ 1100 kb long, and the largest deletion of this region removes ~ 792 kb, including two genes, *USP9Y* and *DBY*. This deletion is caused by the homologous recombination between identical sequence blocks within retroviral sequences in the same orientation [51-53]. Partial AZFa deletions involving only *USPY9* or *DBY* have been reported, and the observed genotype–phenotype correlations have suggested a major role played by *DBY* in the disruption of spermatogenesis [49,54]. Deletions involving the AZFa locus are rare, accounting for ~ 5% of all the Yq deletions, and are associated with a more severe testicular phenotype, with complete Sertoli cell only (SCO) syndrome and azoospermia [44,55-57]. Deletions of the AZFb locus account for ~ 9% of all Yq deletions and cause the loss of several gene families, including *RBMY*, the first gene identified within Yq [45,58]. The phenotype of complete AZFb deletions is generally characterized by azoospermia associated with SCO or pre-meiotic spermatogenic arrest [18,44]. Most Yq microdeletions (~ 75%) involve the AZFc locus, probably because the presence of large palindromes (consisting of long, direct and indirect identical repeats) makes this region especially prone to rearrangement [59,60]. The complete AZFc deletion removes eight gene families, including all members of the *DAZ* gene family, the strongest candidate responsible for the AZFc phenotype [46]. The pathogenic role played in male infertility by other genes mapped within the AZFc locus, such as *BPY2* [50] or *CDY* [61], has not been elucidated yet. The typical AZFc deletion leads to azoospermia or severe oligozoospermia, associated with different spermatogenic phenotypes in the testis.

ART represents the only available therapy for men bearing Y microdeletions. As a consequence, this genetic defect is invariably transmitted to the male offspring, probably affecting their fertility. However, several cases of males with AZFc microdeletions who naturally fathered one or more children have been reported [62-64]. This suggests that AZFc deletions in some cases allow natural fertilization owing to the presence of residual sperms. In the reported families, the AZFc deletion was transmitted to all the sons, who were infertile owing to different defects of spermatogenesis [62-64]. The presence of a different genetic background and/or environmental factor affecting the penetrance of the genetic defect could explain the different phenotypes associated with the identical AZFc deletion within the same family.

No major malformations have been described in the sons of patients with Yq deletions. However, a reduced percentage of normal Y-bearing spermatozoa, a concomitant increase in nullisomic spermatozoa and a significant increase of XY-disomic spermatozoa in patients with AZFc deletions have been reported, suggesting that AZF microdeletions could be considered as 'pre-mutations' for a subsequent complete loss of the Y chromosome, increasing the risk of embryonic X0 cells [18,65,66].

In addition to the complete AZFc deletions, deriving from a rearrangement between the b2 and b4 amplicons, two types of partial AZFc deletion have been identified. The first one is the gr/gr deletion, caused by a homologous recombination between two g or two r amplicons producing a 1.6 Mb DNA segment excision from the AZFc region [67]. The gr/gr deletion has been considered as a significant risk factor for infertility by some authors [68-71], whereas other studies failed to confirm such an association [72-77]. The second partial deletion is the b2/b3 deletion, which removes a 1.8 Mb DNA segment from the AZFc locus. Also for this deletion, the association with male infertility is still debated [72,78-80]. Thus, partial AZFc deletions cannot so far be considered pathogenic for the development of a defect of the spermatogenesis. However, it has recently been suggested that the presence of partial AZFc deletions could represent a risk factor for the complete AZFc deletion [81]. This would confirm the presence of a genetic instability of the rearranged AZFc locus suggested previously by other studies [82].

2.3 Cystic fibrosis

Cystic fibrosis (CF) (OMIM #219700) is the most common lethal autosomal recessive disease in Caucasians, affecting 1:2500 newborns, with a prevalence of healthy carriers of 1 in 25 individuals in the general population. CF is caused by > 1000 different mutations of the CF transmembrane conductance regulator (*CFTR*) gene. The classical form of CF is characterized by obstructive chronic pulmonary disease,

pancreatic dysfunction and elevated concentration of electrolytes in the sweat. However, based on the presence of different CFTR mutations, several 'atypical' or 'mild' CF forms exist, characterized by a less severe phenotype [83]. In fact, CFTR mutations can be classified into 'classic' or 'severe' (CF) and 'mild' (CF^m) mutations. The classic CF with pancreatic insufficiency (PI) is always associated with the presence of two severe mutations (CF/CF genotype), whereas the presence of CF^m/CF^m or CF/CF^m genotypes produces atypical CF, with the phenotype ranging from monosymptomatic diseases (such as idiopathic pancreatitis or disseminated bronchiectasis) to CF with pancreatic sufficiency (PS) (**Figure 1**) [83]. The mildest form of CF is represented by obstructive azoospermia due to congenital bilateral absence of vas deferens (CBAVD) [84]. In this condition, otherwise healthy patients are infertile owing to the complete absence of sperms in their ejaculate caused by the mechanical obstruction. The most frequent CFTR mutation in CBAVD patients is the 5T allele, characterized by the presence of five thymidines within intron 8 and resulting in a reduction of the splicing efficiency of the *CFTR* gene [85]. In 30 – 40% of CBAVD individuals the genetic analysis allows the identification of a compatible genotype (CF/CF^m; CF/5T; $CF^m/5T$; CF^m/CF^m; 5T/5T); the remaining cases showing only one mutation (CF, CF^m or 5T). In these patients the second mutation can be represented by a regional change, specific to the different geographic regions [86,87], or a large rearrangement of the *CFTR* gene [88], both not detectable by conventional mutation screening. As spermatogenesis is normal in CBAVD patients, ART can be easily carried out by retrieving testis sperms. As a consequence, the CFTR mutations responsible for the CBAVD condition can be transmitted to the offspring, with the risk of generating a child with full-blown CF if the female partner is a healthy CF carrier. Thus, it is largely accepted that all CBAVD patients and their female partners should undergo genetic testing of the *CFTR* gene [3]. On the other hand, it has been demonstrated that couples undergoing ART for causes different from CBAVD do not show an increased prevalence of CFTR mutations as compared with the normal population [17,89]. Nevertheless, owing to the high frequency of CF healthy carriers in the general population, it has been suggested that all couples undergoing ART should be tested for the presence of CFTR mutations [3,89].

2.4 Other genetic causes of infertility

Mutations in the androgen receptor (*AR*) gene on the X chromosome cause a variety of defects collectively known as androgen insensitivity syndrome (AIS) (OMIM #300068). The mild form of AIS (MAIS) is characterized by male infertility as the primary or single clinical sign, and in fact *AR* gene mutations have been found in ~ 2% of unselected infertile men, with similar prevalence in azoospermia, severe oligozoospermia and moderate oligozoospermia [90]. The complete AIS (CAIS) is characterized by a full female phenotype in 46,XY subjects, despite the complete testicular differentiation of internal genitalia. In these cases, testes normally produce androgen hormones, but mutations in the *AR* gene cause a complete inactivation of androgen receptors, hampering the male differentiation.

A male karyotype can be detected in infertile females also as a consequence of a 46,XY complete gonadal dysgenesis (CGD) (OMIM #233420), due to mutations of the *SRY*, *NR5A1* or *DHH* genes [91-93]. In both CAIS and CGD, 46,XY females cannot usually produce gametes and are thus not at risk of transmitting the genetic defects. On the other hand, infertile males affected by MAIS, if submitted to ART, can transmit the *AR* gene mutation to the female offspring. Heterozygous daughters are all healthy carriers of the disease, with a 50% risk of generating an affected 46,XY son.

Another genetic condition recently associated with female infertility is represented by the pre-mutation of the *FMR1* gene (Xq23). Full mutations of this gene (represented by an expansion > 200 – 230 repeats of a CGG trinucleotide sequence in the *FMR1* gene) cause the Martin Bell syndrome (OMIM #300624), characterized by mental retardation in affected males. On the other hand, both males and females with a pre-mutation (60 – 200 repeats) are generally healthy but at increased risk for Fragile X tremor/ataxia syndrome (FXTAS) (OMIM #300623), affecting ~ 40% of males with pre-mutations who are over the age of 50 years. Females show a lower risk for FXTAS, but it has been demonstrated that ~ 10 – 15% of women carriers of a pre-mutation develop POF [94]. Therefore, women with POF of unknown origin should be informed of the possibility of having genetic testing for FMR1 pre-mutations, in order to identify a genetic defect segregating in their families [36].

3. Genetic testing in couples undergoing ART protocols

3.1 Karyotype analysis

Owing to the high frequency of chromosome aberrations in infertile couples, cytogenetic investigation represents the main genetic test to be carried out before ART. Nevertheless, there is no general agreement about the usefulness of performing cytogenetic investigation in all couples undergoing ART, independently from the cause of infertility. As a clear association has been demonstrated between the prevalence of chromosomal abnormalities and male infertility, it has been suggested to perform cytogenetic evaluation with a sperm cell count < 20×10^6 cells/ml rather than in every single male entering assisted reproduction programs [5]. However, Robertsonian and reciprocal translocations can also be found in men with normal sperm count, representing a potential risk for generating an unbalanced child affected by a severe genetic disease. In women, the identification of subjects at increased risk of being carriers of a chromosomes abnormality is even more difficult. However,

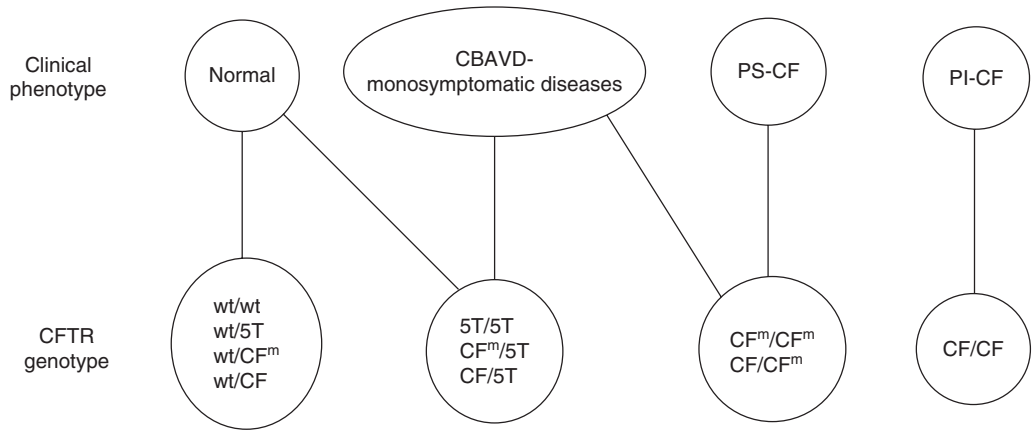

Figure 1. Genotype–phenotype correlation of the different CFTR mutations.
CBAVD: Congenital bilateral absence of vas deferens; CF: Cystic fibrosis; PI: Pancreatic insufficiency; PS: Pancreatic sufficiency.

in cases where the indication for ICSI is a poor reproductive outcome, chromosome analysis for both partners should be considered [95]. The practice of genetic testing for chromosomal aberrations varies between and within countries, with some countries offering testing to both males and females, and others limiting cytogenetic investigations to males with non-obstructive oligozoospermia and azoospermia [1,95].

3.2 Yq microdeletions

The identification of Yq microdeletions as a cause of spermatogenesis disruption has led in the last few years to a wide application of genetic testing for the detection of these rearrangements in infertile males, and specific guidelines for laboratory practices have been developed [96]. The search for Yq microdeletions is recommended in cases of severely impaired spermatogenesis, in particular before ICSI, in patients with normal karyotype, both to identify the etiology of the spermatogenesis disruption and to assess the prognosis for testicular sperm retrieval [97]. In fact, complete AZFa deletions and most AZFb deletions cause the impossibility to retrieve testicular sperm for ICSI, whereas ~ 60% of patients with AZFc deletion have sperms in the ejaculate or in the testis [18]. On the other hand, this analysis is not useful in normozoospermic males or even in patients with mild oligozoospermia (sperm count > 10×10^6 cells/ml) [3]. Genetic counselling after testing is an important point: patients with Yq deletion should be reassured about their condition and about the risk of transmission of the genetic abnormality to the male offspring, as no case of major abnormalities has been reported in > 30 children born by ICSI from Yq-deleted fathers [18]. As the association between male infertility and partial AZFc deletions is still controversial, genetic testing for these rearrangements in the diagnostic workup of couples undergoing ART is not recommended.

3.3 Screening of CFTR mutations

Genetic testing of the *CFTR* gene is mandatory in patients with CBAVD and in their female partners, in order to prevent the birth of a child affected by CF. In this view, a critical point is represented by the identification of both CFTR mutations in the CBAVD patient. In fact, CBAVD patient carriers of CF/CF^m or CF/5T genotypes, in the presence of a severe CFTR mutation in the female partner, have a 25% risk of generating a child with full-blown CF (**Table 3**). Thus, in CBAVD patients showing only a mild mutation or a 5T allele after the first level screening of the *CFTR* gene, a search for the second mutation using specific techniques such as denaturing high-performance liquid chromatography (DHPLC) screening of the entire coding region [98] or multiplex ligation-dependent probe amplification (MLPA) analysis for the detection of large CFTR deletions or duplications [99] should be carried out. On the contrary, in the presence of CF^m/CF^m, CF^m/5T or 5T/5T genotypes there is no risk of generating a child with full-blown CF with PI even in the presence of a severe CF mutation in the female partner (**Table 3**). However, in these cases 50% of the offspring will inherit a CF/CF^m or CF/5T genotype, resulting in variable phenotypes ranging from normal to CBAVD (in males) or atypical CF. Thus, genetic counselling for these couples represents quite a difficult issue [89]. It has been shown that the penetrance of the 5T allele is modified by adjacent coexisting TG repeat and M470V polymorphism in exon 10 (**Figure 2**) [100]. Therefore, analysis of these variants could improve genetic counselling and assessment of the risk of mild CF or CBAVD in the offspring [89].

In couples without CBAVD and not showing familiar history of CF, the genetic testing of the *CFTR* gene is recommended anyway owing to the very high frequency of healthy carriers in the population. In these cases, testing the most frequent CFTR pathogenic mutations in only one

Table 3. Risk of mild and full-blown CF in the offspring of a CBAVD patient and of a female healthy carrier of a severe CFTR mutation, based on the CFTR genotype of the CBAVD patient.

Genotype of the CBAVD patient	Genotype of the female partner	Possible genotypes in the offspring	Possible phenotypes in the offspring
CF/CF^{m}	CF/wt	25% CF/wt 25% CF^{m}/wt 25% CF^{m}/CF 25% CF/CF	Normal, healthy carrier Normal, healthy carrier Atypical CF with variable severity, PS Classic CF, PI
CF/5T	CF/wt	25% CF/wt 25% 5T/wt 25% 5T/CF 25% CF/CF	Normal, healthy carrier Normal, healthy carrier Variable, from normal to mild CF Classic CF, PI
CF^{m}/CF^{m}	CF/wt	50% CF^{m}/wt 50% CF^{m}/CF	Normal, healthy carrier Atypical CF with variable severity, PS
CF^{m}/5T	CF/wt	25% CF^{m}/wt 25% 5T/wt 25% 5T/CF 25% CF^{m}/CF	Normal, healthy carrier Normal, healthy carrier Variable, from normal to mild CF Atypical CF with variable severity, PS
5T/5T	CF/wt	50% 5T/wt 50% 5T/CF	Normal, healthy carrier Variable, from normal to mild CF

CF: Cystic fibrosis; PI: Pancreatic insufficiency; PS: Pancreatic sufficiency.

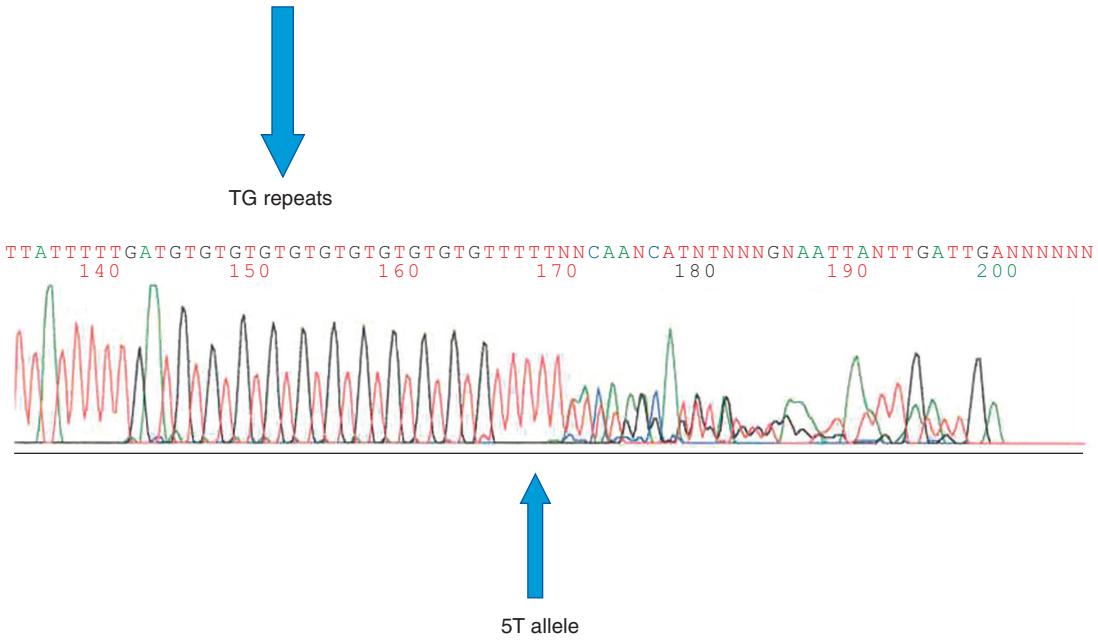

Figure 2. The TG repeats flanking the 5T sequence in the *CFTR* gene.

partner is sufficient in clinical practice. In fact, the negative result of a test with a 75 – 80% detection rate reduces the risk of being a CF carrier to 1/100, and the residual risk of generating a CF-affected child for a couple after the analysis of one partner only can be estimated in 1/10000 [89].

3.4 Other genetic testing

As 2% of infertile males are carriers of mutations of the *AR* gene, the usefulness of mutation screening of this gene in male infertility should be considered. However, screening of the entire *AR* gene coding region is expensive and time-consuming. Previous guidelines had suggested that analysis of the *AR* gene should be limited to selected cases, such as patients with high androgen sensitivity index (ASI) [3]. Nevertheless, more recent reports showed that no clear hormonal or clinical data can be used to preselect patients at higher risk of mutation [90]. As a consequence, the application of genetic screening of the *AR* gene in the clinical practice is still debated.

Analysis of the *FMR1* gene should be carried out in women with unexplained POF, to identify the presence of an *FMR1* pre-mutation segregating in the family of the patient, with a high risk for fertile carriers in the family of transmitting a full *FMR1* to the offspring and generating a child with mental retardation.

All the above-described conditions are characterized by the presence of a constitutional genetic abnormality in the couple, leading to an increased risk of a genetic disease in the offspring. However, it has been shown that other conditions, such as the presence of increased sperm aneuploidies, could play an important role in the determination of the genetic risk for the offspring [6,19], suggesting that sperm FISH analysis should be included in preliminary tests given to infertile couples. Nevertheless, no clear indication for the use of this test as a routine analysis has been provided so far.

4. Expert opinion

The widespread use of ART for the treatment of infertility of couples has raised the question of the risk of transmission to the offspring of genetic defects underlying the condition of infertility and of the need for genetic testing to prevent the birth of affected children. However, genetic testing is a multidisciplinary approach composed not only of laboratory analysis, but also of appropriate counselling carried out by certified genetic counsellors. Although great attention has been given to the laboratory part of genetic testing in couples undergoing ART (e.g., the number of Yq loci to test for the screening of AZF deletions, the number of mutations to analyze for the screening of the CFTR gene etc.), less care has been devoted to genetic counselling, as demonstrated by the fact that one-third of the genetic testing centers surveyed recently were not linked to clinical genetics services [1]. Genetic counselling should be performed in couples undergoing ART before and after any laboratory genetic analysis. In pre-testing counselling, based on the medical and family histories of both partners, a genetic counsellor can assess the specific genetic risks to a pregnancy and suggest the appropriate genetic test that should be carried out to prevent the birth of an affected child. The pre-testing information to the couple should enable decision-making, allowing the patients to decide whether to submit or not to the suggested tests before treatment. Nevertheless, couples enrolled in ART protocols are often asked to undergo genetic testing in the context of a list of routine analysis, without any specific information about the aims and the benefits of the test. This kind of approach to the genetic testing of infertile couple is not acceptable. For each couple, the appropriate genetic testing should be selected based on the cause of the infertility. For a male factor, with a poor sperm count in the male partner, clinical and laboratory data can indicate a testicular, pre-testicular or post-testicular origin of the disease. In the first case, cytogenetic investigation is the first-choice approach, being able to display numerical and structural chromosome abnormalities responsible of the spermatogenesis failure. In the presence of a normal karyotype, genetic testing for Yq microdeletions should be carried out, but only in patients showing <2 million sperm per milliliter in their ejaculate. With a pre-testicular origin of male infertility, screening for mutations of the *AR* gene could be considered, although the limits of this test have been discussed previously. Finally, with a post-testiscular origin of the disease, with a documented obstructive azoospermia, testing for the CFTR is mandatory.

When the infertility of the couple is ascribable to the female partner, a high proportion of cases are due to chromosomal defects, single-gene defects being rare causes of infertility [101]. Although in the future specific testing will probably be developed to detect other potential genetic defects that may be clinically important, at present karyotype and FMR1 testing in POF cases are the only tests to perform in infertile females.

In many cases the infertility of the couple is not related to the presence of damage in the gametogenesis of one or both the partners. In these cases, it is possible anyway to perform genetic testing for diseases not related to the infertility of the couple but showing a high incidence in the population. In recent years, new techniques able to provide a fast, efficient and low-cost screening of the most common genetic diseases have been developed. Thus, the condition of healthy carriers of several recessive and X-linked disorders can easily be investigated in the couple, based on the information provided by a careful examination of the family history of both partners. However, in the absence of well-documented previous cases of genetic diseases in the pedigree of the couple, the usefulness of screening for the most common genetic diseases is debatable, and the cost/benefit ratio should always be considered. Although so far there is general agreement only about the opportunity to carry out *CFTR* gene analysis, in the future genetic testing for other diseases could be considered. An example is spinal muscular atrophy (SMA) (OMIM #253300), a very severe disease

showing a high frequency of healthy carriers in the population (1:50). As with the use of new molecular approaches > 95% of mutations responsible for this disease can de detected by a single test [102], screening of SMA could in future be offered to couples undergoing ART.

In post-testing counselling, the result of the genetic test should be communicated by the counsellor to the couple and it should be discussed together, in order to specify the risk of the offspring being affected by a genetic disease. A crucial point is represented by a clear and detailed description of the disease that could be transmitted to the offspring. In some cases, such as the presence of a balanced translocation in one partner or a classic CTRF mutation in both partners, the offspring could be affected by a severe disease, but in other cases, such as the presence of a sex chromosome trisomy or a Yq microdeletion, the genetic defect will not cause major malformation in the offspring, the only relevant clinical sign being represented by infertility. As a consequence, the genetic counselling of these conditions must be appropriate. Prenatal diagnosis should not invariably follow the detection of a genetic defect. What is the rationale in exploring by prenatal diagnosis the presence of a Yq microdeletion in the male fetus of an infertile male carrier of the same genetic abnormality, as no major malformation is associated with deletions of the AZF loci?

In many cases, the consequence of the identification of a genetic mutation within a couple undergoing ART is the need for further counselling and testing for other family members. In fact, when a severe CFTR mutation or a FMR1 pre-mutation are detected by genetic testing in an infertile couple, the carrier subject should be told that other members of his/her family are at high risk of being healthy carriers of a genetic disease and generating an affected child even with natural fertilization. This is another crucial point of genetic counselling, as the involvement of other members of the family in genetic testing is generally unexpected, causing further stress for the couple.

In the presence of a negative result of genetic testing, couples should be told that, despite the testing procedures, absolutely safe germ cells do not exist, as only a limited number of genetic diseases can be detected and other factors also may affect the outcome. However, this communication must be provided in a way to avoid generating stress in the couple, and it must be specified that a basic genetic risk is also present in natural reproduction.

In conclusion, genetic testing in couples undergoing ART should not be considered as a simple laboratory analysis. Genetic testing involves delicate issues for the couple, such as the risk of generating an affected child, and the emotional impact of different treatment options (e.g., prenatal diagnosis), including anxiety and ethical questions [1]. Great attention should be devoted to the genetic counselling of the couple, before and after analysis. Genetic testing should be performed on the basis of the specific risk shown by each couple and evaluated by genetic counselling, and not as a routine investigation to be carried out in all couples undergoing ART. Finally, in some circumstances, the presence of other healthcare professionals such as a psychologist for emotional support should be considered.

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Declaration of interest

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Affiliation

Liborio Stuppia[†1,2,3] MD, Valentina Gatta[1,2], Ivana Antonucci[1,2], Rossella Giuliani[1], Anna Paola Scioletti[2,4] & Giandomenico Palka[1,5]
[†]Author for correspondence
[1]G. d' Annunzio University, Department of Biomedical Sciences, Via dei Vestini 35 66013, Chieti-Pescara, Italy
Tel: +39 08713554137; Fax: +39 08713554135; E-mail: stuppia@unich.it
[2]Ce.S.I., G. d'Annunzio University Foundation, Ageing Research Center, Via dei Vestini 31, 66013 Chieti, Italy
[3]I.G.M.-C.N.R., Unit of Bologna, c/o IOR, Via di Barbiano 1/10, 40136 Bologna, Italy
[4]G. d'Annunzio University, Department of Clinical Sciences and Bioimaging, Via dei Vestini 31, 66013 Chieti, Italy
[5]Pescara Hospital, Human Genetic Division, Via Renato Paolini 47, 65100, Pescara, Italy