The evolving role of genetic tests in reproductive medicine

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Abstract

Infertility is considered a major public health issue, and approximately 1 out of 6 people worldwide suffer from infertility during their reproductive lifespans. Thanks to technological advances, genetic tests are becoming increasingly relevant in reproductive medicine. More genetic tests are required to identify the cause of male and/or female infertility, identify carriers of inherited diseases and plan antenatal testing. Furthermore, genetic tests provide direction toward the most appropriate assisted reproductive techniques. Nevertheless, the use of molecular analysis in this field is still fragmented and cumbersome. The aim of this review is to highlight the conditions in which a genetic evaluation (counselling and testing) plays a role in improving the reproductive outcomes of infertile couples. We conducted a review of the literature, and starting from the observation of specific signs and symptoms, we describe the available molecular tests. To conceive a child, both partners’ reproductive systems need to function in a precisely choreographed manner. Hence to treat infertility, it is key to assess both partners. Our results highlight the increasing importance of molecular testing in reproductive medicine.

Keywords: Genetic tests, Reproductive medicine, Male infertility, Female infertility, Assisted reproductive technology

Introduction

During the last few decades, there have been a series of striking advancements in reproductive and laboratory medicine that have essentially caused these two fields to become inextricably connected. Laboratory medicine now plays a critical role in all stages of the reproductive process, from diagnostic approaches to the choice of the most complex therapy.

In particular, genetic tests are carried out for three main purposes in reproductive medicine: the identification of the infertility causes, identification of genetic diseases transmissible to offspring, and optimization of the assisted reproductive technology (ART) (Fig. 1).

The overall fertility rate is decreasing; for example, in the US, 12% of women receive fertility treatment over the course of their lifetimes, so it is important to emphasize the fertility journey of couples [1]. The reproductive systems of both partners function in a combined and precisely coordinated way to conceive a child; for this reason, evaluation of both members of the couple is mandatory.

A medical evaluation is indicated when the couple fails to achieve pregnancy after 12 months of regular, unprotected sexual intercourse [2]. Currently, the diagnostic timeline of infertile couples includes biochemical and instrumental analyses that allow for a diagnosis in 65% of cases; in the remaining 35% of cases, which are undiagnosed, genetic tests are performed. Considering that approximately 15% of genetic disorders are associated with infertility and that similar clinical signs can have genetic and nongenetic causes, it is important that an infertility diagnosis be determined by the combination of an accurate medical history and instrument- and laboratory-based evaluations, including targeted genetic tests [3]. Confirmation of the clinical diagnosis through genetic evaluation (counselling and testing) can lead to more specific and targeted medical management.

In addition, genetic tests are also indicated for the identification of genetic diseases that are transmissible to the offspring: preconception screening allows couples who are planning to become pregnant to know...
their reproductive risk a priori. Normally, gametes with genetic or chromosomal alterations have reduced reproductive potential. Thanks to ART, many of these difficulties can be overcome, and therefore, genetic tests (carrier screening, preimplantation and prenatal diagnosis) have the crucial impact of monitoring the possible transmission of these genetic alterations to the offspring \[4, 5\]. Another application field of molecular diagnostics is related to the antenatal diagnosis. To date, the diagnostic options for couples at risk of transmitting a specific
Genetic tests in the identification of the causes of infertility

It has been estimated that every healthy subject is a carrier of 5/8 genetic alterations associated with recessive genetic disorders; therefore, even in the absence of specific symptoms, family planning and reproduction can be risky. Moreover, it has been reported that almost 50% of infertility cases are related to genetic disorders [7, 8]. In the presence or high suspicion of a genetically based reproductive risk, the genetic test provides a more accurate diagnosis of infertility and provides the opportunity to inform the couple about the possible risk of transmission to the offspring. Unfortunately, genetic tests for examining infertility are based on a standard algorithm directed to investigate the most frequent genetic causes without taking into account the patient’s personal or family history. Consequently, the results are quite discouraging: a genetic diagnosis is reached in approximately 4% of all infertile males, and approximately 20% of infertile couples remain undiagnosed. In contrast, an accurate medical and familial history aimed at identifying genetically based syndromes (characterized by typical dimorphisms, associated disabilities and even infertility) could direct patients to specific genetic tests [9].

Starting from this consideration, we examined the genetic disorders related to male and female infertility and subdivided them according to the signs and symptoms observed by the specialist during the first medical examination. Genes associated with specific and rare clinical conditions were not excluded either; they could be useful, in the context of a specific clinical picture, to request an in-depth analysis using a targeted genetic test. Therefore, we provide the main points on the genetic pathology, current test execution modality and management of the patient (Tables 1, 2, 3, 4, 5, 6, 7).

Materials and methods

The literature review was carried out according to PRISMA guidelines. No temporal restrictions were applied. The research was performed using the following keywords: genetic cause of infertility, genetic cause of male infertility, genetic cause of female infertility, mutations and infertility, molecular diagnostics in infertile couples, molecular diagnostics and reproductive medicine, PGT techniques, and PGT and ART. All the papers found were carefully read and evaluated before their inclusion. No unpublished studies were taken into consideration. In addition, the following databases were also consulted to verify gene/phenotype associations: NIH (https://www.nih.gov), OMIM (https://www.omim.org) and OrphaNet (https://www.orpha.net/consor/cgi-bin/index.php).

Results

Genetic tests in the identification of the causes of infertility

Male genetic infertility

Genetic factors have been found in all the etiological categories of male fertility (pre-testicular, testicular and post-testicular): OMIM (Online Mendelian Inheritance in Man) reports more than 200 genetic conditions related to male infertility, ranging from the most common clinical presentations of infertility to the rarest complex syndromes in which signs and symptoms are beyond the reproductive problems [103]. In most cases, infertility is only one of the clinical signs of a complex syndrome; on the contrary, in some genetic conditions, infertility is the main phenotypic feature. Moreover, it is important to monitor these infertile patients over time because a greater morbidity and a lower life expectancy have been described for these infertile patients than for the general population and are most likely caused by the genetic abnormalities involved in male sterility [104].

Today, the presence of alterations in the spermogram is the first indication for genetic tests, particularly in cases of severe oligospermia (<5 million/ml) (further parameters are hormonal levels, malformations, recurrent abortions, and family history) [105]. Interestingly, a recent study by Oud et al. highlighted how the number of genes that are definitively linked to the more common phenotypes of oligozoospermia or azoospermia remains limited (50%); the other half are genes involved in teratozoospermia, although the monomorphic forms of teratozoospermia are extremely rare [106].

Genetic disorders related to male infertility include whole chromosomal aberrations (structural or numerical), partial chromosomal aberrations (i.e., microdeletions of the Y chromosome) (listed in Tables 1, 2) and monogenetic diseases (listed in Tables 3, 4, 5). In particular, abnormalities in sex chromosomes have a greater impact on spermatogenesis, while mutations affecting autosomes
Table 1 The chromosome aberrations related to testicular male infertility: from the first observation to the report

| Indications for genetic test | Genetic condition | Frequency | Test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|----------------------------|-------------------|-----------|------|--------------------------------|-----|-------------|---------------|-----------------------|-------|
| Azoospermia/oligozoospermia; Sertoli cell syndrome type I and type II (presence of some tubules with normal spermatogenesis) and hypospermato genesis diagnosis by histological evaluation | Microdeletion Y chromosome AZFc | 1/2.500; (AZFc 60%, AZFb 15%, AZFb-c 2%, AZFa 3%); 13% of azoospermia cases; 3–7% of oligozoospermia cases | Molecular diagnosis by PCR of STS sequences |Interstitial deletion of AZFc Y region (recombination between palindromes b2 and b4); DAZ, BPY2, PRY2, CDY1 | ✓ | testicular sperm retrieval + ICSI | Y linked | ✓ | Other causes of azoospermia or oligozoospermia | [10] |
| Azoospermia; spermatogenesis arrest by histological evaluation | Microdeletion Y chromosome AZFb | | |Interstitial deletion of AZFb Y region (deletions PS/proximal-P1); RBMY, CDY, HSFY, PRY | | | | | | |
| Azoospermia | Microdeletion Y chromosome AZFb-c | | | | | | | | |
| Azoospermia; Sertoli cell syndrome type I (diagnosis by histological evaluation (i.e., complete absence of germ cells in seminiferous tubules) | Microdeletion Y chromosome AZFa | | |Deletion of AZFa Y (recombination between HERV15yq1 and HERV15yq2) | | | | | |

Database sources: NIH, OMIM and OrphaNet

AZF, azoospermia factor; ✓, yes; x, no; NA, not applicable; donor, heterologous fertilization with sperm donor; STS, sequence tagged sites
| Indications for genetic test | Genetic condition | Frequency | Test | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|----------------------------|-------------------|-----------|------|-----|-------------|---------------|----------------------|-------|
| Hypergonadotropic hypogonadism, ↑FSH ↓LH ↑, azoospermia, oligozoospermia; small testes, infertility, gynecomastia; neurocognitive deficits; metabolic syndrome, type 2 diabetes. Approximately 10% of these subjects have spermatozoa in the ejaculate, and in 30–50% of cases there is intratesticular spermatogenesis. | Klinefelter's syndrome | 1/660 newborns; >5% in severe oligozoospermia, 10% in azoospermia | Karyotype | 47,XY (85–90%); 46,XY/47,XY mosaicism (6–7%); 46,XX/47,XY or multiple X aneuploidy (3–8%); 47,XXY (85–90%) | Testicular sperm retrieval + ICSI | De novo mutation | NA | 46,XX testicular DSD [11, 12] |
| Nonsyndromic 46,XX Testicular Disorders of Sex Development (De la Chapelle syndrome) | | 1/20,000; 0,9% in azoospermia; 1–3% normospermia | FISH or CMA | SRY+ XX (80–90%); SRY− XX (<10%); CNV or rearrangements in SOX9, SOX3, RSPO1 and WNT4 (rare) | Testicular sperm retrieval; ✓ heterologous fertilization | AD | ✓ | Syndromic forms of 46,XX testicular DSD; 45X/46,XY; 47,XXY; 46,XX; sex chromosome mosaics; Prenatal exposure of 46,XX fetuses to androgens [13] |
| Short stature; gynecomastia; male external genitalia; small testes, cryptorchidism, hypospadias, infertility; ↑FSH ↑LH ↓T, azoospermia/oligozoospermia | Penoscrotal hypospadias, cryptorchidism, infertility; ↑FSH ↑LH ↓T, azoospermia/oligozoospermia | | | | | | | |
| Short stature; small testes, infertility; ↑FSH ↑LH ↓T, azoospermia/oligozoospermia | | | | | | | | |

**Table 2** The chromosome aberrations related to pretesticular male infertility: from the first observation to the report.
### Table 2 (continued)

| Indications for genetic test | Genetic condition | Frequency | Test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|-------------------|-----------|------|--------------------------------|-----|-------------|----------------|------------------------|-------|
| Tall stature, delayed development of speech, language or motor skills, autism spectrum disorder, hypotonia, motor tics, clinodactyly, scoliosis, attention deficit hyperactivity disorder, ↑FSH normal or ↓T; from normal to azoospermia; from 0.57 to 77.8% sperm mosaicism, a- or hyperdiploidy | Double Y syndrome (Jacobs syndrome) | 1/1,000; 0.4% in oligozoospermia | Cytogenetics tests | 47,XY; 46,XY/47,XYY mosaics | ✓ | MF or ICSI in case of oligospermic patients | ✓ | 46,XY | [14, 19] |
| Subfertility or uneventful andrological history; oligozoospermia | Balanced structural chromosome aberrations | 5% of infertile men | FISH | t(SRY; X); del(13, 14); del(4, 21); del(14, 15) | ✓ | NA | ✓ | PGT | Other causes of oligozoospermia | [20] |

Database sources: NIH, OMIM and OrphaNet

✓, yes; x, no; NA, not applicable; ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; FISH, fluorescence in situ hybridization; PGT, preimplantation genetic testing; CMA, chromosomal microarray analysis
Table 3 The genetic causes related to pretesticular male infertility: from the first observation to the report

| Genetic disorder | Frequency | Genetic test | Genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis |
|------------------|-----------|--------------|---------------------|-----|-------------|---------------|------------------------|
| Lack of puberty; micropenis, cryptorchidism; prepubertal testicular volume, absence of secondary sexual features, decreased muscle mass, diminished libido, erectile dysfunction, infertility, low testosterone, estradiol | Prevalence: 1/30,000; incidence: 1/8000 | Molecular diagnosis | ANOS1 CHD7, FGFR1, FGFR8, SOX10 FEZF1, PROK2, PROKR2 | ✓ | X-linked | ✓ | Syndromes associated with hypogonadotropic hypogonadism |
| Obesity, retinitis pigmentosa, postaxial polydactyly, kidney dysfunction, behavioral dysfunction, infertility | 1:100,000 North America; 1:160,000 Switzerland; 1:17,500 Newfoundland; 1:13,500 Bedouin, Kuwait | Multigene panel | From BBS1 to BBS19 | ✓ | AR | ✓ | McKusick–Kaufman syndrome (MKS) |
| Adrenal insufficiency; cryptorchidism; delayed puberty; infertility | 1:12,500 | Molecular diagnosis | NR0B1 | ✓ | X-linked recessive pattern | ✓ | 21-hydroxylase deficiency, 11-hydroxylase deficiency |
| Diabetes mellitus, hypothyroidism, alopecia totalis, long, triangular face, hypertelorism; dystonia, dysarthria, dysphagia; infertility | Unknown | Molecular diagnosis | DCAF17 | ✓ | AR | ✓ | Perrault syndrome; Deafness and hereditary hearing loss; Gonadotropin-releasing hormone deficiency |
| Adult-onset neurodegenerative disorder; hypogonadotropic hypogonadism | Unknown | Molecular diagnosis | RNF216, PNPLA6 | ✓ | AR | ✓ | Cerebellar ataxia |
Table 3 (continued)

| Main indications for genetic test | Hypogonadotropic hypogonadism (CHH) | Genetic disorder | Frequency | Genetic test | Genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|----------------------------------|--------------------------------------|------------------|-----------|--------------|--------------------|-----|-------------|----------------|-------------------------|-------|
| Cirrhosis, diabetes, cardiomyopathy, arthritis, skin hyperpigmentation; elevated serum transferrin-iron saturation (TS); elevated serum ferritin concentration; infertility | Hemochromatosis (Hemochromatosis Type 1, HFE-Associated Hemochromatosis, HFE-HH) | 2–5:1000 northern European ancestry; 1:200–400 non-Hispanic whites, North America | Gene-targeted or molecular diagnosis | HFE (typically p.Cys282Tyr and p.His63Asp can be performed first) | ✓ | AR | NA | Rarer primary iron overload disorders and secondary iron overload disorders | [31–34] |
| Azoospermia/oligozoospermia; ↑ LH, normal T, hyperandrogenism; feminization of the external genitalia at birth, abnormal secondary sexual development in puberty, and infertility | Androgen insensitivity syndrome (AIS) | 2–5:100,000 | Screening for AR mutations (>300) | AR | Donor | X-linked recessive | NA | MRKH syndrome; Hypospadias; MAIS; Undermasculinization of external genitalia and pubertal undervirilization | [35, 36] |
| Glucocorticoid and mineralocorticoid deficiencies; hypospadias; ambiguous genitalia, infertility | 3-β-hydroxysteroid dehydrogenase (HSD) deficiency | Unknown | Molecular diagnosis | HSD3B2 | Donor | AR | NA | Ambiguous genitalia | [37] |
| Main indications for genetic test | Hypogonadotropic hypogonadism (CHH) |
|---------------------------------|-------------------------------------|
| Other indications for genetic test | Genetic disorder | Frequency | Genetic test | Genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
| Deficiencies in GH, TSH, LH, FSH, PrL, and ACTH; hypothyroidism; neonatal hypoglycemia; micropenis without hypospadias, with or without cryptorchidism; short stature and delayed bone maturation; absent/delayed/incomplete secondary sexual development; infertility | PROP1-related combined pituitary hormone deficiency | 1:4000 in England and the US | Molecular diagnosis | PROPI | ✓ | AR | ✓ | CPHD; isolated growth hormone deficiency; isolated hypogonadotropic hypogonadism | [38, 39] |
| Ambiguous genitalia or external genitalia that appear female; micropenis and hypospadias; not much facial or body hair; infertility | 5-Alpha reductase deficiency familial incomplete male pseudohermaphroditism, type 2 | Unknown | Molecular diagnosis | SRD5A2 | ✓ | AR | ✓ | Ambiguous genitalia | [40–42] |

Database sources: NIH, OMIM and OrphaNet
✓, yes; x, no; NA, not applicable; donor, heterologous fertilization with sperm donor
| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|------------------|-----------|--------------|--------------------------------|-----|-------------|---------------|----------------------|-------|
| Maldeveloped testes         | Cryptorchidism   | 2% 20% of infertile men; 30/80% of azoospermia | Molecular diagnosis | INSL3; LGR8 | ✓ | AD | ✓ | Hypogonadotropic hypogonadism; Noonan and Prader–Willi syndrome | [43, 44] |
| Hypertension, hypokalemic alkalosis, lack of secondary sexual characteristics, testicular feminization | 17 alpha(α)-hydroxylase/17,20-lyase deficiency | 1 in 1 million | Molecular diagnosis | CYP17A1 | Donor | AR | NA | Ambiguous genitalia | [45] |
| Severe muscular hypotonia, genital hypoplasia, incomplete pubertal development, infertility; cryptorchidism (93%); obesity, mental retardation (late onset) | Prader–Willi syndrome (PWS, Prader–LaBhart–Willi syndrome) | 1:10,000 to 1:30,000 | DNA methylation testing; cytogenetic/FISH/chromosomal microarray findings: deletion in bands 15q11.2-q13 (70%) | 15q11.2 region | Donor | Paternal deletion; maternal uniparental disomy15 | ✓ | Cryptorchidism; craniopharyngioma | [21, 46–48] |
| Short stature, facial dysmorphism, congenital heart defects, skeletal defects, webbed neck, mental retardation, bleeding diathesis; early onset | Noonan syndrome-1 (NS1) | 1:1000–2500 | Gene sequencing starting with PTPN11, followed by SOS1, KRAS and RAF1 | PTPN11 (> 50%), SOS1 (10–15%), KRAS (5%), RAF1 (3–17%) | ✓ | AD | ✓ | Turner syndrome; cryptorchidism; azoospermia | [49] |
| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|------------------|-----------|--------------|--------------------------------|-----|-------------|---------------|------------------------|-------|
| Gonadal dysgenesis, ambiguous genitalia, infertility; increased risk of Wilms tumor | Denys–Drash syndrome | Unknown | Molecular diagnosis | WT1 | – | AD | ✓ | Frasier syndrome | [50, 51] |
| Atrophy of the abdominal muscles, malformations of the urinary tract | Prune–belly syndrome (other names Syndrom of Eagle–Barret; syndrome of Obrinsky) | 1/35,000 and 1/50,000 births and 95% of cases occur in males | Molecular diagnosis | CHRM3 | NA | – | NA | Megacystis/ megaureter or posterior urethral valves | [52, 53] |
| Osteoporosis; hyperglycemia; ambiguous genitalia | Aromatase deficiency | Unknown | Molecular diagnosis | CYP19A1 | ✓ | AR | ✓ | Other condition of estrogen deficiency | [54, 55] |
| Proportionate short stature, delayed closure of fontanelles, prominent forehead, drooping shoulders, abnormal dental development; early onset | Cleidocranial dysplasia | 1:1,000,000 | Molecular diagnosis | RUNX2 (CBFA1) | ✓ | AD; de novo pathogenic variant | ✓ | Pycnodysostosis; mandibulo-ocular dysplasia; CBFB | [21, 56] |

Syndromic without maldescended testes:

| Short stature, telangiectatic erythematous skin lesions, high risk for malignancies; early onset; azoospermia or severe oligospermia | Bloom’s syndrome (Bloom–Torre–Machacek syndrome) | Rare disorder | Molecular diagnosis | BLM | ✓ | AR | ✓ | REOQ-mediated genome instability; Ataxia–telangiectasia; Fanconi anemia; Nijmegen breakage syndrome; Werner syndrome | [21, 57] |
| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|-------------------|-----------|--------------|-------------------------------|-----|-------------|---------------|-----------------------|-------|
| Short stature, macrocephaly, distinctive face (small, triangular face with prominent forehead, narrow chin, small jaw), delayed development, speech and language problems, learning disabilities; digestive system abnormalities; micropenis; early onset | Russell–Silver syndrome | Prevalence: unknown; estimated incidence ranges from 1 in 30,000–1 in 100,000 people | Methylation | Methylation involving H19 and IGF2 | ✓ | Sporadic, uniparental disomy | Usually not possible | Intrauterine growth retardation and short stature | [21, 58, 59] |
| Keratoconus, glaucoma, and myopia as well as from malformations of the brain, skeleton, and kidney; impairment of respiratory functions; infertility (asthenozoospermia and abnormal flagellar morphology) | Primary ciliary dyskinesia (PCD) | Prevalence: 1:16,000, 1:400 in a Volendam population residing in a fishing village of North Holland | Molecular diagnosis | DNAH5 (30%), DNAI1 (10%) and TXNDC3, DNAH11, DNAI2 (rare); 60% gene loci unknown | ICSI | AR | ✓ | Chronic sinusitis and bronchiectasis | [21, 60] |
| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|------------------|-----------|--------------|-------------------------------|-----|-------------|---------------|--------------------|-------|
| Multisystem disorder affecting the skeletal and smooth muscles, the heart, the eyes, and the endocrine and central nervous systems. Mental retardation; infertility | Myotonic dystrophy 1 (Morbus Curretchmann–Steinert, Dystrophia myotonica 1, DM1) | 1 in 8000 | Molecular diagnosis of the CTG repeat expansion in the DMPK gene (> 50 CTG repeats result in DM1) | DMPK | ✓ | AD | ✓ | Prader–Willi syndrome, nemaline myopathy, X-linked centronuclear myopathy; DM2; Hereditary distal myopathies; Hereditary myotonia | [21, 61] |
| Bone marrow failure, hypopigmentation, short stature, physical abnormalities, organ defects (gastrointestinal abnormalities; heart defects; and eye abnormalities; malformed ears and hearing loss; increased risk of certain cancers; and malformations of the reproductive system and infertility) | Fanconi anemia | 1 in 160,000 (more common among people of Ashkenazi Jewish descent, the Roma population of Spain, and black South Africans) | Molecular diagnosis | FANCA, FANCC, and FANCG (90%) | NA | AR; AD/ RAD51-related FA; X-linked: FANCB-related FA | ✓ | Bloom syndrome; ataxia–telangiectasia, NBS; Seckel syndrome; neurofibromatosis 1 | [62, 63] |
| Nonsyndromic infertility | Globozoospermia (spermatogenic failure 9) | Rare (165,000); common in North Africa. 1:100 cases of male infertility | Molecular diagnosis of DPY19L2, followed by SPATA16 | DPY19L2 homozygous deletion; point mutations; SPATA16 | ✓ | AR | ✓ | Spermatogenic failure | [64, 65] |
Table 4 (continued)

| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|------------------|-----------|--------------|-------------------------------|-----|-------------|---------------|----------------------|-------|
| Abnormal sperm cells (abnormally large and misshapen heads, contains extra chromosomes; multiple flagella, most often four) and infertility | Macrozoospermia (spermatogenic failure 5) | Unknown; 1:10,000 males in North Africa | Molecular diagnosis | AURKC mutations (c.144delC, 85%; p.Y248, DR 13%) | Donor | AR | NA | Spermatogenic failure | [66–68] |
| Primary infertility; multiple morphological abnormalities of sperm flagella (absent, short, coiled, bent, and irregular flagella); asthenozoospermia | Multiple morphological abnormalities of the sperm flagella (spermatogenic failure 18) | Unknown | Molecular diagnosis | DNAH1 mutation (c.8626-1G > A; c.3860 T > G) | ✓ ICSI | AR | NA | Ciliary dyskinesia primary | [69] |
| Genital abnormalities; hypoplasia of Leydig cells; micropenis, hypospadias, bifid scrotum, ambiguous genitalia | Leydig cell hypoplasia (hypergonadotropic hypogonadism due to LHCGR defect) | Unknown | Molecular diagnosis | LHCGR | Donor | AR | ✓ | Hyponadotropic hypogonadism | [70, 71] |
| Asthenozoospermia; absence of any other symptoms | CATSPER-related nonsyndromic male infertility | Unknown | Molecular diagnosis | CATSPER1, GALNTL5 | Donor | AR | ✓ | Male infertility | [69, 72, 73] |
Table 4 (continued)

| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|------------------|-----------|--------------|-------------------------------|-----|-------------|------------------|------------------------|-------|
| Normal general physical examination, absence of clinical findings involving other organ systems typical female external genitalia, uterus and fallopian tubes normally formed, gonadal dysgenesis; skeletal abnormalities, campomelic dysplasia | Swyer syndrome (46,XY complete gonadal dysgenesis) | 1 in 80,000 | Molecular diagnosis | SRY (15%), MAP3K1 (18%), DHH and NR5A1 (rare) | ART | De novo; rare AD | ✓ | Ambiguous genitalia and/or sex chromosome-phenotype discordance | [13] |
| Asthenozoospermia; hearing loss | Deafness-infertility syndrome (DIS) | Unknown | CMA/array-CGH | Homozygous deletion at 15q15.3 including CAT-SPER2, STRC | Donor | AR | ✓ | DFN16 | [74, 75] |
| Nonobstructive azoospermia | Small testes and infertility, with severe oligozoospermia or nonobstructive azoospermia due to maturational arrest at the primary spermatocyte stage | Meiotic arrest at primary spermatocyte stage (spermatogenic failure 25) | Molecular diagnosis | TEX11 | Donor | X-linked | NA | Spermatogenic failure | [76–78] |
| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|------------------|-----------|--------------|-------------------------------|-----|-------------|-----------------|------------------------|-------|
| Nonobstructive azoospermia, infertility, testicular biopsy showing absence of spermatogenic cells and a Sertoli cell-only pattern | Spermatogenic failure 32 | Unknown | Molecular diagnosis | SOHLH1 | Donor | AD | NA | Spermatogenic failure | [79] |
| Azoospermia; testicular histology showing arrest of spermatogenesis at the pachytene stage of primary spermatocytes | Spermatogenic failure 4 (SPGF4) | 1% | Molecular diagnosis | SYCP3 (COR1 RPRG14 SCP3 SPGF4) | Donor | AD | NA | Spermatogenic failure | [80] |
| Azoospermia or oligozoospermia | Spermatogenic failure, Y-linked 2 | Unknown | Molecular diagnosis | RBMY1A1, DAZ1–4 | Donor | Y-linked | NA | Spermatogenic failure | [81, 82] |

Database sources: NIH, OMIM and OrphaNet

✓, yes; ×, no; NA, not applicable; donor, heterologous fertilization with sperm donor; AOA, assisted ovarian activation; CMA, chromosomal microarray analysis
Table 5 The genetic causes related to posttesticular male infertility: from the first observation to the report

| Main indications for genetic test | Obstructive azoospermia or severe oligospermia | Genetic disorder | Frequency | Genetic test | Genetic alteration | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|----------------------------------|-----------------------------------------------|------------------|-----------|--------------|--------------------|-----|-------------|---------------|----------------------|-------|
| Abnormalities of seminal vesicles or absence of vas deferens; normal testicular development and function; normal spermatogenesis; a low volume of ejaculated semen with a specific profile (volume < 1.5 ml, pH < 7.0, elevated citric acid concentration, elevated acid phosphatase concentration, low fructose concentration, and failure to coagulate) | Congenital bilateral absence of the vas deferens (CBAVD) | 25–1–2% in infertility | Screening for CFTR mutations | Two CFTR pathogenic variants identified (46%); one CFTR pathogenic variant identified (79%) | ✓ ICSI AR ✓ | ✓ | | | Young syndrome; Hereditary urogenital dysplasia | [83–85] |
| Multisystem disease affecting epithelia of the respiratory tract, exocrine pancreas, intestine, hepatobiliary system, and exocrine sweat glands; obstructive azoospermia and male infertility | Cystic fibrosis | 1:3200; CF occurs with lower frequency in other ethnic and racial populations (1:15,000 African Americans, and 1:31,000 Asian Americans) | Screening for CFTR mutations | Two CFTR pathogenic variants identified | ✓ ICSI AR ✓ | ✓ | | | Asthma; congenital airway anomalies; primary ciliary dyskinesia; Shwachman–Diamond syndrome; Bronchiectasis with or without elevated sweat chloride; Isolated hyperchlorhidrosis; Congenital bilateral absence of the vas deferens (CBAVD) | [84, 86] |

Database sources: NIH, OMIM and OrphaNet
✓, yes; x, no; NA, not applicable; ICSI, intracytoplasmic sperm injection
### Table 6 The genetic causes related to ovarian female infertility: from the first observation to the report

| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|------------------|-----------|--------------|-------------------------------|-----|-------------|----------------|-----------------------|-------|
| POI                         | Short stature, skeletal abnormalities, kidney problems, webbed neck, lymphedema; ovarian hypofunction or premature ovarian failure, infertility | Turner (45,X) (other names monosomy X, TS) | 1 in 2500 | Karyotype | Monosomy X: 45,X0 | ✓-donor | Not inherited | NA | POF | [87] |
| Asymptomatic (only 10% of individuals with trisomy X are actually diagnosed); tall stature, epicanthal folds, hypotonia and clinodactyly; renal and genitourinary abnormalities; psychological problems | Trisomy X | 1/1000 | Karyotype | 47XXX or mosaic | ✓ | NA | ✓ | | |
| Irregular menstrual cycles, early menopause, premature ovarian failure, infertility | Fragile X-associated primary ovarian insufficiency (primary ovarian failure 1) | 1 in 200 (4/6% of all cases of POI) | Molecular diagnosis of premutations in the FMR1 gene on chromosome Xq27.3 (CGG segment is repeated 55 to 200 times) | FMR1 gene | ✓-donor | X-linked | ✓ | POF | [88] |
| Hypogonadotropic hypogonadism; hypotonia, poor feeding, vomiting, weight loss, jaundice; impaired growth; cognitive deficit and cataracts | Galactosemia (galactose-1-phosphate uridyltransferase deficiency) | prevalence unknown; incidence 1/40,000-60,000 | Molecular diagnosis | GAUT, GALK1, and GALE genes (9p13, 17q24, 1p36) | ✓ | AR | ✓ | POF | |
| Indications for genetic test                                                                 | Genetic disorder                                                                 | Frequency                                                                 | Genetic test                                                                 | Chromosome/genetic alterations | ART  | Inheritance | Antenatal test | Differential diagnosis                                                                                   | Refs.       |
|------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------|------|-------------|----------------|--------------------------------------------------------|-------------|
| Chronic mucocutaneous candidiasis, hypoparathyroidism and autoimmune adrenal failure; early onset | Autoimmune polyglandular syndrome (types I)                                      | Prevalence: 1–9 in 1,000,000; 1/25,000 in Finland                         | Molecular diagnosis                                                          | ARE gene (21q22.3)            | ✓    | AR          | ✓             | IPEX syndrome; autoimmune polyendocrinopathy type 2 | [45]        |
| Hypertension, hypokalemia; abnormal sexual development, amenorrhea, infertility              | 17α-hydroxylase deficiency                                                       | 1 in 1 million                                                             | Molecular diagnosis                                                          | CYP17A1 gene                   | Donor| AR          | NA            | Severe congenital adrenal hyperplasias                 | [62, 63]    |
| Mineralization of bones and osteoporosis; hyperglycemia; ambiguous genitalia, ovarian cysts early in childhood, anovulation; hirsutism | Aromatase deficiency                                                             | unknown                                                                   | Molecular diagnosis                                                          | CYP19A1 gene                   | Donor| AR          | NA            | PCOS                                                   | [89]        |
| Ophthalmic disorder associated with premature ovarian failure; early onset                  | Blepharophimosis, ptosis, epicanthus inversus syndrome type I (BPES, type I)    | Prevalence: 1–9/100,000                                                   | Molecular diagnosis                                                          | FOXL2 gene                     | ✓    | AD or de novo | ✓             | PCOS                                                   |             |
| Pre- and postnatal growth retardation, facial sun-sensitive telangiectatic erythema, increased susceptibility to infections, and predisposition to cancer | Bloom syndrome                                                                  | Unknown; 1/48,000 among people of Ashkenazi Jewish descent                 | Cytogenetic or molecular diagnosis                                           | 15q26.1, BLM gene              | ✓    | AR          | ✓             | Silver–Russell syndrome, Rothmund–Thomson syndrome, ataxia–telangiectasia, Cockayne syndrome, and Nijmegen breakage syndrome |             |
| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis |
|-----------------------------|------------------|-----------|--------------|--------------------------------|-----|-------------|---------------|----------------------|
| Ovulation disorders (not POI) | Kallmann | Prevalence: 1/30,000; incidence: 1/8,000 | Molecular diagnosis | Type 1: ANOS1 Type 2 and 6: CHD7, FGFR1, FGF8 and SOX10 Type 3: FEZF1, PROK2, PROKR2 | ✓ | X-linked | ✓ | Syndromes associated with hypogonadotropic hypogonadism |
| Diabetes mellitus, hypothyroidism, alopecia totalis, long, triangular face, hypertelorism; dystonias, dysarthria, dysphagia | Woodhouse–Sakati syndrome | Unknown | Molecular diagnosis | DCAF17 gene | Donor | AR | NA | Diabetes; hypogonadism; deafness-intellectual disability [27] |
| Hearing loss; intellectual disability, ataxia, peripheral neuropathy; ovarian dysgenesis, primary amenorrhea, primary ovarian insufficiency, normal external genitalia, infertility | Perrault syndrome | Rare | Molecular diagnosis | TWNK, CLPP, HARS, LARS2, HSD17B4 | Donor | AR | NA | Gonadal dysgenesis; sensorineural deafness [90] |
Table 6 (continued)

| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|------------------|-----------|--------------|--------------------------------|-----|-------------|-----------------|------------------------|-------|
| Primary amenorrhea, infertility, polycystic ovarian syndrome, hirsutism, ambiguous genitalia | Cytochrome P450 oxidoreductase deficiency | Unknown | Molecular diagnosis | POR gene | Donor | AR | NA | PCOS | [91] |
| Skeletal abnormalities, craniosynostosis, a flattened mid-face, a prominent forehead, and low-set ears; arachnodactyly, choanal atresia; primary amenorrhea, infertility, polycystic ovarian syndrome, hirsutism, ambiguous genitalia | Antley–Bixler syndrome | Unknown | Molecular diagnosis | FGFR2 gene | Donor | AR | NA | PCOS | [92] |
| Obesity, hirsutism, and amenorrhea are clinical correlates of enlarged polycystic ovaries | Polycystic ovary syndrome (PCOS) | 6 to 10% of women worldwide | Molecular diagnosis | AOPEP, AR; DENND1A; ERBB4; FSHB; FSHR; FTO; GATA4; HMG2; INSR; KRR1; LHCG; RAB5B; RAD50; SUMO1P1; SUOX; THADA; TOX3; YAP1 | ✓ | Does not have a clear pattern of inheritance | NA | Amenorrhea | [93, 94] |
| Polycystic ovary syndrome 1 (STEIN–LEVENTHAL SYNDROME HYPERANDROGENEMIA) | Polycystic ovary syndrome | Molecular diagnosis | PCOS1 | ✓ | AD | NA | Amenorrhea; HYPERANDROGENEMIA | [93, 94] |
Table 6 (continued)

| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Chromosome/genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|-----------------------------|------------------|-----------|--------------|-------------------------------|-----|-------------|------------------|-----------------------|-------|
| Hydropic placental villi, trophoblastic hyperplasia, and poor fetal development | Recurrent hydatidiform mole-type I (familial recurrent hydatidiform mole, FRHM) | 1:250 in eastern Asia | Molecular diagnosis | NLRP7 gene (55%); KHDC3L gene (5%) | ✓ | AR | ✓ | Hydatidiform mole | [95, 96] |
| Abnormally developed embryo and placenta that result in the formation of hydatidiform moles | Hydatidiform mole | 1:1500 in USA | Molecular diagnosis | C11 or F80, MEI1, REC114 | ✓ | AR | ✓ | FRHM | [95, 96] |
| Normal general physical examination, absence of clinical findings involving other organ systems, typical female external genitalia, normally formed uterus and fallopian tubes, gonadal dysgenesis, skeletal abnormalities, campomelic dysplasia | Swyer syndrome (46,XY complete gonadal dysgenesis) | 1 in 80,000 | Molecular diagnosis | SRY (15%); MAP3K1 (18%); DHX and NR5A1 (rare) | ART | De novo; rare AD | ✓ | Ambiguous genitalia and/or sex chromosome-phenotype discordance | [69] |

Database sources: NIH, OMIM and OrphaNet
✓, yes; x, no; NA, not applicable; POF, premature ovarian failure; PCOS, polycystic ovarian syndrome
| Indications for genetic test | Genetic disorder | Frequency | Genetic test | Genetic alterations | ART | Inheritance | Antenatal test | Differential diagnosis | Refs. |
|----------------------------|------------------|-----------|--------------|--------------------|-----|-------------|---------------|----------------------|-------|
| Underdeveloped or absent uterus and abnormalities of other reproductive organs; normal female external genitalia, breasts; hyperandrogenism; facial hirsutism; primary amenorrhea; infertility | Müllerian aplasia and hyperandrogenism (other names: Biason–Lauber syndrome, WNT4 deficiency) | Rare | Molecular diagnosis | WNT4 gene | NA | AD or de novo | ✓ | Abnormalities of the reproductive system | [97–99] |
| Vagina and uterus to be underdeveloped or absent; although external genitalia are normal, primary amenorrhea | Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome (type 1) | 1 in 4500 | Molecular diagnosis | ESR1, OXTR, WNT9B | NA | AD | ✓ | Abnormalities of the reproductive system | [100–102] |
| Underdeveloped or absent vagina and uterus, although external genitalia are normal; primary amenorrhea; unilateral renal agenesis; skeletal abnormalities; hearing loss or heart defects | Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome (type 2) | | | | | | | |
| Bone marrow failure, hypopigmentation, short stature, physical abnormalities, organ defects (gastrointestinal abnormalities; heart defects; eye abnormalities; malformed ears and hearing loss), and an increased risk of certain cancers; abnormal genitalia or malformations of the reproductive system and infertility | Fanconi anemia (Fanconi pancytopenia) Fanconi pancytopenia Fanconi panmyelopathy | 1 in 160,000 (more common among people of Ashkenazi Jewish descent, the Roma population of Spain, and black South Africans) | Molecular diagnosis | FANCA, FANCC and FANCG (90%) | NA | AR, AD (RAD51-related FA); X-linked (FANCB-related FA) | ✓ | Bloom syndrome; ataxia–telangiectasia; Nijmegen breakage syndrome (NBS); Suckel syndrome; neurofibromatosis 1; POI | [62, 63] |

Database sources: NIH, OMIM and OrphaNet
✓, yes; x, no; NA, not applicable; POI, primary ovarian insufficiency
are more related, for example, to hypogonadism, terato-
sperma or asthenozoospermia and to familial forms of
obstructive azoospermia.

Currently, the main genetic tests routinely used for the
diagnosis of male infertility are the karyotype, the study
of chromosome Y microdeletions, and the analysis of the
CFTR gene. Since it has been reported that several
mutations are related to male infertility, it is not surprising
that in ~40% of all cases of male infertility, the underlying
genetic pathogenesis remains unknown [107, 108]. It
must also be considered that the role of de novo mutations
should be further investigated, especially in light of what
happens for Klinefelter syndrome and AZF deletions that occur almost exclusively de novo [106].
Therefore, to improve and personalize the entire diagnostic–therapeutic pathway of male infertility, targeted
genetic tests should be performed in the presence of specific
clinical pictures, always after appropriate genetic coun-
selling: (1) for diagnostic purposes, (2) during clinical
decision-making to establish the most appropriate ART strategy (for example, in the presence of deletions of the AZFa and AZFb regions, the possibility of sperm recovery using testicular biopsy is extremely low), and (3) for prognostic purposes (to establish the risk of trans-
mitting the pathology and plan a prenatal or preimplantation
diagnostic procedures).

**Whole chromosomal aberrations** The prevalence of chromosomal alterations varies from 1.05 to 17% (this gap depends on the characteristics of the studied group) but is 0.84% in newborns [109]. Structural chromo-
somal rearrangements are more common with respect to numerical abnormalities; this does not apply to sex chromosomes whose abnormalities, accounting for approximately 4.2% of all whole chromosomal aberrations, are represented by sex chromosome aneuploidies in 84% of cases and by structural rearrangements of chromosome Y in the remaining 16% of cases. Klinefelter syndrome (karyotype 47, XXY) is the most frequent type of sex chromosome aneuploidy detected in infertile men [11, 12]; the second most frequent gonosomal abnormality is Double Y syndrome or Jacobs syndrome, characterized by the presence of Y chromosome disomy [14, 110]. In addition to reduced reproductive potential, carriers of chromosomal abnormalities have an increased risk of abortion or generate a child with an abnormal karyotype. For this reason, Table 1 shows the main chromosomal aberrations that could interfere with healthy reproduction, the relative information on the phenotypic aspect, the laboratory tests to highlight them and the indications for antenatal genetic testing.

**Partial chromosomal aberrations** Microdeletions in the long arm of the Y chromosome (Yq), named the AZF (Azoospermia Factor) region, have been found in 8–12% of azoospermic men and 3–7% of oligozoospermic men [106], resulting in the most common molecular genetic cause of male infertility [110]. The AZF region includes three groups of genes (AZFa, AZFb and AZFc) that are most responsible for spermatogenesis, so partial or complete deletions in this area may impair reproductive capacity. Indications for AZF deletion screening are based on sperm count (< 5 × 10⁶ spermatozoa/ml) associated with primary testiculopathy, and ICSI is required to overcome infertility [111].

Male offspring will carry the same father’s Yq microdele-

tions or even a worse one; therefore, genetic counselling
is mandatory [112]. Parents should be aware of the
risk of having a child affected by Turner’s syndrome (45,
X0) or other phenotypic anomalies associated with sex chromosome mosaicism [113].

The rearrangement of the AZFc zone is responsible
for 60% of all Yq microdeletions [114]. The AZFc region
(3.5 Mb) contains several copies of five repeats (b1, b2, b3, b4, and gr), whose similarity and large size predispose an individual to a relatively high incidence of de novo deletions via homologous recombination [115]. The most common is the loss of the whole b2/b4 region, which includes the DAZ family (Deleted in Azoospermia) and leads to spermatogenesis deterioration [115, 116]. More details about AZF are reported in Table 1.

**Single gene mutations** This section will focus on the noteworthy single gene disorders that have clinical relevance for male infertility (Tables 3, 4, 5). Although thousands of genes are involved in male infertility, today, only a handful of genetic diseases are routinely investigated (e.g., cystic fibrosis) [117, 118]. As shown by several studies, the approaches to identify a single causative gene are not useful considering that more than 2300 genes are expressed in the testis alone and that hundreds of them influence reproductive functions and can contribute to male infertility. Even if nearly 50% of infertility cases are due to single or multiple genetic defects, the genetic causes remain unexplained for 20% of patients [3]. Furthermore, the increasingly widespread use of tools, such as NGS (next-generation sequencing), for both diagnostic and research purposes will allow us to rapidly expand our knowledge of this field [4].

Starting from the clinical and laboratory evaluation, as shown in Tables 3, 4, 5, the main genetic conditions that could interfere with healthy reproduction are reported with the aim of improving the targeted genetic test in the presence of specific clinical pictures.

**Female genetic infertility** In contrast to male infertility, little is known about the genetic bases of female infertility. Accordingly, fewer specific tests are routinely recommended to infertile females.
to investigate the presence of chromosomal disorders or single-gene defects related to their clinical phenotypes. Indeed, isolated infertility due to genetic causes is rare; more commonly, syndromic diseases contribute to female infertility. To date, genetic tests are mainly used for patients with POI, limited to chromosomal aberrations and FMR1 premutations. We therefore focused on the description of these two conditions; however, as shown in Tables 6, 7, more details have been reported concerning the main chromosomal and genetic alterations that could interfere with healthy reproduction; for each of them, the main phenotypic presentations and the laboratory tests that are available in the pre- and postnatal periods are reported.

**Whole chromosomal aberrations** Considering that chromosomal disorders significantly impact fertility and the miscarriage risk, karyotype analysis is always advisable [119]. The most clinically important structural disorders in infertile females are translocations, both reciprocal (exchange of two terminal segments from different chromosomes) or Robertsonian (centric fusion of two acrocentric chromosomes) responsible for blocks of meiosis and structural alterations of the X chromosome. Patients with reciprocal translocations are at a significantly increased risk of infertility, including hypogonadotropic hypogonadism with primary or secondary amenorrhea or oligomenorrhea. The balanced rearrangements do not create health problems for their carriers because they cause neither loss nor duplication of genetic information, but they can give rise to gametes in which the genetic information is unbalanced and can thus become a cause of infertility or multiple miscarriage. Some abnormalities, such as the XXX karyotype, could not be clearly associated with infertility.

Women with a normal karyotype produce a variable percentage of oocytes with chromosomal abnormalities due to errors occurring during crossing-over and/or meiotic nondisjunction [120, 121]. The three main classes of abnormalities are 45X, trisomy and polyploidy. It is well known that these events increase with maternal age [122]. It is possible to analyze gametes or embryos while undergoing ART thanks to PGT. The efficacy of the technique is increased after screening for aneuploid embryos and transferring only euploid embryos [123, 124].

**Fragile X syndrome** Fragile X syndrome is an autosomal dominant genetic disorder caused by the presence of over 200 repetitions of the CGG triplet sequence in the FMR1 (Fragile X Mental Retardation 1) gene or by a deletion affecting the FMR2 (Fragile X Mental Retardation 2) gene. Carriers of the female FMR1 premutation (when the number of CGG repeats falls between 55 and 200) or FMR2 microdeletion show menstrual dysfunction, diminished ovarian reserve, and premature ovarian failure [125, 126].

In addition to the family history, in the case of women with these clinical manifestations, the possibility of a molecular test should be considered. The most common genetic contributors to POI are X-chromosome-linked defects. In rare cases, the cause is an alteration in an autosomal chromosome [88]. Identifying the mutations in a timely fashion is of paramount importance to managing the reproductive options and, if necessary, choosing a preimplantation genetic diagnosis program: the aim is to identify the specific clinical pictures in which a targeted genetic test could guide a personalized diagnostic–therapeutic treatment approach.

**Molecular approaches in the identification of genetic diseases that are transmissible to offspring**

It is well known that in 20–25% of cases, perinatal mortality is caused by inherited chromosomal or genetic alterations [127]. Thanks to medical awareness in recent decades, preconception carrier screening has become widely requested. The identification of couples at risk of transmitting a specific inherited disorder to their offspring offers the possibility of making informed reproductive choices to future parents. If the reproductive partner happens to carry a gene alteration for one of the genetic conditions, the pregnancy would be at risk for a child with that disease.

The American College of Obstetricians and Gynecologists has issued standard recommendations for ethnic and general population genetic screening in couples based on reproductive age [128]. Testing is available for more than 2000 genetic disorders, including common diseases, such as sickle-cell anemia, cystic fibrosis, and spinal muscular atrophy, or more complex conditions, such as mental retardation and congenital heart disease.

In this context, genetic counselling is crucial for recognizing the genetic risk, referring patients appropriately and informing patients about genetic issues that are relevant to decision-making [129]. In fact, preconception carrier screening provides genetic information for multiple disorders; thus, all carrier couples can make reproductive decisions based on their results. The tailored genetic test is a crucial tool to improve short-term and long-term outcomes for mothers and their babies [130, 131].

Currently, during the antenatal period, a variety of techniques are available to identify a transmissible disorder to the offspring in the presence of carrier or affected couples. Each of these techniques can be applied only during a specific time period of pregnancy or at different embryo stages in the IVF protocol.
Invasive PND is usually performed on DNA extracted from fetal cells obtained by chorionic villus sampling (CVS) (between the 11th and 13th weeks of gestation) or from amniocytes (from the 15th to the 20th week), and the result is obtained in 7 or 15 days, respectively [132]. The molecular diagnosis for monogenic disease, as we detailed in a previous publication, is carried out by direct mutation analysis when the parental mutations are known or by linkage analysis when the parental mutations are unknown [5, 132]. Paternity verification and contamination analysis are always performed in addition to the specific analytic phases [5].

An increasing amount of interest has been shown regarding the noninvasive prenatal diagnosis (NIPD) of monogenic disease that is able to detect fetal genetic alterations in maternal blood at an early gestational age (approximately 10 weeks). However, although noninvasive prenatal testing (NIPT) of cell-free fetal DNA (cffDNA) for the screening of chromosomes 21, 18, 13, X and Y has been clinically adopted, NIPD remains a challenge [133]. Very recently, NIPD for clinical use has been adopted in cases of sex-linked disorders and RHD [134]. Several studies have tested the application of NIPD in monogenic diseases, such as β-thalassemia, congenital adrenal hyperplasia, and Duchenne and Becker muscular dystrophy [135–137]. The disruptive technology of NGS together with the haplotyping strategy is driving the possibility of using NIPD in clinical cases.

PGT has the same diagnostic motivation as the traditional PND, with the advantage of advancing the timing of diagnosis at the embryo stage. Only disease-free embryos are transferred to the mother, avoiding recourse to therapeutic abortion. Even for couples who are able to conceive naturally, PGT requires the application of IVF techniques, including (a) the collection of gametes from both partners; (b) the fertilization of the oocyte by intracytoplasmic sperm injection (ICSI); (c) the embryo biopsy, which allows one or more cells from the blastomere or trophectoderm to be taken 3 or 5 days, respectively, postfertilization; (d) molecular analysis and (e) the embryo transfer.

PGT protocols are set to start from a small amount of biological sample, ranging from 1 to 10 cells from the embryo at the cleavage stage or blastocyst stage. Conflicting opinions are reported on the detrimental effects of embryo biopsy and mosaicism events between cleavage-stage and blastocyst embryos. Linan et al. demonstrated that the concordance of diagnosis in embryos that were double biopsied on D3 and D5 is 67.8% lower than previously reported, supporting the use of blastocyst biopsies instead of cleavage-stage embryo biopsies [138]. Recently, data from the Preimplantation Genetic Diagnosis International Society (PGDIS) in 2018 showed no difference in the detrimental effects between the embryo stages if experienced operators performed the biopsies [139, 140]. PGT includes whole genome amplification (WGA) to obtain a sufficient quantity of genomic DNA for one or more molecular investigations [141, 142]. Several types of WGA can be used depending on the downstream application [142]. The most used technique for PGT is still represented by “multiplex polymerase chain reaction” (PCR) and capillary electrophoresis analysis for the direct identification of the causative mutation of the disease and the analysis of at least two informative polymorphic markers [the most used are the “short tandem repeats” (STR), microsatellites characterized by short tandem nucleotide repeats] or the analysis of at least 3 polymorphic markers in the event that the causative mutation is not known [5, 143]. However, since PGT tailored to a disease is a laborious and expensive procedure, which is time-consuming in the preliminary phase for the study of the family, several laboratories use genome-wide approaches to analyze gene markers throughout the genome. Alan Handyside tested “karyomapping” based on a single nucleotide polymorphism (SNP) array able to determine the genotype of an individual by analyzing thousands of SNPs distributed throughout the genome [144]. The “karyomapping” involves a “linkage” analysis: with a comparison of the SNPs associated with the causative mutation of the disease to be investigated, that are present in the index case and that are therefore in the parents’ chromosomes, to the SNPs present in the embryo cells, it is possible to identify the presence or absence of the mutation carrier [144–146]. In addition, the density of the SNPs allows a higher resolution in the case of crossings between chromosomes close to the mutated gene. Finally, it is possible to use “karyomapping” in families with combinations of more monogenic alterations or that require HLA compatibility, truly demanding investigations to be carried out with conventional methods. “Karyomapping”, however, loses its effectiveness when it is not possible to establish which parental allele is linked to the genetic alteration; for quantitative analysis of mitotic abnormalities (mosaicsisms), “karyomapping" does not directly analyze the mutation and cannot detect de novo mutations. The aim of most new approaches is to use a targeted SNP analysis to detect single mutations or groups of common mutations combined with quantitative haplotype analysis or chromosome count. Recently, a genome-wide protocol using NGS has been tested for the identification of family mutations together with cytogenetic screening in embryo biopsies [147–149]. The protocol is based on an enlarged panel of disease-associated genes (approximately 5000 genes) and enables, in a single workflow, (a) the direct detection of family mutations and the indirect detection through linkage analysis of heterozygous SNPs (PGT-M);
(b) a chromosomal translocation (PGT-SR) analysis; and (c) testing for aneuploidies. However, the limitations of a single NGS protocol are related to its inability to detect haploidies, polyplodies, and mosaicisms. In addition, the analysis of consangunieous families is not recommended. Finally, other limitations regarding the limit of detection or the size of the translocation supported by the protocol could be overcome using haplotyping in the presence of the index case.

**Molecular approaches for the optimization of art techniques**

Human embryos that are developed in vitro show a great deal of acquired chromosomal abnormalities; for this reason, PGT for aneuploidy (PGT-A) has been developed to select euploid embryos that are suitable for transfer [150].

PGT-A is primarily indicated for couples with advanced maternal age, recurrent implantation failure, recurrent abortions, or severe male infertility. Meiotic errors are one of the main causes of the low success rate (~30%) of in vitro fertilization techniques. Randomized studies and meta-analyses have shown that the PGT-A technique does not increase the live birth rate but decreases the miscarriage rate and increases the efficiency of IVF techniques [123, 151].

The evolution of PGT-A techniques started with a limited number of chromosomes analyzed by fluorescence in situ hybridization (FISH) in 1995 [152]. It was soon overcome by the analysis of the whole chromosome set by using different genetic platforms, such as metaphase Comparative Genomic Hybridization (mCGH), array-based Comparative Genomic Hybridization (aCGH), single nucleotide polymorphism (SNP) microarray, quantitative polymerase chain reaction (qPCR), and, most recently, NGS.

Currently, the most commonly used technique is NGS. This method involves the amplification of the genome from a single cell by WGA, the preparation of a DNA library, starting directly from the amplified DNA, and the subsequent sequencing of a pool of libraries in parallel, each identified by a specific “barcode” sequence. Finally, an analysis software that compares the sequences obtained in each sample with respect to the “human haplotype reference genome” allows the identification of the possible presence of chromosome aneuploidies [153]. Literature data confirm that NGS can be successfully applied to the diagnosis of a variety of genetic abnormalities, even in single cells isolated from human embryos following WGA, and has numerous advantages over the technologies traditionally used for PGT-A [153–156].

However, it was soon clear that the gold standard was to develop a method for the analysis of both monogenic diseases and PGT-A at the same time. Indeed, as previously discussed, the very recent innovation for this purpose is the use of NGS to analyze single gene mutations and chromosomal copy number variations to select euploid disease-free embryos [157]. Currently, only a novel mutation continues to be a challenge [140].

**Discussion**

Healthy reproduction can be affected by unhealthy environmental and lifestyle factors, increasing paternal and/or maternal age, anatomical or genetic anomalies, systemic or neurological diseases, infections, trauma, and antibody development [158, 159]. As a consequence, infertility can be the result of nongenetic and genetic factors, and it is often multifactorial, polygenic or a combination of both. Presumably, hundreds of genes must interact in a precise manner during sex determination, gametogenesis, complex hormone actions/interactions, embryo implantation, and early development to generate healthy offspring. Indeed, known genetic causes of infertility include chromosomal aberrations, single gene variants and phenotypes with multifactorial inheritance. To date, specific genes and mutations have been confirmed to be associated with infertility phenotypes in males, females or both, and our knowledge regarding the molecular basis of infertility is continually growing. Confirmation of a clinical diagnosis through genetic testing may lead to personalized medical management. Similar clinical symptoms may be the result of different genetic variations. Specifically, in more rare clinical situations, genetic evaluations (counseling and testing) can contribute to the specific identification of the disease or to the confirmation of a suspected diagnosis. The combination of the detailed clinical information provided and the identified genetic cause will allow the development of a personalized diagnostic–therapeutic strategy.

The first stage in assessing a couple with potential fertility problems involves the substantial synergy between the patient’s medical history, physical examination, instrumentation analysis (pelvic ultrasound in women) and laboratory tests (sperm analysis in males) to identify any underlying pathology and possible risk factors. Although this evaluation should be performed simultaneously for both partners, the male partner analysis is required only in 18% of cases [160]. Figure 2 shows how the integration of couples’ information can stratify patients on the basis of different morpho-functional aspects and outline already differential diagnostic–therapeutic approaches. When a complete and simultaneous assessment of the couple is not performed, the fertility prognosis is compromised, and the opportunity to improve the health outcomes of each subject is lost. For this reason, it is essential to accurately collect, for each member of the couple, data regarding the personal history (life habits,
smoking, alcohol use, drug use, sports activities, etc.) and the medical history (genital characteristics at birth and in the first years of life, pubertal development, and any past disease affecting the genital system). The medical history evaluation must focus on the presence of possible metabolic, endocrine, and genetic disorders with the aim of highlighting elements that can lead to subsequent, specific diagnostic tests. At this stage, it is important to identify patients who can benefit from predictive genetic testing: the use of targeted multigene panels can become a useful tool that, by allowing the identification of further causes of infertility, may improve genetic and reproductive counselling and patient stratification. In fact, some cases of infertility are due to unknown genetic mutations; for this reason, collecting data is mandatory to increase the detection rate (which is currently 80%) and to reduce the percentage of idiopathic infertility.

Many couples learn that they are at high risk of having a child that is affected by a genetic disease only when the woman is already pregnant or after giving birth. Thus, a detailed family history for a couple planning to undergo ART is a useful tool for identifying genetically at-risk couples and can improve the medical care of these patients. A number of important factors must be considered when collecting a detailed family history in the context of family planning [129]. These include the history of the patient's pregnancy (e.g., multiple abortions may indicate a chromosomal abnormality), the degree of kinship (particular attention should be given to first- and second-degree relatives who may have a history of mental retardation, learning difficulties, progressive muscle weakness, early cataracts, infertility, motionless birth, recurrent miscarriages, and coagulation disorders), the consanguinity of the couple [129], and the ethnicity of
both groups of grandparents. Furthermore, references to other specialists (e.g., fetal maternal medicine, reproductive endocrinology, gynecology) should be considered to exclude maternal/paternal infertility causes. In the case of identification of pathologies, in one or both partners, standard diagnostic algorithms will be used, while in specific situations, integration will need to be made with specific diagnostic procedures that require integration with eligibility criteria to access ART.

NGS-based strategies offer the opportunity not only to optimize genetic testing in reproductive medicine (since in one step, it may be possible to analyze the potential causes of infertility, perform carrier screening, and support ART) but also to tailor the therapeutic decision on the basis of the specific genomic features of the patients. Common observation reveals that patients with the same diagnosis may respond differently to therapies, and it is becoming increasingly evident that this differential responsiveness may be due to specific DNA variations at the genomic level. The challenge for future medicine is to move from a population-based view to an individually based one. Novel technologies are driving this revolution. In the context of reproductive medicine, for example, it has been established that specific single nucleotide variants in the genes encoding for hormonal receptors are able to influence the efficacy of hormonal therapies in inducing ovulation [161]. In addition, other molecular features of the patients should be taken into account before starting therapeutic protocols to avoid potential side effects. An opportunity comes from the example of the BRCA test. Indeed, BRCA1 and BRCA2 are the most common genes related to hereditary breast and ovarian cancers. Molecular screening for these genes has greatly increased in recent years due to technological simplification and the availability of specific drugs that are suitable for patients with mutations in these genes. As a consequence, several affected and nonaffected young females have been found to be carriers of a BRCA mutation. This information should be taken into account when reproductive choices are planned, considering the potential risk of treating these patients with hormonal therapy.

Conclusions
Currently, both the entire diagnostic pathway and the effectiveness of genetic analysis for infertility suffer from an approach that is ineffective: only a few genetic variables are studied, each through a specific molecular diagnostic procedure. This makes the process of genetic investigation fragmented and cumbersome, with a negative impact on the couple, in addition to the psychological distress caused by infertility. Therefore, despite the large potential of genetic tests to provide real insights into infertility causes, this crucial tool is still used without a method tailored to diagnostic needs regarding infertility. However, recent developments in new sequencing technologies have made it possible to compact one or more tests into a single NGS-based analysis, thus reducing diagnostic costs and time. The European Society of Human Genetics (ESHG) and the European Society of Human Reproduction and Embryology (ESHRE) have recently issued a recommendation for the development and introduction of extended carrier screening [162]. Although it is difficult to predict at this time how much the diagnostic yield of genetic tests for the different subtypes of male and female infertility will increase, it is realistic to expect a decrease in the current percentage of idiopathic infertility.

The general state of health in the reproductive environment is gaining increasing attention and clinical relevance. Therefore, reproductive specialists have the task of evaluating infertile couples by considering both their general and reproductive health, since the relative conditions of comorbidity can influence their reproduction. Medicine is undergoing an important transformation from a reactive to a preventive approach: the future will focus on the integrated diagnosis, treatment and prevention of diseases in individual patients.

Abbreviations
aCGH: array-based Comparative Genomic Hybridization; AOA: assisted oocyte activation; ART: assisted reproductive technology; AZF: azoospermia factor; cffDNA: cell-free fetal DNA; CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; CVS: chronic villus sampling; DAZ: Deleted in Azoospermia; DNA: deoxyribonucleic acid; ESHG: European Society of Human Genetics; ESHRE: European Society of Human Reproduction and Embryology; FISH: fluorescence in situ hybridization; FMR: Fragile X Mental Retardation; HLA: Human Leukocyte Antigen; ICSI: Intracytoplasmic Sperm Injection; IVF: in vitro fertilization; mCGH: metaphase Comparative Genomic Hybridization; NGS: next generation sequencing; NPT: non-invasive prenatal testing; OMIM: Online Mendelian Inheritance in Man; PCR: polymerase chain reaction; PGD: preimplantation genetic diagnosis; PGDIS: Preimplantation Genetic Diagnosis International Society; PGT-A: Preimplantation Genetic Test for Aneuploidy; PGT: Preimplantation Genetic Testing; PND: prenatal diagnosis; POI: primary ovarian insufficiency; qPCR: quantitative polymerase chain reaction; SNP: single nucleotide polymorphism; STR: short tandem repeat; STS: sequence tagged site; WGA: whole genome amplification.

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