Genetics in human reproduction

Vivian de Oliveira Rodrigues¹, Fernanda Polisseni², Gabriel Duque Pannain¹, Miralva Aurora Galvão Carvalho¹

¹Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil
²Surgery Department, Medical School - Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil

ABSTRACT
Approximately 50% of the causes of infertility are of genetic origin. The objective of this study was to analyze the role of genetics in human reproduction by reviewing the main genetic causes of infertility and the use of preimplantation genetic testing in Brazil. This literature review comprised articles in English and Portuguese published on databases PubMed, Scielo, and Bireme from 1990 to 2019. Randomized clinical trials and specialized guidelines were given preference whenever possible. Genetic cause can be traced back to up to 20% of the cases of severe azoospermia or oligozoospermia. Subjects with these conditions are good candidates for genetic screening. In women, genetic causes of infertility (fragile X syndrome, X-trisomy, and Turner’s syndrome, some of which diagnosed with karyotyping) culminate with premature ovarian failure. Genetic screening helps advise couples of the risk of experiencing early reproductive capacity loss and of the chances of their offspring carrying genetic disorders. In addition to enhancing the prevention of serious diseases in the offspring of couples at increased risk of genetic diseases, preimplantation genetic screening improves the success rates of assisted reproduction procedures by allowing the selection of euploid embryos for transfer. The interface between genetics and human reproduction has gained significant relevance, but discussions are still needed on which procedures are clinically and ethically acceptable and how they should be regulated.

Keywords: genetics, male infertility, female infertility, preimplantation genetic diagnosis, preimplantation genetic screening

INTRODUCTION
According to the World Health Organization, infertility is a disease of the reproductive system defined by the inability of sexually active couples to get pregnant within a period of one year, without the use of contraceptive methods. In women over 35 years of age or couples with known infertility-related comorbidities, this period is six months (Marshburn, 2015; Zegers-Hochschildd et al., 2009).

Genetic and environmental factors may be related to infertility, which often has a multifactorial etiology. It has been estimated that approximately 50% of the causes of infertility are related to genetic factors. Hundreds of experimental studies with animal models have demonstrated an association between infertility and single or multiple gene defects. Despite these advances, translating these results into clinical trials has been challenging. At present, only a small number of genes and genetic changes have been unequivocally associated with primary infertility. This situation has been changing since the conclusion of the genome project and the progress of personalized medicine. In fact, ten to 15 new genetic tests are added to the roster of genetic tests annually (Zorrilla & Yatsenko, 2013).

The main known genetic causes of infertility include chromosomal aberrations, monogenic diseases, and phenotypes with multifactorial inheritance. The physiology of reproduction involves several paracrine, autocrine, and endocrine processes. They are regulated by a plethora of genes and any discrepancy in these processes can lead to infertility (Venkatesh et al., 2014).

In men, fertility criteria include normal spermatogenesis, complete sperm maturation during passage through accessory organs of the reproductive system, patency of accessory organs, adequate production of seminal fluid, ability to deposit semen into the vagina, adequate sperm cell mobility and morphology so it can reach the oocyte in the uterine tubes and penetrate it (Travaglini et al., 2006).

The main genetic causes of male infertility are chromosomal abnormalities, mutation in the cystic fibrosis transmembrane receptor (CFTR) gene, and microdeletion on the Y chromosome. Genetic cause can be traced back to up to 20% of the cases of severe azoospermia or oligozoospermia. Subjects with these conditions are good candidates for genetic screening. Although the detection of genetic alterations does not substantially alter treatment, they must be analyzed for two main reasons: to achieve a conclusive causal diagnosis and assess the genetic risk to the offspring in case of successful treatment (Kara & Simoni, 2010).

In women, genetic causes of infertility (fragile X syndrome, X-trisomy, and Turner’s syndrome, some of which diagnosed with karyotyping) culminate with premature ovarian failure. Complex multifactorial conditions such as endometriosis and polycystic ovary syndrome have been associated with gene alterations (Zorrilla & Yatsenko, 2013).

Recurrent miscarriage, defined as three or more pregnancy losses before 20 weeks of gestation, may also have a genetic etiology. Between a quarter and 51% of the cases of recurrent miscarriage have been associated with chromosomal anomalies of the fetus. Karyotyping of miscarriage products should be performed to determine the cytogenetic reasons for the pregnancy loss. Karyotyping of the parents is also recommended (Kara & Simoni, 2010).

Genetic testing has applications not only in the investigation of infertile couples, but also in preimplantation analysis before in vitro fertilization (IVF). Preimplantation genetic testing (PGT) is a clinical application of genetics that enables the examination of a limited number of embryonic cells during their in vitro development (Harper et al., 2018; Zegers-Hochschildd et al., 2009).

Many recent studies in the field of genetics have mentioned the transition from traditional "monogenic genetics" to comprehensive testing of the human genome through the integration of Next Generation Sequencing (NGS), which allows complete DNA sequencing in a single day with bioinformatics techniques. Novel technologies have shed light on the variations of the human genome and extended the application of genetics. Recent technological advances are already being used to investigate the underlying causes of male and female infertility and in preimplantation genetic testing, both subjects of this paper (Harper et al., 2018).
MATERIAL AND METHODS
This literature review comprised articles in English and Portuguese published on databases PubMed, Scielo, and Bireme from 1990 to 2019. Additional references were collected from relevant studies. Randomized clinical trials and specialized guidelines were given preference whenever possible. The search yielded 122 papers from randomized controlled trials, guidelines of renowned medical societies in the field, or review articles, all of which read in full. The search used keywords genetics, male infertility, female infertility, preimplantation genetic test, preimplantation genetic diagnosis, and preimplantation genetic screening.

RESULTS
Male infertility and genetics
Keywords genetics and male infertility found 11,985 matches. Only papers whose full version was available and recent publications from renowned authors on relevant themes were read. Table 1 lists the main monogenic diseases associated with male infertility (Asero et al., 2014).

Klinefelter syndrome
Klinefelter syndrome (KS) is the most common cause of male infertility of genetic origin. Its prevalence reaches 5% in men with severe oligozoospermia and increases up to 10% in subjects with spermogram-documented azoospermia. Infertility occurs due to changes in spermatogenesis and testicular injuries in individuals with progressive syndrome, progressive fibrosis, degeneration of germinal cells and Sertoli cells (Bonomi et al., 2017; Klinefelter et al., 1942; Lanfranco et al., 2004; Piomboni et al., 2014; Wosnitzer & Paduch, 2013).

There is consensus around the correlation between testicular phenotype severity and frequency of chromosomal abnormalities such as KS. For this reason, G-banding is recommended in Europe for men with sperm concentrations of less than 10 million/mL. This cut-off point was established based on the fact that the incidence of chromosomal alterations is ten times higher in these patients compared with the general population (Jungwirth et al., 2012; Krausz & Chianese, 2014).

The American Society for Reproductive Medicine recommended karyotyping to screen males with non-obstructive azoospermia (absence of azoospermia) or severe oligozoospermia (concentration <5 million/mL) for KS, especially before ICSI (ASRM, 2015).

Peripheral blood karyotyping and identification of KS are fundamental in the differential diagnosis of obstructive azoospermia (sperm production is normal, but there is no sperm in the ejaculate due to obstruction or absence of the vas deferens) and non-obstructive azoospermia (no or minimal sperm production as in KS). In this case, genetic testing is a valuable tool to achieve proper patient management (Wosnitzer et al., 2014).

The treatment of this syndrome requires a multidisciplinary approach and the involvement of assisted reproduction specialists. A small proportion of men with KS maintain capacity for spermatogenesis at levels to allow the presence of spermatozoa in ejaculate. However, men with KS may have residual preserved spermatogenesis and testicular sperm extraction (TESE) may retrieve tubules with active spermatogenesis. The combination of TESE and ICSI, in which the spermatozoa are injected directly into the egg, allowed men with KS previously considered sterile to father children (Vloebberghs et al., 2018).

Robertsonian and reciprocal translocations
Robertsonian translocations are structural chromosomal abnormalities that cause infertility by altering the genetic pattern of spermatozoa. They result from the fusion of the long arms of two acrocentric chromosomes (13, 14, 15, 21, 22) to form an anomalous chromosome. A reciprocal translocation, on the other hand, occurs when genetic material is exchanged usually between non-homologous chromosomes (Asero et al., 2014).

Balanced reciprocal translocations do not cause changes in carrier phenotype. However, in some cases they may cause decreases in testicular volume and testosterone levels, impacting spermatogenesis and resulting in azoospermia or oligozoospermia (Godo et al., 2013).

Individuals with severe oligozoospermia and azoospermia and couples experiencing recurrent miscarriages should undergo karyotyping to find possible chromosomal alterations. Furthermore, when a spermatozoan carrying a chromosome translocation fertilizes an ovum, the resulting embryo will carry the translocation, generating a genetic imbalance. The associated phenotype will depend on the exact region of the chromosome involved, which may result in mental retardation, malformations, and death of the fetus. When a translocation is detected in one of the spouses, the couple may choose to order preimplantation genetic screening of the embryos, embryo biopsy, or PGT to rule out the presence of the translocation in the embryos (Asero et al., 2014; Yin et al., 2017).

Y chromosome microdeletions
Azoospermia factor (AZF) was identified in the long arm of the Y chromosome in 1996 and deletions in this region were identified in 13 of 370 men with severe oligozoospermia or azoospermia. Although microdeletions are too small to be found in conventional karyotyping, they can be diagnosed via polymerase chain reaction (ASRM, 2015; Vogt et al., 1996).

AZF regions are divided into AZFa (proximal), AZFb (central), and AZFc (distal) and contain many of the genes needed in spermatogenesis. Males with deletions in the AZFc region may have sperm in ejaculate. Some with the same deletion are azoospermic, but may have sufficient sperm production to allow TESE. TESE is contraindicated for patients with deletions in the AZFa and AZFb regions, since their chances of having a successful sperm extraction procedure are extremely slim (Hopps et al., 2003; Oates et al., 2002).

The frequency of Y chromosome microdeletions ranges from 1% to 58% in published studies, and more specifically from 15% to 20% in males with idiopathic non-obstructive azoospermia; 7-10% in males with idiopathic oligozoospermia (sperm counts of less than 5 million/mL); and 2-3% in ICSI candidates. Differences in frequency might

| Table 1. Main genes associated with male infertility |
| --- | --- | --- |
| Gene | Disease | Clinical aspects |
| CFTR | congenital bilateral absence of the vas deferens | Obstructive azoospermia |
| KAL-1 | Kallmann syndrome | Hypogonadotropic hypogonadism and changes in spermatogenesis |
| AR | Androgen insensitivity syndrome | Decreased androgen sensitivity and changes in spermatogenesis |
| INS13-RXFP2 | Cryptorchidism | Changes in spermatogenesis |

Source: Asero et al., 2014.
be due to poor patient selection, differences in the ethnicity of the studied population, sample size, and differences in study design (Li et al., 2008; Rives, 2014; Suganthi et al., 2014). Thus, analysis of Y chromosome microdeletions based on peripheral blood should be offered to men with non-obstructive azoospermia before ICSI. In cases of non-obstructive azoospermia, the test may not only identify the origin of spermatogenesis impairment, but also predict the probability of sperm retrieval after TESE (ASRM, 2015; Rives, 2014).

In addition, considering that sperm counts may be significantly reduced with aging in men with Y chromosome microdeletions, sperm cryopreservation should be offered at the time of diagnosis when sperm cells are present in the ejaculate (Rives, 2014).

Consequently, when ICSI is performed in patients with Y chromosome microdeletions, the couple should be advised of the risk of transmitting the condition to their male offspring along with its negative effects on spermatogenesis. Therefore, couples should be instructed to order semen analysis (spermogram) for their adolescent children to consider the possibility of cryopreserving sperm as a measure of fertility preservation (ASRM & SMRU, 2018; Rives, 2014).

**Cystic fibrosis and other monogenic diseases**

The main known monogenic disease is congenital bilateral absence of the vas deferens (CBAVD) with obstructive azoospermia. Mutations in the CFTR gene are found in more than 90% of the patients with agenesis of the vas deferens (Zorrilla & Yatsenko, 2013).

The CFTR gene is located on chromosome 7. In its homozygous form, the gene causes cystic fibrosis, one of the most common and severe autosomal recessive diseases to affect Caucasians. One in 2,500 individuals is affected and one in 25 is an asymptomatic carrier of mutation. The presence of mutations that do not completely impair the expression of the CFTR gene causes CBAVD in men, with consequent obstructive azoospermia. CBAVD is found in 6% of the patients with obstructive azoospermia and in about 2% of infertile individuals. Infertility caused by obstructive azoospermia is observed in more than 95% of the males with cystic fibrosis, while 60-70% of the patients with CBAVD have mutations on the CFTR gene without manifesting clinical symptoms of cystic fibrosis (Asero et al., 2014; Claustrès et al., 2000).

Individuals with obstructive azoospermia are candidates for mutation testing on the CFTR gene through peripheral blood analysis, since they may present a congenital malformation of the Wolff ducts, which are precursors of the vas deferens, epididymis, and seminal vessels during fetal development (Tüttelmann & Simoni, 2008). Most of these patients have normal spermatogenesis observed in testicular biopsy. Thus, there is a significant chance that these men might have children through ICSI. However, since the offspring of these couples is at risk of cystic fibrosis when the female partner is heterozygous for the CFTR gene, screening for this mutation is imperative in humans before attempting assisted reproduction technology procedures (Field & Martin, 2011; Tüttelmann & Simoni, 2008).

**Female infertility and genetics**

The search for papers under this item used keywords genetics and female infertility and yielded 8805 matches. However, the authors read only papers available in full and prioritized recent publications with relevant themes and authors.

**Premature ovarian failure (POF)**

The end of a woman’s reproductive life is marked by the occurrence of menopause, defined as the last menstruation, caused by the exhaustion of the ovarian reserve. In the general female population, menopause occurs at 50-52 years of age. However, changes in ovulation may cause a pathogenic depletion of the ovarian follicles, resulting in early menopause. Menopause before the age 40 is the definition of POF (Perry et al., 2013; Shelling, 2010).

POF has been described as the premature cessation of ovarian function. The condition is characterized by 4-6 months of amenorrhea, increased levels of FSH (above 40,000/L), and hypoestrogenism. It occurs in 1% of all women and in 0.1% of women under the age of 30. POF has been associated with increased risk of osteoporosis, osteoarthritis, and cardiovascular disease, all of which related to hypoestrogenism. In addition to experiencing typical postmenopausal symptoms, women with POF suffer from early loss of reproductive capacity. Therefore, women at high risk for POF and who delay pregnancy to after the age of 30 may experience difficulty conceiving and maintaining pregnancy to term (Chapman et al., 2015; Perry et al., 2013).

A 2011 study showed that 50-90% of the causes of POF are idiopathic and probably have a significant genetic contribution. A genetic etiology of premature ovarian failure has been reinforced by estimates that between 44-65% of the daughters of mothers with FOP also have the condition. Recent reports have described various age-related genetic loci in natural menopause identified through broad genomic association studies. It is also important to note that in 10-30% of idiopathic cases a first-degree relative is affected. In addition, daughters of mothers with POF have a sixfold risk of manifesting the disease. The genetic causes of POF include chromosomal abnormalities, gene mutations (Table 2), and gene polymorphisms (Cordts et al., 2011; He et al., 2009; Pu et al., 2014; Qin et al., 2012; Stolk et al., 2009). However, the genetic alterations identified to date account a small proportion of the cases of POF. The disease has a diverse and heterogeneous etiology, involving the interaction of multiple genes, environmental factors, and associations with autoimmune conditions (Dixit et al., 2010; Qin et al., 2012).

Given the multifactorial etiology of POF, patients suspected for the disease should not undergo genetic testing. The associations described between the condition and a few noteworthy genetic diseases - fragile X syndrome (mutation in the FMR-1 gene on X chromosome at Xq27), chromosome X trisomy (47,XXX), and Turner’s syndrome (monosomy of chromosome X; 45,X0), to name a few - are still controversial. (Abir et al., 2001; Barasoain et al., 2013; CFM, 2017; De Geyter et al., 2014; Gleicher et al., 2010; Kawamura et al., 2016; Lubs, 1969; Schufreider et al., 2015; Sullivan et al., 2005; Tartaglia et al., 2010).

**Other Causes**

Other important causes of female infertility also appear to be linked to genetic alterations, including the likes of polycystic ovarian syndrome (PCOS) and endometriosis.

**Table 2. POF-associated genes**

| Genes involved in ovarian function | Genes involved in oogenesis |
|-----------------------------------|-----------------------------|
| FSH / FSHR | NOBOX LH / LHR LHX8 |
| CYP17 | NANOS CYP19 |
| BMP15 | |
| GDF9 | |
| GPR3 | |

Source: Kara & Simoni, 2010.
Although PCOS appears to follow a pattern of dominant inheritance, no specific gene has been linked to the disease. Therefore, genetic testing for this condition loses its purpose. There is a known association between PCOS and the following genes: FBG3, FST, INS, INSR, TCF7L2, CAPN10, FTO, SHBG, PCOS1, SRD5A1, SRD5A2, and CYPI1A. However, they have also been associated with obesity, diabetes, and insulin resistance, alterations commonly associated with PCOS. To date, only the insulin receptor gene (INSR) has demonstrated a more significant association with susceptibility to PCOS as described in the GWAS study (Chen et al., 2011; Kosova & Urbanek, 2013).

The presence of disease in first-degree relatives increases the risk of endometriosis by five to eight times. In 1999, a study described gene changes in three chromosomal regions (1p36,7p22.1 and 22q1) in patients with endometriosis (Bulun, 2009; Gogusev et al., 1999; Painter et al., 2011; Trelaro et al., 2005; Uno et al., 2010). It has been recently suggested that retinoid deficiency plays a causal role in the etiology of endometriosis. Abnormal methylation of the promoters of genes such as GATA6, ESR2 and NR5A1 in endometrial implants leads to local increases in estrogen and prostaglandin levels, causing inhibition of progesterone receptors. This, in turn, results in the reduction of retinoid synthesis and absorption. These molecular abnormalities have detrimental effects on cell differentiation and produce excessive inflammation, which might result in the development of endometriosis (Bulun et al., 2015).

Embryo biopsy and preimplantation genetic testing

A search using keywords preimplantation genetic diagnosis and preimplantation genetic screening yielded 221 matches. However, we read only the papers available in full text format. Priority was given to more recent texts of prominent authors discussing relevant themes.

Genetic analysis is not valuable only in the investigation of infertile couples; it also allows the analysis of embryo diseases before implantation via assisted reproduction procedures. Preimplantation genetic testing (PGT) is a clinical application of genetics that allows the examination of a limited number of embryo cells harvested by biopsy during in vitro embryo development. Assisted reproduction procedures involve the in vitro management of oocytes, spermatozoa or human embryos with the objective of achieving pregnancy (Zegers-Hochschild et al., 2009).

There currently are two types of PGT: preimplantation genetic testing for monogenic diseases (PGT-M) and preimplantation genetic testing of aneuploidies (PGT-A). PGT-M is designed to diagnose a specific Mendelian genetic disorder in the embryo for which the parents are at high risk, as in the case of multiple sclerosis. PGT-A is used to detect chromosomal aneuploidies (chromosome number alterations) and select embryos free from conditions such as Down syndrome (trisomy 21) (Farquhar & Marjoribanks, 2018; Traeger-Synodinos, 2017). Likewise, there have been significant advances in techniques used for genetic and chromosomal analysis using small amounts of DNA, including polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH), single nucleotide polymorphism (SNP) microarrays, comparative genomic hybridization (aCGH), and new generation sequencing (NGS) (Sullivan-Pyke & Dokras, 2018).

Embryo biopsy techniques

Polar body biopsy

The procedure involves the removal of the first and second polar bodies (cells resulting from meiosis I and II, which occur during oogenesis) prior to the initiation of embryo cleavage. Although polar body biopsy precludes the removal of embryo cells, it is limited by the fact that only maternal genes and chromosomes can be analyzed, thus excluding possible paternal contributions to the embryo. In addition, the amount of material obtained from a single cell is small and subject to limitations. For these reasons, this technique is not widely used (Sullivan-Pyke & Dokras, 2018; Verlinsky et al., 1990).

Blastomere biopsy from cleavage-stage embryos

The procedure consists of the removal of one or two cells (blastomeres) from an embryo in the cleavage stage, which has between six and eight cells. This technique is more advantageous than polar body biopsy, since maternal and paternal contributions can be analyzed. However, its limitations include the small amount of genetic material available for study and the presence of mosaicism (Treff & Fransasiak, 2017).

The presence of mosaicism in early-stage embryos is significant. A 1994 study described a 50% rate of mosaicism in cleavage-stage embryos. Recent studies claim that the proportion may be as high as 60%. Therefore, it is possible that the cell biopsied and tested during PGT-A might not represent the ploidy status of other embryo cells. Mosaicism potentiates the occurrence of diagnostic error and undesired clinical outcomes even in cases where a precise cellular genetic diagnosis has been performed (Brezina et al., 2016a; 2016b; Capalbo et al., 2013; Munné et al., 1994).

In addition to the high rates of mosaicism, removing cells at the cleavage stage may delay the development of the embryo to the blastocyst stage and decrease implantation rates and pregnancy (Scott et al., 2013a, 2013b).

Trophectoderm biopsy

The first birth after trophoderm biopsy and blastocyst-stage embryo transfer was reported in 2005, a decade after the first reports of births from blastomere biopsies (Kokkali et al., 2005). The development of sequential culture media allowed the success of embryo culture at the blastocyst stage (extended culture) and improved gestation rates after blastocyst transfer. The introduction of trophoderm biopsy in clinical practice has allowed the analysis of hundreds of cells with consequent accuracy improvements, since there is excellent genetic agreement between the internal cell mass of the embryo and the trophoderm. Although lower rates of mosaicism have been described in blastocyst-stage biopsies compared with cleavage-stage biopsies, mosaicism confined within this layer as described in the placenta in later stages, or variations within the trophoderm itself may lead to erroneous results (Gardner et al., 1998).

PGT-A

Aneuploidy is a common event in developing human embryos. It has been defined as any number of chromosomal copies other than diploidy affecting any of the 23 pairs of chromosomes. Examples include trisomy (an extra copy of a chromosome) and monosomy (one copy less). It is currently believed to occur in most embryos. Frequency of aneuploidy increases with maternal age. Aneuploidies are the most common cause of early miscarriage and usually halt embryo development before implantation (Breznia et al., 2012; Brezina & Kutteh, 2015; Ginsburg et al., 2011; Maxwell et al., 2016).

Pregnancy rates from IVF may be improved with the transfer of only euploid embryos to the uterus, resulting in higher implantation and lower miscarriage rates. PGT-A is an option for patients undergoing IVF and a particularly useful tool for couples with an aging female partner, individuals with a history of recurrent first trimester pregnan-
cy loss, and subjects with recurrent implantation failure in previous cycles of IVF (Sullivan-Pyke & Dokras, 2018).

ISH was initially used with in single-cell biopsies to evaluate a limited number of chromosomes most frequently associated with aneuploidy. However, in 2007 a prospective study reported that PGT-A did not increase pregnancy rates. Other authors have since found no benefit from PGT-A in terms of improved pregnancy outcomes. Therefore, the American Society for Reproductive Medicine (ASRM), the American College of Obstetrics and Gynecology (ACOG), and the European Society for Human Reproduction and Embryology (ESHRE) issued formal opinions discouraging the use of PGT-A (ACOG, 2009).

However, the development of single-cell genome amplification allowed the use of new technologies to quantify all 24 chromosomes, known as comprehensive chromosome testing (CCT), which includes microarrays with SNP matrices, aCGH, PCR, and NGS (Table 3) (Brezina et al., 2016a; 2016b; Sullivan-Pyke & Dokras, 2018). The clinical validation of the technologies involved in PGT-A must include an assessment of pregnancy and live birth rates (Sullivan-Pyke & Dokras, 2018).

A 2017 study showed that the transfer of euploid blastocysts identified after PGT-A by aCGH increased implantation rates (52.8% vs. 27.6%) and live birth (64.8% vs. 27.4%) compared with untested transferred blastocysts (Rubio et al., 2017). A retrospective study published in 2012 showed that the transfer of a single euploid embryo identified by trophoderm biopsy with qPCR resulted in higher pregnancy (55% vs. 41.8%) and lower miscarriage (10.5% vs. 24.8%) rates compared with untested embryo transfers (Forman et al., 2012). In a randomized clinical trial with 72 patients submitted to qPCR biopsy, implantation rates were higher in the case (79.8%) than in the control group (63.2%) (relative risk [RR] 1.26; 95% CI 1.04-1.39, p=0.001) (Scott et al., 2013a, 2013b).

There are advantages and disadvantages to screening for aneuploidies in embryos. The procedure is known to reduce the risk of aneuploidies detected during pregnancy and after birth. In addition, the identification and subsequent disposal of aneuploid embryos may decrease the cost of excess frozen embryos (ASRM & SMRU, 2018). When used to identify euploid embryos, PGT-A may shorten the time to pregnancy and allow the selection of embryos with greater chances of implantation. The procedure benefits older women, couples willing to have more children, and cancer patients. In addition, it offers the possibility of selecting the sex of the embryo (ASRM & SMRU, 2018). Potential drawbacks of the method include the need for increased resources and the use of up to eight cumulative hours of work for the embryology team in each biopsy. Not every embryo survives in culture media to the blastocyst stage required for trophoderm biopsy. However, they might have hypothetically resulted in live births if they had been transferred in the initial cleavage or blastocyst stage (Alikani et al., 2014; ASRM & SMRU, 2018).

Given the uncertainties around the supposed ability embryos have to autocorrect, the false-positive rates of PGT-A, and the accuracy of diagnoses of mosaicism, there is concern that embryos that might result in healthy babies are being discarded (Greco et al., 2015). A number of authors have looked into factors such as cost-effectiveness, time to gestation, use in specific subgroups of patients (recurrent miscarriage, previous implantation failure, advanced maternal age), and cumulative success rates tied to PGT-A. The American Society of Reproductive Medicine does not recommend the routine use of PGT-A in infertile patients (ASRM & SMRU, 2018). However, some patient subpopulations benefit from PGT-A, including couples experiencing unexplained recurrent pregnancy loss, couples with recurrent aneuploidy as the cause of miscarriage, couples with repeated implantation failures in IVF cycles, men with severe male factor infertility, couples undergoing PGT-M, and couples in fertility treatment looking for single-embryo transfers (Sullivan-Pyke & Dokras, 2018).

### PGT-M

Preimplantation genetic diagnosis determines whether embryo cells carry genetic anomalies associated with specific disorders known to affect one or both parents (Brezina & Kutteh, 2015). Gardner & Edwards (1968) were the first to publish on biopsies of trophectoderm cells of blastocyst-stage rabbit embryos to identify sex. This seminal animal study set the stage for further studies involving human embryo biopsy and PGT-M (Gardner & Edwards, 1968; Sullivan-Pyke & Dokras, 2018).

The first successful case of preimplantation genetic testing in humans was in fact a PGT-M. In 1990, the test was performed for adrenoleukodystrophy, an X-linked recessive condition. The cleavage-stage embryos were biopsied and PCR was performed to distinguish between male and female embryos. The female embryos were transferred and yielded two pregnancies. In 1992, PGT-M was performed after cleavage-stage embryo biopsy to detect a specific mutation associated with cystic fibrosis, an autosomal recessive disease, resulting in a live birth. Since then, PGT-M has been used to decrease the chances of propagation of known genetic diseases (Handyside et al., 1990; 1992; Zhao et al., 2011).

In monogenic diseases, PGT-M is used to detect specific pathogenic variations in the gene sequence associated with certain phenotypes. An example is the association of

| Method | Duration | Anomalies | Limitations |
|--------|----------|-----------|-------------|
| Array Comparative Genomic Hybridization (aCGH) | 12 hours | Aneuploidies Translocations | False positives. It does not detect mosaics. |
| Single Nucleotide Polymorphism Array (SNP) | 72 hours | Aneuploidies Translocations Parental Origin | Does not detect balanced translocations and mosaics. |
| Quantitative Polymerase Chain Reaction (qPCR) | 4 hours | Aneuploidies | Does not detect segmental aneuploidies, translocations and mosaics. |
| Next-Gen Sequencing (NGS) | < 24 hours | Aneuploidies Mosaics Monogenic Diseases Translocations | Limited capacity to detect balanced translocations |

Source: Sullivan-Pyke & Dokras, 2018.
the ΔF508 mutation and the development of cystic fibrosis (Berger & Baker, 2014). Many genetic variations produce heterogeneous phenotypes in different people due to variable penetrance and expression. However, it is appropriate to offer PGT-M when a parent is known to have a specific DNA variation that may have deleterious effects on the phenotype of their offspring (Brezina & Kutteh, 2015).

Before performing the test, the inheritance pattern of the genetic variation in question must be defined. For example, cystic fibrosis is an autosomal recessive disorder. Therefore, if one spouse has a single mutation ΔF508 and the other does not have any known genetic variation that predisposes their offspring to cystic fibrosis, PGT-M is not indicated. However, if both parents carry a mutation for cystic fibrosis, PGT-M is indicated. In contrast, dominant autosomal disorders usually require testing, even if only one parent has the disease - the case in Huntington's disease. Similarly, women with X-linked recessive disorders should also be counseled about the availability of the test (Berger & Baker, 2014; Janssens et al., 2014; Van Rij et al., 2012; Verlinsky et al., 1992; 2004).

**Monogenic diseases**

Genotyping and direct sequencing are the most common methods used to identify monogenic diseases. As only one or a few cells are harvested during biopsy, both techniques require DNA amplification. This has traditionally been done through PCR protocols (Berger & Baker, 2014). More recently, however, some centers have achieved high-quality DNA amplification for monogenic diseases and a screening of 23 pairs of chromosomes using a modified genome-wide amplification protocol (Rechitsky et al., 2013).

A recent technology called karyomapping uses broad genomic linkage analysis to compare SNPs of the couple to SNPs of family members with known genetic statuses to identify the combination of SNP alleles associated with a chromosome carrying the genetic mutation. In this method, a monogenic disease can be identified without knowledge of the specific associated genetic mutation. Karyomapping has shown high accuracy and presents 97.7% agreement with conventional PGT-M without the need to design specific tests for any disease (Natesan et al., 2014).

In addition to the detection of cystic fibrosis and Huntington's disease, Table 4 lists other possible diseases to be analyzed with PGT-M (Sullivan-Pyke & Dokras, 2018).

**Chromosomal alterations**

PGT-M can also be used in parents with known structural chromosome aberrations. These aberrations may be present in the form of translocations (reciprocal or Robertsonian) or inversions (mainly pericentric, but also paracentric to a lesser degree) (Escudero et al., 2008).

Reciprocal translocations usually involve the breaking and reunion of two different chromosomes with exchange of the acentric terminal segments. Robertsonian translocations involve the fusion of two acrocentric chromosomes and the loss of the short arms of these chromosomes. It is worth mentioning that the short arms of acrocentric chromosomes (pairs 13, 14, 15, 21, and 22) supposedly contain little genetic information of relevance. Chromosome inversions are two breaks in the same chromosome, either in the same arm (paracentric) or one in each arm (pericentric), with inversion of the segment between the points of interruption (Escudero et al., 2008; Lim et al., 2008).

People with such structural chromosomal aberrations usually have a normal phenotype because all the necessary genetic coding is present, though not organized in the standard way. These aberrations are therefore referred to as translocations or balanced inversions or inversions. However, the descendants of these individuals are at higher risk of unbalanced translocations or inversions (Escudero et al., 2008; Lim et al., 2008).

The chances of a child having an unbalanced karyotype depend on the type of structural chromosomal aberration of the parents and, possibly, the gender of the parent carrier. Unbalanced translocations in offspring usually result in pregnancy loss or severe birth defects (Bint et al., 2011).

Structural chromosomal aberrations are present in less than 1% of phenotypically normal adults, but are detected in one partner in 2-5% of couples with a history of recurrent pregnancy loss. However, most American specialists and medical societies recommend parental karyotyping as part of the diagnostic investigation of couples with recurrent miscarriages (ASRM, 2012; Brezina & Kutteh, 2014).

**Table 4. Monogenic diseases diagnosed by PGT-M**

| Dominant Autosomal Diseases | Recessive Autosomal Diseases | X-Linked Diseases |
|----------------------------|----------------------------|------------------|
| Familial adenomatous polyposis | Sickle-cell anemia | Duchenne muscular dystrophy |
| Huntington’s disease | Spinal muscular atrophy | Becker muscular dystrophy |
| Breast cancer (BRCA1/BRCA2) mutations | Joubert syndrome | Chronic granulomatous disease |
| Retinoblastoma | Osteogenesis imperfecta | Fragile X syndrome |
| Kell antigen system | Gaucher disease | X-linked adrenoleukodystrophy |
| Myotonic dystrophy | Fanconi syndrome | |
| Peutz-Jeghers syndrome | Propionic acidemia | |
| Dilated cardiomyopathy | Cystic fibrosis | |
| Lynch syndrome | Homocystinuria | |
| Crouzon syndrome | Usher syndrome | |
| Polycystic kidney disease | Familial dysautonomia | |
| Brugada syndrome | Methylmalonic acidemia | |
| Multiple endocrine neoplasia | Alpha-1 antitrypsin deficiency | |
| Hereditary multiple osteochondromas | | |

Source: Sullivan-Pyke & Dokras, 2018.
Conventional techniques for detecting chromosomal aberrations use FISH, but the method has severe limitations. These include errors resulting from hybridization and errors tied to the complexity of the testing procedure, which increase significantly with the test not performed by a trained specialist. In addition, FISH generally does not assess the ploidy status of chromosomes that are not part of the known structural aberration. In many patients with such aberrations, embryos may be balanced for the chromosome in question, but still harbor aneuploidies in other chromosomes. Therefore, technologies based on SNP and NGS are currently preferred (Brezina & Kutteh, 2014; Harper & Sengupta, 2012; Sullivan-Pyke & Dokras, 2018).

Although carriers of recessive conditions and carriers of balanced chromosome rearrangements do not have genetic disease, their offspring may be at increased risk of being affected. PGT-M can help individuals in this situation to have the same chance of bearing a healthy child as the general population. The technique is an option among other available reproductive options, which also include gamete donation and adoption (Brezina & Kutteh, 2014). The use of PGT-M to prevent the spread of parental disorders of genetic origin is recognized by professionals and international societies as an appropriate medical procedure (Ferraretti et al., 2013; Ginsburg et al., 2011; Harton et al., 2011).

The number of patients eligible for the test is likely to increase in the coming decades, as the number of diseases with an identifiable genetic cause continues to increase. Currently, many of the mutations assessed by PGT-M lead to specific syndromes, such as cystic fibrosis. However, it is now known that many common diseases, such as diabetes mellitus, hypertension, and breast cancer, are associated with certain gene sequences or mutations. In the future, the test might be used to detect genetic sequences or mutations that predispose to certain diseases (Brezina, 2013; Cirulli & Goldstein, 2010; Harper et al., 2013).

The ethics of embryo biopsy in Brazil
Resolution 2168/2017 of the Brazilian Board of Medicine regulates preimplantation genetic testing at a federal level. These technologies cannot be used to select the sex of any other biological characteristic of the future child, except in cases in which this is done to avoid diseases in the offspring. After the embryos have been selected for transfer, the remaining embryos can be discarded or donated for research upon written consent by the couple. Preimplantation genetic testing can also be used to type embryo HLA in order to select embryos that are HLA-compatible with a sibling affected by a disease which treatment is stem cell transplantation (CFM, 2017).

CONCLUSION
The interface between genetics and human reproduction has become increasingly larger, as knowledge about the genetic causes of infertility grows and the availability of genetic testing in daily clinical practice increases. Genetic tests often enable the identification of the cause of infertility and increase the success rate of fertility treatments. It is also an important tool in counseling individuals at risk of early loss of reproductive capacity and couples with genetic alterations. Genetic testing should also be considered in investigations of recurrent pregnancy loss and as part of gamete donor screening procedures. Preimplantation genetic testing is fundamental to avoid the occurrence of severe diseases in children of couples at increased risk. However, ordering tests and performing treatment must always be based on sound research performed to evaluate efficacy, safety (including long-term), and cost-effectiveness. This continually evolving field requires close communication between clinical genetics, IVF teams, and patients to ensure that everyone is fully informed and able to make well thought out choices.

The success rates of assisted reproductive technology procedures are increasing, and genetic diagnosis is a fundamental element in the treatment of infertile couples. Further discussions are required about which procedures are clinically and ethically acceptable and how they should be regulated.

CONFLICT OF INTEREST
None.

Corresponding author:
Vivian de Oliveira Rodrigues
Federal University of Juiz de Fora
Juiz de Fora, MG, Brazil.
E-mail: vivian.oro@outlook.com

REFERENCES
Abir R, Fisch B, Nahum R, Orvieto R, Nitke S, Ben Rafael Z. Turner's syndrome and fertility: current status and possible putative prospects. Hum Reprod Update. 2001;7:603-10. PMID: 11727869 DOI: 10.1093/humupd/7.6.603

ACOG Committee Opinion No. 430: preimplantation genetic screening for aneuploidy. Obstet Gynecol. 2009;113:766-7. PMID: 19300349 DOI: 10.1097/AOG.0b013e31819e9f05

Alikani M, Go KJ, McCaffrey C, McCulloh DH. Comprehensive evaluation of contemporary assisted reproduction technology laboratory operations to determine staffing levels that promote patient safety and quality care. Fertil Steril. 2014;102:1350-6. PMID: 25226853 DOI: 10.1016/j.fertnstert.2014.07.1246

Asero P, Calogero AE, Condorelli RA, Mongioi’ L, Vicari E, Lanzafame F, Crisci R, La Vignera S. Relevance of genetic investigation in male infertility. J Androl. 2014;37:415-27. PMID: 24458834 DOI: 10.1007/s40618-014-0053-1

ASRM - Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012;98:1103-11. PMID: 22835448 DOI: 10.1016/j.fertnstert.2012.06.048

ASRM - Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril. 2015;103:e18-25. PMID: 25597249 DOI: 10.1016/j.fertnstert.2014.12.103

ASRM - Practice Committee of the American Society for Reproductive Medicine; SMRU - the Society for Male Reproduction and Urology. Evaluation of the azoospermic male: a committee opinion. Fertil Steril. 2018;109:777-82. PMID: 29778371 DOI: 10.1016/j.fertnstert.2018.01.043

Bararsoain M, Barrenetxea G, Huerta I, Télez M, Carrillo A, Pérez C, Criado B, Arrieta I. Study of FMR1 gene association with ovarian dysfunction in a sample from the Basque Country. Gene. 2013;521:145-9. PMID: 23537988 DOI: 10.1016/j.gene.2013.03.032

Berger VK, Baker VL. Preimplantation diagnosis for single gene disorders. Semin Reprod Med. 2014;32:107-13. PMID: 24515905 DOI: 10.1055/s-0033-1363552
Bint SM, Ogilvie CM, Flinter FA, Khalaf Y, Scriven PN. Meiotic segregation of Robertsonian translocations ascertained in cleavage-stage embryos-implications for preimplantation genetic diagnosis. Hum Reprod. 2011;26:1575-84. PMID: 21441546 DOI: 10.1093/humrep/der080

Bonomi M, Rochira V, Pasquali D, Balerca G, Jannini E, Ferlin A; Klinefelter ItaliaN Group (KING). Klinefelter syndrome (KS): genetics, clinical phenotype and hypogonadism. J Endocrinol Invest. 2017;40:123-34. PMID: 27644703 DOI: 10.1007/s40618-016-0541-6

Brasil. Conselho Federal de Medicina - CFM. Resolução nº 2.168/2017. Brasília: Diário Oficial da União; 2017.

Brezina PR, Brezina DS, Kearns WG. Preimplantation genetic testing. BMJ. 2012;345:e5908. PMID: 22990995 DOI: 10.1136/bmj.e5908

Brezina PR. Preimplantation genetic testing in the 21st century: uncharted territory. Clin Med Insights Reprod Health. 2013;7:17-21. PMID: 24453515 DOI: 10.4137/CMRH.S10914

Brezina PR, Kutteh WH. Classic and cutting-edge strategies for the management of early pregnancy loss. Obstet Gynecol Clin North Am. 2014;41:1-18. PMID: 24491981 DOI: 10.1016/j.ogc.2013.10.011

Brezina PR, Kutteh WH. Clinical applications of preimplantation genetic testing. BMJ. 2015;350:g7611. PMID: 25697663 DOI: 10.1136/bmj.g7611

Brezina PR, Anchan R, Kearns WG. Preimplantation genetic testing for aneuploidy: what technology should you use and what are the differences? J Assist Reprod Genet. 2016a;33:823-32. PMID: 27299602 DOI: 10.1007/s10815-016-0740-2

Brezina PR, Kutteh WH, Bailey AP, Ke RW. Preimplantation genetic screening (PGS) is an excellent tool, but not perfect: a guide to counseling patients considering PGS. Fertil Steril. 2016b;105:49-50. PMID: 26493116 DOI: 10.1016/j.fertnstert.2015.09.042

Bulun SE. Endometriosis. New Engl J Med. 2009;360:268-79. PMID: 19144942 DOI: 10.1056/NEJMr0804690

Bulun SE, Monsivais D, Kakinuma T, Furukawa Y, Bernardi L, Pavone ME, Dyson M. Molecular biology of endometriosis: from aromatase to genomic abnormalities. Semin Reprod Med. 2015;33:220-4. PMID: 26036904 DOI: 10.1055/s-0035-154053

Capalbo A, Wright G, Elliott T, Ubaldi FM, Rienzi L, Nagy ZP. FISH reanalysis of inner cell mass and trophectoderm samples of previously array-CGH screened blastocysts shows high accuracy of diagnosis and no major diagnostic impact of mosaicism at the blastocyst stage. Hum Reprod. 2013;28:2298-307. PMID: 23739221 DOI: 10.1093/humrep/det245

Chapman C, Cree L, Shelling AN. The genetics of premature ovarian failure: current perspectives. Int J Womens Health. 2015;7:799-810. PMID: 26445561 DOI: 10.2147/IJWH.S64024

Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, Li Z, You L, Zhao J, Liu J, Liang X, Zhao X, Zhao J, Sun Y, Zhang B, Jiang H, Zhao D, Bian Y, Gao X, Geng L, et al. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16. 3, 2p21 and 9q33. 3. Nat Genet. 2011;43:55-9. PMID: 21151128 DOI: 10.1038/ng.732

Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet. 2010;11:415-25. PMID: 20479773 DOI: 10.1038/nrg2779

Claustres M, Guittard C, Bozon D, Chevalier F, Verlingue C, Ferec C, Girodon E, Cazeneuve C, Bienvenu T, Lalau G, Dumur V, Feldmann D, Bieth E, Blayau M, Clavel C, Creveaux I, Malinge MC, Monnier N, Malzac P, Mitter H, et al. Spectrum of CFTR mutations in cystic fibrosis and in congenital absence of the vas deferens in France. Hum Mutat. 2000;16:143-56. PMID: 10923036 DOI: 10.1002/1098-1004(20000816)16:2<143::AID-HUMU7>3.0.CO;2-j

Cordts EB, Christofolini DM, dos Santos AA, Bianco B, Barbosa CP. Genetic aspects of premature ovarian failure: a literature review. Arch Gynecol Obstet. 2011;283:635-43. PMID: 21188402 DOI: 10.1007/s00404-010-1815-4

De Geyter C, M’Rabet N, De Geyter J, Zürcher S, Moffat R, Bösch N, Zhang H, Heinimann K. Similar prevalence of expanded CGG repeat lengths in the fragile X mental retardation 1 gene among infertile women and among women with proven fertility: a prospective study. Genet Med. 2014;16:374-8. PMID: 24113347 DOI: 10.1038/gim.2013.146

Dixit H, Rao L, Padmalatha V, Raseswari T, Kapu AK, Pandba B, Murthy K, Tosh D, Nallari P, Deenadayal M, Gupta N, Chakrabarty B, Singh L. Genes governing premature ovarian failure. Reprod Biomed. 2010;20:724-40. PMID: 20382564 DOI: 10.1016/j.rbmo.2010.02.018

Escudero T, Estop A, Fischer J, Munne S. Preimplantation genetic diagnosis for complex chromosome rearrangements. Am J Med Genet A. 2008;146A:1662-9. PMID: 18536046 DOI: 10.1002/ajmg.a.32286

Farquhar C, Marjoribanks J. Assisted reproductive technology: an overview of Cochrane Reviews. Cochrane Database Syst Rev. 2018;8:CD010537. PMID: 30117155 DOI: 10.1002/14651858.CD010537.pub5

Ferraretti AP, Goossens V, Kupka M, Bhattacharya S, de Mouzon J, Castilla JA, Erb K, Korsak V, Nyboe Andersen A; European IVF-Monitoring (EIM) Consortium for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2009: results generated from European registers by ESHRE. Hum Reprod. 2013;28:2318-31. PMID: 23842560 DOI: 10.1093/humrep/det278

Field PD, Martin NJ. CFTR mutation screening in an assisted reproductive clinic. Aust N Z Obstet Gynaecol. 2011;51:536-9. PMID: 21875427 DOI: 10.1111/j.1479-828X.2011.01348.x
Forman E, Tao X, Ferry K, Taylor D, Treff NR, Scott RT Jr. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum Reprod. 2012;27:1217-22. PMID: 22343551 DOI: 10.1093/humrep/des020

Gardner R, Edwards RG. Control of the sex ratio at full term in the rabbit by transferring sexed blastocysts. Nature. 1968;218:346-9. PMID: 5649672 DOI: 10.1038/218346a0

Gardner DK, Vella P, Lane M, Wagley L, Schlenker T, Schoolcraft WB. Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. Fertil Steril. 1998;69:84-8. PMID: 9457939 DOI: 10.1016/S0015-0282(97)00438-X

Ginsburg ES, Baker VL, Racowsky C, Wantman E, Goldfarb J, Stern JE. Use of preimplantation genetic diagnosis and preimplantation genetic screening in the United States: a Society for Assisted Reproductive Technology Writing group paper. Fertil Steril. 2011;96:865-8. PMID: 21872229 DOI: 10.1016/j.fertnstert.2011.07.1139

Gleicher N, Weghofer A, Barad DH. Ovarian reserve determinations suggest new function of FMR1 (fragile X gene) in regulating ovarian ageing. Reprod Biomed. 2010;20:768-75. PMID: 20378415 DOI: 10.1016/j.rbmo.2010.02.020

Godo A, Blanco J, Vidal F, Anton E. Accumulation of numerical and structural chromosome imbalances in spermatozoa from reciprocal translocation carriers. Hum Reprod. 2013;28:840-9. PMID: 23250926 DOI: 10.1093/humrep/des431

Gogusev J, Bouquet de Jolinière J, Telvi L, Doussau M, du Manoir S, Stojkoski A, Levardon M. Detection of DNA copy number changes in human endometriosis by comparative genomic hybridization. Hum Genet. 1999;105:444-51. PMID: 10598811 DOI: 10.1007/s004399900174

Greco E, Minasi MG, Fiorentino F. Healthy Babies after Intrauterine Transfer of Mosaic Aneuploid Blastocysts. N Engl J Med. 2015;373:2089-90. PMID: 26581010 DOI: 10.1056/NEJMct1500421

Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature. 1990;344:768-70. PMID: 2330030 DOI: 10.1038/344768a0

Handyside AH, Lesko JG, Tarin JJ, Winston RM, Hughes MR. Birth of a normal girl after in vitro fertilization and preimplantation diagnostic testing for cystic fibrosis. N Engl J Med. 1992;327:905-9. PMID: 1381054 DOI: 10.1056/NEJM199209243271301

Harper JC, Sengupta SB. Preimplantation genetic diagnosis: state of the art 2011. Hum Genet. 2012;131:175-86. PMID: 21748341 DOI: 10.1007/s00439-011-1056-z

Harper JC, Geraedts J, Borry P, Cornel MC, Dondorp W, Gianaroli L, Hartog G, Milichich T, Kääriäinen H, Liebaers I, Morris M, Sequeiros J, Sermon K, Shenfield F, Skirton H, Soini S, Spits C, Veiga A, Vermeshech JR, Viville S, et al. Current issues in medically assisted reproduction and genetics in Europe: research, clinical practice, ethics, legal issues and policy. European Society of Human Genetics and European Society of Human Reproduction and Embryology. Eur J Hum Genet. 2013;21:S1-21. PMID: 24225486 DOI: 10.1038/ejhg.2013.219

Harper JC, Aittomäki K, Borry P, Cornel MC, de Wert G, Dondorp W, Geraedts J, Gianaroli L, Kettersen K, Liebaers I, Lundin K, Mertes H, Morris M, Pennings G, Sermon K, Spits C, Soini S, van Montfoort APA, Veiga A, Vermeshech JR, et al. Recent developments in genetics and medically-assisted reproduction: from research to clinical applications. Eur J Hum Genet. 2018;26:12-33. PMID: 29199274 DOI: 10.1038/s41431-017-0016-z

Harton G, Magli M, Lundin K, Montag M, Lemmen J, Harper JC; European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium/Embryology Special Interest Group. ESHRE PGD Consortium/Embryology Special Interest Group-best practice guidelines for polar body and embryo biopsy for preimplantation genetic diagnosis/screening (PGD/PGS). Hum Reprod. 2011;26:41-6. PMID: 20966459 DOI: 10.1093/humrep/der265

He C, Kraft P, Chen C, Buring JE, Paré G, Hankinson SE, Chanock SJ, Rider PM, Hunter DJ, Chasan DI. Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. Nat Genet. 2009;41:724-8. PMID: 19448621 DOI: 10.1038/ng.385

Hops CV, Mielenk A, Goldstein M, Palermo GD, Rosenwaks Z, Schlegel PN. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum Reprod. 2003;18:1660-5. PMID: 12871878 DOI: 10.1093/humrep/deq348

Janssens S, De Paepe A, Borry P. Attitudes of health care professionals toward carrier screening for cystic fibrosis. A review of the literature. J Community Genet. 2014;5:13-29. PMID: 23275180 DOI: 10.1007/s12687-012-0131-z

Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Schlegel PN. Detection of sperm in men with Y chromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum Reprod. 2003;18:1660-5. PMID: 12871878 DOI: 10.1093/humrep/deq348

Kara E, Simoni M. Genetic screening for infertility: When should it be done? Middle East Fertil Soc J. 2010;15:139-45. DOI: 10.1016/j.mefs.2010.06.002

Kawamura K, Kawamura N, Hsueh AJ. Activation of dormant follicles: a new treatment for premature ovarian failure? Curr Opin Obstet Gynecol. 2016;28:217-22. PMID: 27022685 DOI: 10.1097/GCO.0000000000000268
Klinefelter HF Jr, Reifenstein EC Jr, Albright F Jr. Syndrome Characterized by Gynecomastia, Aspermatogenesis without A-Leydigism, and Increased Excretion of Follicle-Stimulating Hormone. J Clin Endocrinol Metab. 1942;2:615-27.

Kokkali G, Vrettou C, Traeger-Synodinos J, Jones GM, Cram DS, Stavrou D, Trounson AO, Kanavakis E, Pantos K. Birth of a healthy infant following trophoderm biopsy from blastocysts for PGD of beta-thalassaemia major. Hum Reprod. 2005;20:1855-9. PMID: 15878929 DOI: 10.1093/humrep/deh893

Kosova G, Urbanek M. Genetics of the polycystic ovary syndrome. Mol Cell Endocrinol. 2013;373:29-38. PMID: 23079471 DOI: 10.1016/j.mce.2012.10.009

Krausz C, Chianese C. Genetic testing and counseling for male infertility. Curr Opin Endocrinol Diabetes Obes. 2014;21:244-50. PMID: 24739313 DOI: 10.1097/MED.0000000000000058

Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter’s syndrome. Lancet. 2004;364:273-83. PMID: 15262106 DOI: 10.1016/S0140-6736(04)6678-6

Li Z, Haines CJ, Han Y. “Micro-deletions” of the human Y chromosome and their relationship with male infertility. J Genet Genomics. 2008;35:193-9. PMID: 18439975 DOI: 10.1016/S1673-8527(08)60027-2

Lim CK, Cho JW, Song IO, Kang IS, Yoon YD, Jun JH. Estimation of chromosomal imbalances in preimplantation embryos from preimplantation genetic diagnosis cycles of reciprocal translocations with or without acrocentric chromosomes. Fertil Steril. 2008;90:2144-51. PMID: 18440525 DOI: 10.1016/j.fertnstert.2007.10.035

Lubs HA. A marker X chromosome. Am J Hum Genet. 1969;21:231-44. PMID: 5794013

Marshburn PB. Counseling and diagnostic evaluation for the infertile couple. Obstet Gynecol Clin North Am. 2015;42:1-14. PMID: 25681836 DOI: 10.1016/j.ogc.2014.10.001

Maxwell SM, Colls P, Hodes-Wertz B, McCulloh DH, McCaffrey C, Wells D, Munné S, Grifo JA. Why do euploid embryos miscarry? A case-control study comparing the rate of aneuploidy within presumed euploid embryos that resulted in miscarriage or live birth using next-generation sequencing. Fertil Steril. 2016;106:1414-9.e5. PMID: 27692437 DOI: 10.1016/j.fertnstert.2016.08.017

Munné S, Weier HU, Grifo J, Cohen J. Chromosome mosaicism in human embryos. Biol Reprod. 1994;51:373-9. PMID: 7803609 DOI: 10.1095/biolreprod51.3.373

Natesan SA, Bladen AJ, Coskun S, Qubbaj W, Prates R, Munne S, Coonen E, Dreesen JC, Stevens SJ, Paulussen AD, Stock-Myer SE, Wilton LJ, Jaroudi S, Wells D, Brown AP, Handyside AH. Genome-wide karyomapping accurately identifies the inheritance of single-gene defects in human preimplantation embryos in vitro. Genet Med. 2014;16:838-45. PMID: 24810687 DOI: 10.1038/gim.2014.45

Oates RD, Silber S, Brown LG, Page DC. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. Hum Reprod. 2002;17:2813-24. PMID: 12407032 DOI: 10.1093/humrep/17.11.2813

Painter JN, Anderson CA, Nyholt DR, Magregor S, Lin J, Lee SH, Lambert A, Zhao ZZ, Roseman F, Guo Q, Gordon SD, Wallace L, Henders AK, Visscher PM, Kraft P, Martin NG, Morris AP, Treloar SA, Kennedy SH, Misserm SA, et al. Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis. Nat Genet. 2011;43:51-4. PMID: 21151130 DOI: 10.1038/ng.731

Perry JR, Corre T, Esko T, Chasman DJ, Fischer K, Franceschini N, He C, Kutilak Z, Mangino M, Rose LM, Vernon Smith A, Stolk L, Sulem P, Weedon MN, Zhuang W, Arnold A, Ashworth A, Bergmann S, Buring JE, Burri A, et al. A genome-wide association study of early menopause and the combined impact of identified variants. Hum Mol Genet. 2013;22:1465-72. PMID: 23307926 DOI: 10.1093/hmg/dd51

Piomboni P, Stendardi A, Gambela L. Chromosomal aberrations and aneuploidies of spermatozoa. Adv Exp Med Biol. 2014;791:27-52. PMID: 23955671 DOI: 10.1007/978-1-461-7783-9_3

Pu D, Xing Y, Gao Y, Gu L, Wu J. Gene variation and premature ovarian failure: a meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2014;182:226-37. PMID: 25445105 DOI: 10.1016/j.ejogrb.2014.09.036

Qin Y, Sun M, You L, Wei D, Sun J, Liang X, Zhang B, Jiang H, Xu J, Chen ZJ. ESRI, HK3 and BRSK1 gene variants are associated with both age at natural menopause and premature ovarian failure. Orphanet J Rare Dis. 2012;7:5. PMID: 22248077 DOI: 10.1186/1750-1172-7-5

Rechitsky S, Verlinsky O, Kulev A. PGD for cystic fibrosis patients and couples at risk of an additional genetic disorder combined with 24-chromosome aneuploidy testing. Reprod Biomed Online. 2013;26:420-30. PMID: 23523379 DOI: 10.1016/j.rbmo.2013.01.006

Rives N. Y chromosome microdeletions and alterations of spermatogenesis, patient approach and genetic counseling. Ann Endocrinol (Paris). 2014;75:117-26. PMID: 24786699 DOI: 10.1016/j.ando.2014.04.001

Rubio C, Belliver J, Rodrigo L, Castillón G, Guillén A, Vidal C, Giles J, Ferrando M, Cabanillas S, Remohi J, Pellicer A, Simón C. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril. 2017;107:1122-9. PMID: 28433371 DOI: 10.1016/j.fertnstert.2017.03.011

Schulbreider A, McQueen DB, Lee SM, Allon R, Uhler ML, Davie J, Feinberg EC. Diminished ovarian reserve is not observed in infertility patients with high normal CGG repeats on the fragile X mental retardation 1 (FMR1) gene. Hum Reprod. 2015;30:2686-92. PMID: 26345686 DOI: 10.1093/humrep/dev220
Scott RT Jr, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, Tao X, Treff NR. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril. 2013a;100:697-703. PMID: 23731996 DOI: 10.1016/j.fertnstert.2013.04.035

Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril. 2013b;100:624-30. PMID: 23773313 DOI: 10.1016/j.fertnstert.2013.04.039

Shelling AN. Premature ovarian failure. Reproduction. 2010;140:633-41. PMID: 20716613 DOI: 10.1530/REP-09-0567

Stolk L, Zhai G, van Meurs JB, Verbiest MM, Visser JA, Estrada K, Rivadeneira F, Williams FM, Cherkas L, Deloukas P, Soranzo N, de Keyzer JJ, Pop VJ, Lips P, Lebrun CE, van der Schouw YT, Grobbee DE, Witteman J, Hofman A, Pols HA, et al. Loci at chromosomes 13, 19 and 20 influence age at natural menopause. Nat Genet. 2009;41:645-7. PMID: 19448619 DOI: 10.1038/ng.387

Suganthi R, Vijesh VV, Vandana N, Fatimah Ali Benazir J. Y chromosomal microdeletion screening in the workup of male infertility and its current status in India. Int J Fertil Steril. 2014;7:253-66. PMID: 24520494

Sullivan AK, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, Yadav-Shah M, Sherman SL. Association of FMR1 repeat size with ovarian dysfunction. Hum Reprod. 2005;20:402-12. PMID: 15608041 DOI: 10.1038/humrep.deh635

Sullivan-Pyke C, Dokras A. Preimplantation Genetic Screening and Preimplantation Genetic Diagnosis. Obstet Gynecol Clin North Am. 2018;45:113-25. PMID: 29428279 DOI: 10.1016/j.ogc.2017.10.009

Tartaglia NR, Howell S, Sutherland A, Wilson R, Wilson L. A review of trisomy X (47,XXX). Orphanet J Rare Dis. 2010;5:8. PMID: 20459843 DOI: 10.1186/1750-1172-5-8

Traeger-Synodinos J. Pre-implantation genetic diagnosis. Best Pract Res Clin Obstet Gynaecol. 2017;39:74-88. PMID: 28099679 DOI: 10.1016/j.bpobgyn.2017.10.009

Treff NR, Franasiak JM. Detection of segmental aneuploidy and mosaicism in the human preimplantation embryo: technical considerations and limitations. Fertil Steril. 2017;107:27-31. PMID: 27816233 DOI: 10.1016/j.fertnstert.2016.09.039

Trelloar SA, Wicks J, Nyholt DR, Montgomery GW, Bahlo M, Smith V, Dawson G, Mackay IJ, Weeks DE, Bennett ST, Carey A, Ewen-White KR, Duffy DL, O’connor DT, Barlow DH, Martin NG, Kennedy SH. Genome wide linkage study in 1,176 affected sister pair families identifies a significant susceptibility locus for endometriosis on chromosome 10q26. Am J Hum Genet. 2005;77:365-76. PMID: 16080113 DOI: 10.1086/432960

Tüttelmann F, Simoni M. Current Recommendations for Genetic Testing in Male Infertility. Eur Urol Rev. 2008;3:88-92.

Uno S, Zembutsu H, Hirasawa A, Takahashi A, Kubo M, Akahane T, Aoki D, Kamatani N, Hirata K, Nakamura Y. A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese. Nat Genet. 2010;42:707-10. PMID: 20601957 DOI: 10.1038/ng.612

Van Rij MC, De Rademaeker M, Moutou C, Dreessen JC, De Rycke M, Liebbers I, Geraedts JP, De Die-Smulders CE, Viville S; BruMastra PGD working group. Preimplantation genetic diagnosis (PGD) for Huntington’s disease: the experience of three European centres. Eur J Hum Genet. 2012;20:368-75. PMID: 22071896 DOI: 10.1038/ejhg.2011.202

Venkatesh T, Suresh PS, Tsutsuji R. New insights into the genetic basis of infertility. Appl Clin Genet. 2014;7:235-43. PMID: 25506236 DOI: 10.2147/TACG.S40809

Verlinsky Y, Ginsberg N, Lifchez A, Valle J, Moise J, Strom CM. Analysis of the first polar body: preconception genetic diagnosis. Hum Reprod. 1990;5:826-9. PMID: 2266156 DOI: 10.1093/oxfordjournals.humrep.a137192

Verlinsky Y, Rechtsik Y, Eviskov S, White M, Cieslak J, Lifchez A, Valle J, Moise J, Strom CM. Preconception and preimplantation diagnosis for cystic fibrosis. Prenat Diagn. 1992;12:103-10. PMID: 1553355 DOI: 10.1002/pd.1970120205

Verlinsky Y, Cohen J, Munne S, Gianaroli L, Simpson JL, Ferraretti AP, Kuliev A. Over a decade of experience with preimplantation genetic diagnosis: a multicenter report. Fertil Steril. 2004;82:292-4. PMID: 15302270 DOI: 10.1016/j.fertnstert.2003.09.082

Vloeborghs V, Verheyen G, Santos-Ribeiro S, Staessen C, Verpoest W, Gies I, Tournaye H. Is genetic fatherhood within reach for all azoospermic Klinefelter men? PLoS One. 2018;13:e0200300. PMID: 30044810 DOI: 10.1371/journal.pone.0200300

Vogt PH, Edelmann A, Kirsch S, Henegariu O, Hirschmann P, Kiesewetter F, Köhn FM, Schill WB, Farah S, Ramos C, Hartmann M, Hartschuh W, Meschede D, Behre HM, Castel A, Nieschlag E, Weidner W, Grüne HJ, Jung A, Engel W, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum Mol Genet. 1996;5:933-43. PMID: 8817327 DOI: 10.1093/hmg/5.7.933

Wosnitzer MS, Paduch DA. Endocrinological issues and hormonal manipulation in children and men with Klinefelter syndrome. Am J Med Genet C Semin Med Genet. 2013;163C:16-26. PMID: 23335092 DOI: 10.1002/ajmg.c.31350

Wosnitzer M, Goldstein M, Hardy MP. Review of Azoospermia. Spermatogenesis. 2014;4:e28218. PMID: 25105055 DOI: 10.4161/spmg.28218

Yin B, Zhu Y, Wu T, Shen S, Zeng Y, Liang D. Clinical outcomes for couples containing a reciprocal chromosome translocation carrier without preimplantation genetic diagnosis. Int J Gynaecol Obstet. 2017;136:304-8. PMID: 28099679 DOI: 10.1016/ijgo.12062
Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, Sullivan E, van der Poel S; International Committee for Monitoring Assisted Reproductive Technology; World Health Organization. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. Hum Reprod. 2009;24:2683-7. PMID: 19801627 DOI: 10.1093/humrep/dep343

Zhao Y, Brezina P, Hsu CC, Garcia J, Brinsden PR, Wallach E. In vitro fertilization: four decades of reflections and promises. Biochim Biophys Acta. 2011;1810:843-52. PMID: 21605628 DOI: 10.1016/j.bbagen.2011.05.001

Zorrilla M, Yatsenko AN. The Genetics of Infertility: Current Status of the Field. Curr Genet Med Rep. 2013;1:247-60. PMID: 24416713 DOI: 10.1007/s40142-013-0027-1