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Review

To Opt or Not to Opt for Preimplantation and/or Prenatal Genetic Testing?

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Abstract:

Both preimplantation genetic testing (PGT) and prenatal diagnosis (PND) are powerful tools to tackle the transmission of inherited disorders carried by families from generation to generation. Here, we present an overview of the current landscape of both methods and identify the pros and cons of each of these reproductive options. In prenatal testing, non-invasive diagnostic methods have become available as an alternative for invasive diagnosis. Different genome sequencing strategies have now been introduced that enable early detection of trisomies as well as inherited chromosomal and monogenic abnormalities. This development is attractive for some couples who would have opted for PGT previously. PGT is still a safe, albeit less efficient, approach to preventing the transmission of chromosomal, Mendelian and mitochondrial disorders. Furthermore, in some Mendelian disorders, assisted reproduction for female carriers can result in maternal complications. The choice between PGT and PND is not always straightforward, particularly in the case of disorders with a variable phenotype. For some people, this does not justify termination of the pregnancy, while others prefer to undergo imaging during the pregnancy because it can reveal better phenotypic information.

Keywords
Preimplantation genetic testing; prenatal genetic testing

1. Introduction

Genetic and reproductive counseling should be offered to couples confronted with a diagnosis of a genetic disease, which can be transmitted and affect future generations. This will allow the prospective parents to arrive at an autonomous decision regarding the option most suited to their clinical needs. The couple may decide to refrain from having children, to adopt or to opt for gamete donation. If the couple are committed to becoming the biological parents of a child free of the genetic disease for which they are at risk of transmitting, this can be achieved by natural conception and prenatal diagnosis (PND), although this carries the risk of facing a selective termination of pregnancy. Finally, they may opt for embryo selection, which has become possible after the introduction of in vitro fertilization (IVF) and other methods of assisted reproduction for prospective parents who cannot conceive children naturally. One or both partners may be subfertile or infertile because of a genetic condition or other type of congenital birth defect, childhood infection, cancer therapy or accident, although in most cases the reason for infertility is undetermined. If the couple is also at risk of transmitting an inherited disease and want to avoid termination of the pregnancy, they can opt for preimplantation genetic testing (PGT) and selective transfer of embryos without the disease-causing mutations. This option was originally developed for fertile couples at risk of transmitting a genetic disease, who had experienced one or more pregnancy terminations after PND.

In this review, we compare the pros and cons of PGT and PND in terms of factors including the safety, efficiency and other aspects of both approaches to preventing the transmission of chromosomal, Mendelian and mitochondrial disorders.

2. Prenatal Diagnosis

The earliest form of PND, amniocentesis, was introduced in the 1960s and was performed from the 16th week of pregnancy. This was initially conducted blind, with the puncture site chosen by external palpation of the uterus. In the 1970s, ultrasound scanning became available, which drastically improved safety by providing the gynecologist with the ability to identify the optimal puncture site using a monitor. Before genetic analysis, it was necessary to pre-culture the cells isolated from the amniotic fluid. The whole procedure took approximately 3 weeks, thus delaying a decision to terminate the pregnancy until approximately 19 weeks into the pregnancy, which is very late and causes considerable anxiety for the parents.

Chorionic villus sampling (CVS) for PND was introduced in the 1980s. This technique allowed an earlier diagnosis during the first trimester, allowing earlier termination of the pregnancy in an outpatient clinic. Furthermore, first trimester termination is much safer for the mother. Given the clear advantage of earlier diagnosis, widespread use of CVS could be expected; however, CVS is a technically more demanding procedure than amniocentesis, and some centers have had difficulties in its implementation. CVS and amniocentesis are equally safe, but the impact of

operator experience cannot be underestimated [1]. Procedure-related fetal loss is one of the reasons why patients do not opt for genetic diagnosis during pregnancy.

As an alternative to invasive diagnosis, a non-invasive diagnostic method later became available based on the analysis of cell-free fetal DNA (cffDNA) in the maternal plasma [2]. Different genome sequencing strategies have now been introduced that enable the detection of trisomies, including trisomy 21 [3]. Inherited chromosomal abnormalities can also be detected in this way [4]. Even in the relatively early stages (8 weeks) of pregnancy, standard instrumentation is sufficient for non-invasive PND of single gene disorders based on a simple blood sample [5].

3. Preimplantation Genetic Diagnosis (PGD)

In 1990, about 12 years after the birth of the first IVF baby, Louise Brown, methods for selecting embryos in vitro were introduced in the clinic by Handyside and colleagues [6]. In the first instance, PGD was developed to avoid the need for selective abortion after PND. In a number of cases, patients may have religious or ethical reasons for refusing a pregnancy termination. However, some couples with no objections on these grounds also opted for PGD after one or more rounds of PND with affected pregnancies. Finally, infertile couples can opt for PGD based on a need for assisted reproduction in addition to an increased risk of transmitting a genetic disease.

In addition to PGD, in which assisted reproduction is used to select an embryo for transfer that is free of a specific disorder, preimplantation genetic screening (PGS) has been introduced with the aim of selecting embryos with the highest developmental potential. In other words, this approach was used as a genetic screening method to improve the results of assisted reproduction. The application of newer and more comprehensive methods allowed the combination of PGD and PGS. The most recent terminology introduced to reflect the current possibilities of preimplantation genetic testing (PGT) distinguishes PGT for monogenic diseases (PGT-M), PGT for structural rearrangements (PGT-SR) and PGT for aneuploidy screening (PGT-AS) [7].

Karyomapping is a reliable, efficient and accurate procedure that combines PGT-M or PGT-SR with PGT-A. This technique can be used to minimize the risks of miscarriage and implantation failure [8].

The percentage of transferable embryos after PGT-M remains slightly higher than 40% and is highest in cases of an autosomal recessive disease, followed by X-linked and autosomal-dominant disorders. A proportion of pregnancies end in miscarriage following PGT, but fortunately the number of terminations due to genetic misdiagnosis is extremely low [9, 10]. Based on a previously published definition of major malformation—that is, malformations that generally cause functional impairment or require surgical correction—the rate of malformations after PGD is not significantly different from children born as a result of intracytoplasmic sperm injection (ICSI) without PGD [11]. Embryo biopsy, therefore, does not introduce additional risk factors for congenital malformations. Avoidance of multiple pregnancy is recommended and achieved by single embryo transfer.

PGT-M has some general specific maternal and embryonic concerns as well as a few that are more specific. General concerns include the need to use assisted reproduction for fertile couples, the high costs of both IVF and PGT if it is not (fully) reimbursed by national healthcare systems and the “slippery slope” of eugenics, in which genetic disorders that are not currently regarded as an indication for PND might be accepted for PGT. Finally, the suboptimal pregnancy rates certainly

discourage couples from opting for PGT. The age of the woman at the time of treatment is the most important variable, although on average the success of treatment is approximately 20% per started cycle and approximately 25% per embryo transferred [12].

During the first 25 years of PGD, biopsies were performed at the cleavage stage at day 3 when the embryo consists of about eight cells. In this procedure, one or two cells were usually taken for genetic analysis. Recently, it has been shown that this is not the optimal stage for biopsy, since a high percentage of embryos show postzygotic mosaicism at this stage [13]. Furthermore, early biopsy significantly impairs the implantation potential of the embryo, whereas biopsy at the blastocyst stage does not [14]. An advantage of the latter is that multiple trophectoderm cells are available at day 5 or 6, which improves the accuracy of the genetic diagnosis. Furthermore, the rate of mosaicism is apparently lower at the blastocyst stage [15]. However, there are concerns that culturing embryos to the blastocyst stage might cause long-term and transgenerational effects on development and disease risk [16].

Ovarian hyperstimulation syndrome (OHSS) is a maternal risk and complication of IVF and of PGT, in particular. Since the principle of PGT is based on embryo selection, it requires a relatively higher number of embryos for selection than IVF. This is achieved by more pronounced hormonal stimulation of follicle growth, which can cause OHSS in approximately 2% of women. This condition varies from mild to severe and, in very rare cases, can lead to death. There are certain factors which predispose women to OHSS, including younger age, low body mass index, polycystic ovarian syndrome, history of OHSS, high follicle count, and elevated serum estradiol at the end of controlled ovarian stimulation [17].

4. Chromosomal Indications

Since it has become possible to obtain amniotic fluid for genetic analysis of the embryo, chromosomal abnormalities have been the main indication for invasive PND. Sporadic numerical abnormalities, with a higher risk in patients of increased maternal age, have always been the most frequent reason for referral. Thanks to the widespread use of non-invasive prenatal testing (NIPT), a vast amount of data has demonstrated the high degree of accuracy of whole-genome sequencing of maternal plasma DNA. The sensitivity and specificity of the detection of the three common trisomies [3, 4, 6] have not yet been shown to be 100% in systematic reviews. For this reason, NIPT is still not regarded as diagnostic and follow-up invasive testing is still recommended in cases of positive NIPT [3, 18]). Common trisomies have never been an indication for PGT, because of the low (recurrence) risk.

However, both PND and PGT-SR can be offered if a structural or numerical chromosome abnormality is diagnosed in one of the prospective parents, possibly as the cause of infertility, in which case PGT-SR is obviously preferred.

From the point of view of preconception counseling and risk management, Klinefelter syndrome, structural chromosomal abnormalities and Y-chromosomal microdeletions are the most important genetic causes of male infertility and azoospermia (or severe oligozoospermia), with Klinefelter syndrome being the most frequent genetic cause of infertility in men. Paternity can be achieved through ICSI with spermatozoa recovered from ejaculated semen if present, or from the testes with testicular sperm extraction (TESE). If sufficient sperm cells are obtained, this procedure can easily be combined with PGT, although this combination is rarely used. In cases of

Y-chromosome microdeletion, genetic counseling is mandatory to provide information about the risk of conceiving a son with impaired spermatogenesis after ICSI. Preimplantation selection of female embryos is an option to prevent the transmission of the fertility problems [19]. If a couple with a known balanced chromosomal translocation or inversion conceives naturally, the couple should be offered diagnostic testing during the first or second trimester to assess fetal chromosomal abnormalities.

Structural chromosome abnormalities are also a cause of recurrent pregnancy loss. A recent systematic review demonstrated similar live birth rates, time to subsequent conception and miscarriage rates in couples receiving PGT-SR for recurrent miscarriage and carrying a structural chromosome rearrangement compared with the rates associated with natural conception. However, well-designed randomized controlled trials are still needed for a direct comparison of reproductive outcomes after PGT-SR in this subgroup of patients and those associated with natural conception [20].

5. Mendelian Disorders

In principle, fertile couples can opt for PGT-M and/or PND in cases of any Mendelian disorder, provided it is possible to track the causative mutation. If natural conception is not possible and assisted reproduction is needed, some disorders may complicate PGT-M. Furthermore, in some Mendelian diseases, pregnancy will also be complicated.

In Mendelian diseases showing an X-linked inheritance pattern, female carriers are often affected, but more mildly than males because of the protective effect of the second X-chromosome. Adverse outcomes can be prevented by careful counseling combined with assessment and management of risks.

For example, cardiac involvement has been reported in carriers of dystrophin mutations, which give rise to Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). Furthermore, similar to patients affected by DMD/BMD, these cardiac abnormalities in DMD/BMD carriers are progressive [21]. Consequently, examination are necessary in some cases, and IVF treatment should be suspended in cases of severe abnormalities.

Before offering PGT-M for Fragile X syndrome, it is important to know that females carrying in between 55 and 200 CGG repeats in the 5'UTR of the FMR1 gene show an increased rate of premature ovarian failure (POF). Furthermore, these premutation alleles tend to expand to full mutations when inherited from the mother. The increased number of trinucleotide repeats in the FMR1 gene acts as a risk factor for POF and also for diminished ovarian reserve [22].

In hemophilia A and B, female carriers have an increased bleeding tendency and intraperitoneal bleeding may occur during, or shortly after oocyte retrieval for IVF; therefore, an assessment of the coagulation profile and factor VIII/IX (increasing drug) administration are required prior to this procedure. Pregnancy and delivery in carriers should be managed by a multidisciplinary team in a comprehensive treatment center [23].

In addition, mothers affected by a dominant disease can experience maternal complications of IVF/PGT and pregnancy. Preconception evaluation is necessary for many conditions, and for IVF without PGT, because an adult-onset dominant disease in the mother can complicate IVF treatment and pregnancy.

Marfan syndrome is a heritable connective tissue disorder involving the ocular, cardiovascular, and skeletal systems as well as others. Eighty percent of patients have some form of cardiovascular involvement and the risk of aortic dissection is substantially increased during pregnancy and in the post-partum period. The strategies for the management of pregnant women with Marfan syndrome are based on the size of the aorta [24].

Myotonic dystrophy type 1 (DM1) is a progressive, autosomal-dominant disorder with myotonia as its cardinal feature. Although there is no treatment for the underlying disease, therapy targeting the symptoms (cardiac arrhythmias, respiratory issues) is available. Women with DM1 have a decreased reproductive fitness and when pregnancy does occur, these women are at higher risk of complications. General anesthesia should be avoided if possible [25].

Tuberous sclerosis complex (TSC) is associated with multiple maternal complications of reproduction. Patients with TSC with a high disease burden, including those with significant TSC-associated neuropsychiatric disorders, have severe reproductive problems. However, the phenotypic spectrum of TSC varies and can also present with a wide variety of milder features that are less life-limiting. Therefore, given that TSC is a multisystem disorder, pregnancy management should be performed by a multidisciplinary team including obstetricians, nephrologists, neurologists, pulmonologists, intervention radiologists, and anesthesiologists [26].

Information on postoperative fertility problems in female patients with familial adenomatous polyposis (FAP) is scarce. The risk of developing postoperative fertility problems is not significantly associated with the type of surgery, indication for surgery, complications, or other comorbid conditions. Postoperative fertility problems are more common among women who had their first surgical procedure at a younger age [27].

Sometimes the choice between PGT and PND is not straightforward, particularly in cases with disorders showing a variable phenotype, such as holoprosencephaly (HPE). Almost one third of carriers of the sonic hedgehog (SHH) mutations commonly associated with HPE may be clinically unaffected, even in affected families [28]. For some, the prenatal detection of SHH mutations does not justify pregnancy termination; therefore, it has been proposed that preimplantation genetic diagnosis is a more attractive option for couples at risk of having a child with HPE [28]. However, prenatal diagnosis of the phenotype by magnetic resonance imaging (MRI) enables accurate evaluation of HPE in utero, which is important because the severity of the imaging findings correlates with postnatal morbidity and mortality in this condition. On this basis families can receive appropriate genetic counseling during the pregnancy [29].

Patients with Huntington's disease (HD) can be identified as carriers by presymptomatic testing. In such cases, straightforward genetic testing can be performed both before and after implantation. However, PND or PGT for HD is complicated for other reasons. It can be argued that selective abortion is unacceptable since a child born carrying the HD mutation might still expect many years of disease-free life. Moreover, patients may decide not to be tested despite wanting a child without HD. In itself, the result of a PND test has implications for the right of an at-risk parent to remain unaware. An alternative to the direct approach is exclusion testing, which allows a parent with a 50% risk of HD to have children without risk. This approach involves screening for the grandparental chromosome 4, which is transmitted by the parent at risk, using polymorphic markers closely linked to the HD locus. If chromosome 4 is inherited from the grandparent with the disease, the fetus has a 50% risk of having HD—the same risk as the asymptomatic prospective patient. However, since half of the fetuses carrying the grandparental allele would be unaffected,

pregnancy termination is problematic. Furthermore, late reversal of a previous intention to terminate pregnancy occurs with relatively high frequency. When the parent develops the disease, the child will ultimately become affected too and will receive this knowledge early in life [30]. This is avoided by exclusion testing through PGD. The alternative, which is non-disclosure PGT, whereby embryos are tested for the presence of the HD mutation without revealing results to the patients, is not without complications [31]. Therefore, many couples favor exclusion PGD as the better alternative. However, couples can reconsider their choices in every subsequent pregnancy based on their previous experience, personal beliefs and the gender of the at-risk partner [32].

Patients with recessive disorders do normally do not have an increased risk of complications; however, due to the intensive care received by patients affected by genetic disorders, many survive long enough to have children and if their partner is a carrier, half of the children will also be affected.

Women with CF can have severe reproductive problems. However, there is only a slight adverse effect on maternal health if the woman is in good general health [33].

6. Mitochondrial Disease

Mitochondrial diseases, which are a group of disorders with exclusive maternal inheritance, are caused by a defect in mitochondrial oxidative phosphorylation. PND and PGT have been used to avoid the transmission of mitochondrial DNA (mtDNA) mutations, which are heteroplasmic, and symptoms arise only if the number of mutated mitochondria exceeds a certain threshold. Both PGT and PND decrease the risk of a severely affected child.

Although PND is technically possible, it is usually not applicable because of limitations in predicting the phenotype. For de novo mtDNA point mutations, recurrence risks are low and PND can be offered to provide reassurance regarding fetal health. PND is also the best option for female carriers with low-level mutations demonstrating skewing to 0% or 100%. PGD is suitable if the selection of embryos for transfer is based on a mutant-load below a mutation-specific or general expression threshold of 18%. PGD is currently the best reproductive option for familial heteroplasmic mtDNA point mutations. Nuclear genome transfer and genome editing techniques are currently being investigated and might offer additional reproductive options for specific mtDNA diseases [34].

7. Conclusion

Both PGT and PND are powerful tools to tackle the transmission of inherited disorders in families carrying the diseases from generation to generation. In prenatal testing, non-invasive diagnostic methods have become available as an alternative to invasive diagnosis. Different genome sequencing strategies have now been introduced that enable early detection of inherited chromosomal and monogenic abnormalities as well as trisomies. This development is attractive for some couples who would have opted for PGT previously. Nevertheless, PGT is still a safe, albeit less efficient approach to preventing the transmission of chromosomal, Mendelian and mitochondrial disorders.

Author Contributions

Joep Geraedts conceived and designed the manuscript and wrote it entirely.

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Competing Interests

The authors have declared that no competing interests exist.

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