PREIMPLANTACIONO GENETIČKO TESTIRANJE

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REVIEW ARTICLE

PREIMPLANTATION GENETIC TESTING

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SAŽETAK

Primena preimplantacionog genetičkog testiranja (PGT) otpočela je kasnih osamdesetih godina prošlog veka. Preimplantaciono genetičko testiranje, kao najraniji mogući vid prenatalne dijagnostike, omogućava selekciju zdravih embriona sa normalnim kariotipom za embriotransfer.

Upotreba preimplantacionog genetičkog testiranja se pokazala korisnom metodom kod tri grupe naslednih bolesti, i to: monogenskih bolesti, bolesti trinukleotidnih ponovaka i hromozomskih aberacija.

Stopa uspeha vantelesne oplodnje (VTO) značajno je porasla nakon što je PGT uveden u kliničku praksu.

U ovom radu dat je pregled literature, sa ciljem jasnog utvrđivanja uloge PGT-a u selekciji embriona pre embriotransfera, kao i uloge ove vrste testiranja u povećanju stope uspeha VTO-a. Jedan od ciljeva rada je i osvrt na razvoj molekularno genetičkih metoda koje su trenutno, ili su ranije bile, u rutinskoj upotrebi prilikom izvođenja PGT-a.

Aktuelna literatura pokazatelj je razvoja i napretka tehnika molekularne genetike koje se primenjuju u PGT-u. Istovremeno daje mogućnost i podsticaj za dalja opsežna istraživanja koja će dovesti do unapređenja samog preimplantacionog genetičkog testiranja, a samim tim povećati stopu uspeha vantelesne oplodnje.

Ključne reči: preimplantaciono genetičko testiranje – PGT, vantelesna oplodnja – VTO, embrion

ABSTRACT

The application of preimplantation genetic testing (PGT) began in the late 1980s. Preimplantation genetic testing, as the earliest possible method of prenatal diagnosis, enables the selection of embryos with a normal karyotype for embryo transfer.

The use of preimplantation genetic testing has proven to be a useful method in the following three groups of inherited diseases: monogenic disorders (single gene defects), trinucleotide repeat disorders, and chromosomal abnormalities.

The success rate of in vitro fertilization (IVF) has increased significantly since the introduction of PGT into clinical practice.

This paper presents a literature review with the aim of clearly determining the role of PGT in embryo selection before embryo transfer, as well as the role of this type of testing in increasing the success rate of IVF. One of the goals of the paper is also to review the development of molecular genetic methods that are currently, or have once been, in routine use when performing PGT.

The current literature is an indicator of the development and progress of molecular genetics techniques applied in PGT. At the same time, it provides an opportunity and an incentive for further extensive research that will lead to the improvement of preimplantation genetic testing and thus increase the success rate of in vitro fertilization.

Key words: preimplantation genetic testing – PGT, in vitro fertilization – IVF, embryo

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UVOD

Preimplantaciono genetičko testiranje (PGT) obuhvata procedure koje su ranije bile poznate kao preimplantaciona genetička dijagnostika (PGD) i preimplantacioni genetički skrining (PGS). Prema Konzorcijumu *ESHRE* (engl. *European Society for Human Reproduction and Embryology*), PGT se definiše kao test koji se sprovodi kako bi se analizirala DNK iz oocita (polarnih tela) ili embriona (embriona na stadijumu brazdanja ili blastocista) za HLA (humani leukocitni antigen) tipizaciju ili za determinaciju genetičkih abnormalnosti [1].

PGT, kao najraniji mogući oblik prenatalne dijagnostike, primenjuje se kod parova koji nose rizik prenošenja nasledne bolesti na potomstvo [2]. Sve do razvoja PGT-a, kasnih osamdesetih godina prošlog veka, invazivna i neinvazivna prenatalna dijagnostika mogla je samo da konstatuje da plod u utrobi majke ima, ili će razviti neku naslednu bolest. Tada su se parovi suočavali sa jednom od najtežih odluka u životu, da li takvu trudnoću prekinuti ili nastaviti, uprkos saznanju da njihovo dete neće biti zdravo. Pojava PGT-a je ovakvim parovima omogućila dragocenu alternativu [3] budući da se metoda primenjuje na preimplantacionim embrionima. To omogućava da se samo zdravi embrioni sa normalnim kariotipom selektuju za embriotransfer [3].

Preimplantacioni genetički skrining (PGS), koji se često nazivao PGD niskog rizika, primenjivao se kod infertilnih parova koji nose nizak rizik od transmisije nasledne bolesti na potomstvo i koji se podvrgavaju VTO-u, a sa ciljem povećanja stope uspeha u ostvarivanju trudnoće i rađanju zdravog deteta.

Preduslov za PGT je vantelesna oplodnja (VTO). U stimulisanim ciklusima, ona ima za cilj dobijanje što većeg broja jajnih ćelija i embriona. Ovo je veoma važno za parove koji se suočavaju sa problemom steriliteta, jer pruža mogućnost selekcije samo zdravih embriona [3]. Ukoliko postoje indikacije za primenu PGT-a prilikom VTO-a, izbegava se klasična *in vitro* fertilizacija (*IVF*), jer PGT podrazumeva korak DNK amplifikacije [4]. Iz tog razloga pristupa se metodi intracitoplazmatične injekcije spermatozoida (engl. *intracytoplasmic sperm injection – ICSI*). Ovo je metoda prvog izbora jer se njenom primenom isključuje mogućnost kontaminacije neželjenom DNK spermatozoida [2].

PGT je prvi put primenjeno u kliničkoj praksi 1990. godine, prilikom određivanja pola embriona *PCR*-om (engl. *polymerase chain reaction*) usled sumnje na X-vezano oboljenje [2]. Nekoliko godina kasnije, primena fluorescentne *in situ* hibiridizacije (FISH) postaje standardna metoda za selekciju embriona prema polu, za detekciju numeričkih i strukturnih hromozomskih aberacija. Danas se ove metode više ne koriste. Njih su zamenile novije, sofisticiranije, osetljivije i specifičnije

INTRODUCTION

Preimplantation genetic testing (PGT) encompasses procedures, previously known as preimplantation genetic diagnostics (PGD) and preimplantation genetic screening (PGS). According to the PGT Consortium of the ESHRE (European Society for Human Reproduction and Embryology), PGT is defined as a test that is carried out in order to analyze the DNA of oocytes (polar bodies) or embryos (cleavage stage embryos or blastocyst stage embryos) for HLA (human leukocyte antigen) typing or for the determination of genetic abnormalities [1].

PGT, as the earliest possible form of prenatal diagnostics, is applied in couples carrying a risk of passing on a hereditary disease onto their children [2]. Until the late 1980s, when PGT was developed, invasive and noninvasive prenatal diagnostics could only ascertain that the embryo in the mother's womb was indeed afflicted with, or would develop, a hereditary disease. Couples were then faced with one of the most difficult decisions of their lives, whether to terminate or continue with the pregnancy, despite the knowledge that their child would not be healthy. The development of PGT provided a valuable alternative [3], as the method is applied on preimplantation embryos. This provides that only healthy embryos with a normal karyotype are selected for embryo transfer [3].

Preimplantation genetic screening (PGS), previously often referred to as low risk PGD, was applied in infertile couples who were carrying a low risk of passing on a hereditary disease onto their offspring, and who were undergoing IVF, for the purpose of increasing the success rate of achieving pregnancy and producing a healthy child.

The precondition for PGT is in vitro fertilization (IVF). In stimulated cycles, its aim is to produce the maximum number of ova and embryos. This is very important for couples faced with the problem of sterility, as it offers the opportunity of selecting only healthy embryos [3]. If there are indications for the application of PGT during IVF, classic *in vitro* fertilization (IVF) is avoided, as PGT implies the step of DNA amplification [4]. This is why the method of intracytoplasmic sperm injection (ICSI) is employed. This is the method of choice, as its application excludes the possibility of contamination with unwanted sperm DNA [2].

PGT was first applied in clinical practice in 1990, for determining the sex of the embryo, with the aid of PCR (polymerase chain reaction), due to suspected X-linked disease [2]. Several years later, the application of fluorescence *in situ* hybridization (FISH) became the standard method for the selection of embryo by sex, for the purpose of detecting numerical and structural

metode, kao što su *micrroarray CGH* (engl. *comparative genomic hybridization*) i *NGS* (engl. *next generation sequencing*).

Na osnovu podataka iznetih u Uvodu, definisani su ciljevi ovog rada:

1. Utvrđivanje uloge PGT-a u selekciji zdravih euploidnih embriona pre embriotransfera;
2. Utvrđivanje uloge PGT-a u povećanju stope uspeha VTO-a;
3. Pregled korišćenih genetičkih metoda u preimplantacionom genetičkom testiranju.

PREIMPLATACIONO GENETIČKO TESTIRANJE

Prema Konzorcijumu *ESHRE*, PGT delimo na:

1. preimplantaciono genetičko testiranje na aneuploidije (PGT-A)
2. preimplantaciono genetičko testiranje na prisustvo strukturnih hromozomskih rearanžmana (PGT-SR)
3. preimplantaciono genetičko testiranje na prisustvo monogenskih oboljenja (PGT-M) [1].

INDIKACIJE

PGT se primenjuje kod parova koji nose rizik za dobijanje potomstva sa naslednom bolešću ili hromozomskim aberacijama, i to u slučaju: starije životne dobi žene [5], teškog oblika muškog infertiliteta [6], ponovljenih neuspeha implantacije embriona nakon VTO-a [3], rekurentnih pobačaja [5], postojanja već bolesnog deteta ili člana porodice [3], kada je jedan od partnera nosilac monogenskih bolesti, hromozomskih aberacija i mitohondrijalnih DNK mutacija, postojanja genetičke predispozicije za malignitet, i kod HLA tipizacije [2].

STARIJA ŽIVOTNA DOB ŽENE

Poznato je da postoji jasna pozitivna korelacija između numeričkih hromozomskih aberacija, najčešće aneuploidija, sa životnom dobi majke. Studije pokazuju da do 70% oocita žena starijih od 40 godina imaju numeričke hromozomske aberacije [5]. Jedno od objašnjenja niže stope implantacije, uključujući prirodna začeća i VTO, jeste veći procenat embriona sa aneuploidijama. PGT-A embriona je pokazala da su aneuploidije česte i da se njihov procenat značajno povećava sa godinama žene. U takvim slučajevima, moguće je uraditi biopsiju polarnog tela ili biopsiju trofoektoderma blastociste [5].

HLA TIPIZACIJA

PGT je našao primenu pri HLA tipizaciji u reproduktivnoj medicini. Kod para koji ima dete kome je potrebna transplantacija hematopoetskih stem ćelija, PGT-om se vrši selekcija embriona koji nisu nosioci mutacije za

chromosome aberrations. Today, these methods are no longer in use. They have been replaced by newer, more sophisticated, more sensitive and more specific methods, such as micrroarray CGH (comparative genomic hybridization) and NGS (next generation sequencing).

Based on the data depicted in the Introduction, the goals of the present paper have been defined. These are:

1. Determining the role of PGT in the selection of healthy euploid embryos before embryo transfer;
2. Determining the role of PGT in increasing the success rate of IVF;
3. Review of genetic methods applied in preimplantation genetic testing.

PREIMPLANTATION GENETIC TESTING

According to the PGT Consortium of the ESHRE, PGT is classified into the following categories:

1. preimplantation genetic testing for aneuploidies (PGT-A)
2. preimplantation genetic testing for chromosomal structural rearrangements (PGT-SR)
3. preimplantation genetic testing for monogenic disorders (PGT-M) [1].

INDICATIONS

PGT is applied in couples at risk of having children with hereditary disease or chromosome aberrations, in the following cases: older maternal age [5], severe form of male infertility [6], repeated failure of embryo implantation after IVF [3], recurrent miscarriage [5], the occurrence of disease in a child already born to the couple or in a family member [3], when one of the partners is a carrier of a monogenic disorder (single gene defect), chromosome aberrations, and mitochondrial DNA mutations, when there is a genetic predisposition to malignancy, and in HLA typing [2].

OLDER MATERNAL AGE

It is well known that there is a clear positive correlation between numerical chromosome aberrations, most frequently aneuploidies, and the maternal age. Studies have shown that up to 70% of oocytes of women past the age of 40 have numerical chromosome aberrations [5]. One of the explanations for a decreased implantation rate, including both natural conception and IVF, is a higher percentage of embryos with aneuploidies. PGT-A of embryos has shown that aneuploidies are frequent and that their percentage significantly increases with maternal age. In such cases, it is possible to perform polar body biopsy or trophectoderm biopsy of the blastocyst [5].

bolest, a kompatibilni su donori za bolesnog brata ili sestru. Ovaj pristup se prvi put uspešno koristio kod Fankoni anemije [2].

Poznato je da se nasleđuje predispozicija za razvoj nekih tipova maligniteta (tumori dojke i jajnika, tumori želuca i debelog creva, nekih leukemija), te PGT nalazi svoju primenu i u ovoj sferi [7].

GENETIČKI UZROK MUŠKOG INFERTILITETA

Genetički uzrok muškog infertiliteta je vrlo heterogen, te se primenjuju sve tehnike PGT-a. Do sad je identifikovano preko 2.300 gena čije mutacije mogu dovesti do razvoja muškog infertiliteta, dok su hromozomske aberacije potvrđeni uzrok infertiliteta u 20% slučajeva. Oba slučaja praćena su lošim parametrima spermograma [6]. Ovaj deo infertilne populacije ima veliku korist od PGT-a, uz primenu dodatnih uroloških procedura ili bez njih.

Klinefelterov sindrom (47, XXY) karakteriše abonormalna spermatogeneza, a često je prisutna i azoospermija. Pokazano je da se, u bar 50% slučajeva, spermatozoidi mogu dobiti postupkom testikularne ekstrakcije spermatozoida (TESE) [6]. Kod muškaraca sa ovim sindromom postoji povećana učestalost aneuploidija kod njihovog potomstva, pa je primena PGT-A metode neprocenjiva [6].

Zahvaljujući primeni PGT-SR metode, nosioci heterologih Robertsonovih translokacija mogu povećati šanse za dobijanje zdravog potomstva. Kod parova sa ovom indikacijom, PGT-SR omogućava selekciju zdravih embriona kojih je svega 25% [6].

PGT-SR i PGT-M se primenjuju ukoliko postoji mikrodelecija AZF-c regiona Y hromozoma [6], kod pacijenata sa Kartagenerovim sindromom [7], kod pacijenata sa globozoospermijom [8], kod velikog broja numeričkih i strukturnih hromozomskih aberacija i pojedinih monogenskih bolesti.

MITOHONDRIJALNE BOLESTI

Mitohondrijalne bolesti su relativno česti poremećaji metabolizma, a u 15% slučajeva uzrokovane su mutacijama u mitohondrijalnoj DNK (mtDNK) majke [9]. Budući da one dovode do ozbiljne fenotipske ekspresije (gubitak neuroloških funkcija, respiratonih i srčanih problema itd.), primena PGT-M metode kod pacijenata sa ovom indikacijom omogućava selekciju zdravih embriona tokom VTO-a.

Prema Konzoricjumu *ESHRE,* sve patogenetske varijante u mtDNK koje se javljaju u pojedinačnim blastomerama reprezentativne su za ceo embrion, što se i očekuje, budući da se do stadijuma brazdanja, mtDNK ne replikuje. Na stadijumu blastociste počinje mtDNK replikacija, što dovodi do pojave različitih novih varijanti [1].

HLA TYPING

PGT has found its application in HLA typing in reproductive medicine. In a couple who has a child in need of hematopoietic stem cell transplantation, PGT is applied in order to select embryos that are not carriers of the mutation related to disease and are also compatible donors for their sick brother or sister. This approach was first successfully applied in Fanconi anemia [2].

It is a known fact that predisposition towards certain types of malignancy is hereditary (breast and ovarian tumors, tumors of the gaster and colon, certain types of leukemia), which is why PGT is applied in this sphere as well. [7].

GENETIC CAUSE OF MALE INFERTILITY

The genetic cause of male infertility is very heterogenous, which is why all PGT techniques are applied. Thus far, more than 2,300 genes, whose mutations can lead to the development of male infertility, have been identified, while chromosome aberrations have been confirmed as the cause of male infertility in 20% of cases. Both types of cases are characterized by poor semen analysis parameters [6]. This part of the infertile population benefits greatly from PGT, along with additional urological procedures, or without them.

Klinefelter syndrome (47, XXY) is characterized by abnormal spermatogenesis, and azoospermia is also often present. It has been determined that, in at least 50% of cases, spermatozoa can be obtained by means of testicular sperm extraction (TESE) [6]. In men with this syndrome, there is an increased incidence of aneuploidies in their offspring, which is why the application of PGT is invaluable [6].

Owing to the application of the PGT-SR method, carriers of heterologous Robertsonian translocations may increase their chances of having healthy children. In couples with this indication, PGT-SR enables the selection of healthy embryos, of which there are only 25% [6].

PGT-SR and PGT-M are applied in the following cases: if there is a microdeletion of the AZF-c region of the Y chromosome [6], in patients with Kartagener's syndrome [7], in patients with globozoospermia [8], in a large number of numerical and structural chromosome aberrations, and in certain monogenic disorders.

MITOCHONDRIAL DISEASES

Mitochondrial diseases are relatively common metabolic disorders, and, in 15% of cases, they are caused by maternal mitochondrial DNA (mtDNA) mutations [9]. Since they lead to severe phenotypic expression (loss of neurological function, respiratory and cardiac problems, etc.), the use of the PGT-M method in

BIOPSIJA

Biopsija podrazumeva uzimanje ćelija, čiji će genetički materijal biti analiziran, nekom od metoda molekularne dijagnostike. Razlikujemo: biopsiju polarnog tela na stadijumu oocite ili zigota; biopsiju blastomera embriona trećeg dana; biopsiju trofoektoderma embriona petog ili šestog dana tj. biopsiju blastociste [3].

Biopsija počinje ablacijom na glikokaliksnom omotaču (lat. *zona pellucida)*. Nekada se to radilo mehaničkim, zatim hemijskim putem, a od 2003. godine, isključivo primenom lasera. Kad se napravi otvor u zoni pelucidi i oslobodi prolaz, aspirira se polarno telo blastomera ili ćelije trofoektoderma, čiji se genetički materijal analizira [10].

BIOPSIJA POLARNOG TELA

Biopsija polarnog tela je prvi put primenjena 1990. godine za detekciju cistične fibroze. Ova procedura je razvijena sa ciljem da se smanji invazivnost biopsije blastomera. Genetički materijal polarnog tela predstavlja samo DNK iz oocite, pa je biopsija polarnog tela posebno korisna za detekciju maternalno nasleđenih monogenskih bolesti, numeričkih i strukturnih hromozomskih aberacija. Nedostatak ove metode je nemogućnost dobijanja informacija o DNK oca i DNK embriona [10]. Danas se ova metoda uglavnom koristi da bi se prevazišli etički problemi u zemljama gde nije dozvoljena biopsija embriona.

BIOPSIJA BLASTOMERA

Biopsija blastomera embriona trećeg dana izvodi se 66 – 72 sata nakon primene *ICSI* metode, kada embrion ima 6 – 8 blastomera koje su još uvek totipotentne i između njih se jasno uočavaju granice [2].

Nedostaci ove metode su: relevantnost rezultata dobijenih analizom pojedinačne ćelije, imajući u vidu visok procenat mozaicizma, koji se javlja kod embriona, kao i nedostatak informacija o negativnom uticaju uklanjanja blastomere ili blastomera na dalji razvoj embriona [10].

Biopsija koja bi se izvodila na ranijem stadijumu, na nivou četvoroćelijskog embriona, može narušiti odnos buduće unutrašnje ćelijske mase (engl. *inner cell mass – ICM*) i trofoektoderma (TE).

Imajući u vidu sve prethodno navedeno, glavna strategija za primenu ove metode je biopsija embriona trećeg dana, koji u tom momentu ima 6 do 8 blastomera [2]. Problem kod ovog tipa biopsije su blastomere koje mogu lako da liziraju, što bi dovelo do gubitka genetičkog materijala, te bi bila potrebna nova blastomera za analizu.

Kompakcija, koja se dešava na nivou između osmoćelijskog embriona i stadijuma morule, dodatno

patients with this indication enables the selection of healthy embryos during IVF.

According to the PGT Consortium of the ESHRE, all pathogenic mtDNA variants, which occur in individual blastomeres, are representative of the entire embryo, which is to be expected, as mtDNA does not replicate until the cleavage stage. At the stage of the blastocyst, mtDNA replication begins, which leads to the occurrence of different new variants [1].

BIOPSY

Biopsy entails extracting cells, whose genetic material is to be analyzed, by means of one of the molecular diagnostics methods. There are different types of biopsy, namely: biopsy of the polar body at the oocyte or zygote stage; blastomere biopsy on day three of embryo development; trophectoderm biopsy of the embryo on day five or day six of development, i.e., biopsy of the blastocyst [3].

Biopsy begins with ablation on the glycocalyx coat (Lat. *zona pellucida*). In the past, this used to be done mechanically, and then chemically, and as of 2003, it has been done exclusively with the use of the laser. Once an opening is made in the *zona pellucida*, and a pathway is made, the polar body of the blastomere or trophectoderm cell, whose genetic material is analyzed, is aspirated [10].

POLAR BODY BIOPSY

Polar body biopsy was first employed in 1990 for detecting cystic fibrosis. This procedure was developed with an aim to decrease the invasiveness of blastomere biopsy. Genetic material of the polar body represents only the DNA from the oocyte, which is why polar body biopsy is especially useful for the detection of maternally inherited monogenic disorders and numerical and structural chromosome aberrations. The drawback of this method is that there is no possibility of obtaining any information on the DNA of the father or the DNA of the embryo [10]. Today, this method is mainly used to overcome ethical issues and concerns in countries where embryo biopsy is not allowed.

BLASTOMERE BIOPSY

Blastomere biopsy on day three of embryo development is performed between 66 and 72 hours after the application of the ICSI method, when the embryo has 6 – 8 blastomeres, which are still totipotent, and the boundaries between them are clearly visible [2].

The drawbacks of this method are the following: the relevance of the results obtained through the analysis of an individual cell, bearing in mind the high percentage of mosaicism that occurs in embryos; as well as the lack of information on the negative effect that

komplikuje PGT. Za vreme kompakcije, granice između ćelija se gube i nije moguće razlikovati pojedinačne ćelije, te je teško izdvojiti samo jednu blastomeru [11].

Pre same biopsije blastomera, neophodna je inkubacija embriona u medijumima bez kalcijuma i magnezijuma, da bi se usporilo stvaranje međućelijskih veza i olakšala biopsija. Kada se genetički materijal blastomere šalje na *PCR* analizu, preporučena metoda fertilizacije oocita je *ICSI*. U slučaju da je metoda fertilizacije klasičan IVF može doći do kontaminacije i amplifikacije DNK spermatozoida umesto DNK embriona, pa se ova metoda ne preporučuje.

BIOPSIJA TROFOEKTODERMA

Biopsija trofoektoderma (TE) blastociste se može izvoditi petog ili šestog dana od oplodnje. Prednost metode je mogućnost biopsije većeg broja ćelija trofoektoderma (5 do 10 ćelija) [10], bez narušavanja *ICM* [12]. Analiza većeg broja ćelija ima prednost u dijagnostici monogenskih bolesti [2]. Ovaj broj ćelija se može smatrati reprezentativnim za ceo embrion, sem u slučaju placentnog mozaicizma [3], koji je primećen u više od 1% trudnoća [13]. Studije pokazuju da blastocistu karakteriše visok nivo mozaicizma, pa se iz tog razloga ćelije TE ne mogu smatrati pogodnim za PGT analizu [3].

Biopsija blastociste sa krioprezervacijom je već neko vreme postala standard pri izvođenju neke od PGT metoda [2]. Zamrzavanje blastociste metodom vitrifikacije nakon biopsije daje vremena za sve neophodne analize [2]. Glavni problem biopsije blastociste sastoji se u tome što će samo ograničeni broj embriona dostići dati stadijum i odgovarajući kvalitet, uprkos usavršavanju medijuma za kultivaciju. Embrioni koji ne dostignu stadijum blastociste mogu imati visok procenat aneuploidija, koje uključuju hromozome X, Y, 16, 18 i 21 [10].

GENETIČKE ANALIZE

Metode koje su se nekada češće koristile za analizu genetičkog materijala dobijenog biopsijom su: *PCR*, FISH (engl. *fluorescence in situ hybridization*), *CGH*, *SNP* (engl. *single nucleotide polymorphism*), a danas ih zamenjuje metoda *NGS* [10]. Izbor odgovarajuće metode zavisi od medicinskih indikacija.

PCR

PCR metoda se koristila za detekciju mutacija na nivou gena, za detekciju broja trinukleotidnih ponovaka i za određivanje pola embriona [2]. Dva glavna problema *PCR* metode u PGT-M testiranju su: kontaminacija uzorka i *allele dropout* [10].

PCR jedne ćelije je osetljiva metoda budući da postoji opasnost amplifikacije strane DNK (DNK ćelija kumulusa ili DNK spermatozoida). Da bi se ovaj problem

the removal of a blastomere, or blastomeres, may have on the further development of the embryo [10].

Biopsy that would be performed at an earlier stage, at the level of a four-cell embryo, could damage the relation between the future inner cell mass (ICM) and the trophectoderm (TE).

Bearing in mind all of the above, the main strategy for the application of this method is day three embryo biopsy, with the embryo comprising 6 to 8 blastomeres at that moment [2]. The problem with this type of biopsy are the blastomeres which can easily lyse, which would lead to the loss of genetic material, necessitating a new blastomere for analysis.

Compaction occurring at the level between the stage of the eight-cell embryo and the morula stage additionally complicate PGT. During compaction, the cell-cell boundaries disappear, and it becomes impossible to differentiate individual cells, which is why it is difficult to extract only one blastomere [11].

Before the actual blastomere biopsy, the incubation of the embryo in mediums devoid of calcium and magnesium is necessary, in order to slow down the creation of bonds among cells and facilitate the biopsy. When the genetic material of the blastomere is sent for PCR analysis, the recommended method of oocyte fertilization is ICSI. In case the fertilization method is classic IVF, contamination and amplification of the sperm DNA instead of the embryo DNA may occur, which is why this method is not recommended.

TROPHECTODERM BIOPSY

Trophectoderm biopsy (TE) of the blastocyst can be performed on the fifth or sixth day upon fertilization. The advantage of this method is the possibility of performing a biopsy of a higher number of cells of the trophectoderm (5 to 10 cells) [10], without damaging the ICM [12]. Analysis of a greater number of cells is the preferred method in the diagnostics of monogenic disorders [2]. This number of cells can be considered representative for the entire embryo, except in the case of placental mosaicism [3], which has been observed in over 1% of pregnancies [13]. Studies have shown that blastocytes are characterized by a high level of mosaicism, which is why TE cells cannot be considered suitable for PGT analysis [3].

Blastocyst biopsy with cryopreservation has been the standard for performing some of the PGT methods for a certain period of time now [2]. Blastocyst freezing by means of the vitrification method upon biopsy provides time for all the necessary analyses [2]. The main problem of blastocyst biopsy is the fact that only a limited number of embryos reaches this stage and the appropriate quality, despite the refined cultivation

izbegao, procedura se izvodi u posebnoj *PCR* sobi sa pozitivnim pritiskom, a metoda fertilizacije je isključivo *ICSI* [2]. Rešenje ovog problema bi bio multipleks *PCR* kojim bi se identifikovala sva 4 parentalna alela, čime bi se osiguralo da ampifikovana DNK bude isključivo embrionskog porekla [2].

Drugi problem je DNK *allele dropou*t ili preferencijalna amplifikacija, pri čemu se jedan od dva alela preferencijalno amplifikuje u odnosu na drugi, što kod dominantnih heterozigota može dati lažno negativan ili lažno pozitivan rezultat [10].

FLUORESCENTNA *IN SITU* HIBRIDIZACIJA - FISH

Fluorescentna *in-situ* hibridizacija je prvi put u PGT-u počela da se primenjuje 1991. godine, za analizu hromozoma embriona radi određivanja pola i hromozomskih aberacija, pre svega aneuploidija [2].

Za razliku od *PCR* metode, kod FISH-a ne postoji rizik od kontaminacije uzorka i nismo ograničeni samo na *ICSI* metodu pri fertilizaciji oocita. Hromozomi koji se najčešće analiziraju su X, Y, 13, 16, 18, 21, i 22 [7]. Ponavljanjem ciklusa i uključivanjem većeg broja hromozoma u analizu smanjuje se efikasnost procedure i povećava verovatnoća lažno pozitivnih i lažno negativnih rezultata. Kako se FISH-om mogu analizirati samo određeni hromozomi i kako je broj prijavljenih lažno pozitivnih i lažno negativnih rezultata visok, ova metoda se više ne koristi u preimplantacionom genetičkom testiranju [14].

KOMPARATIVNA GENOMSKA HIBRIDIZACIJA *I MICROARRAY-CGH*

U međuvremenu su se razvijale nove genetičke metode koje omogućavaju simultanu analizu svih hromozoma sa daleko većom preciznošću. Jedna od tih metoda je metafazna komparativna genomska hibridizacija (engl. *metaphase comparative genomic hybridization – mCGH*) [2]. Iako je ova molekularna citogenetička metoda pouzdano detektovala aneuploidije, nedostatak je bilo vreme neophodno za analizu (3 do 5 dana) te se embriotransfer nije mogao vršiti u vremenski predviđenom intervalu. Prelazak sa tadašnjeg metoda zamrzavanja, sporog zamrzavanja (engl. *slow freezing*) na usavršen metod vitrifikacije koji se i danas koristi (preživljavanje blastocista nakon odmrzavanja preko 96%) [15] omogućilo je primenu *mCGH* . Vitrifikacija nakon biopsije obezbeđuje dovoljno vremena za genetičku analizu i tumačenje rezultata i dozvoljava da se embriotransfer uradi u trenutku optimalne receptivnosti endometrijuma [14].

Kasnije je *mCGH* zamenjen metodom *microarray-CGH*. Prednost ove metode u odnosu na prethodne je smanjenje vremena analize na jedan dan, povećanje broja hromozoma koji se analizira, kao i preciznija detekcija aneuploidija [2].

mediums. The embryos that do not reach the blastocyst stage may have a high percentage of aneuploidies, which include chromosomes X, Y, 16, 18 and 21 [10].

GENETIC ANALYSES

Methods which were more frequently used in the past for the analysis of genetic material obtained through biopsy are the following: PCR, FISH (fluorescence *in situ* hybridization), CGH, SNP (single nucleotide polymorphism), while today these are being replaced by the NGS method [10]. The selection of the appropriate method depends on medical indications.

PCR

The PCR method was used for the detection of gene-level mutations, for the detection of the number of trinucleotide repeats, and for determining the sex of the embryo [2]. The two main problems of the PCR method in PGT-M testing are the following: sample contamination and allele dropout [10].

PCR of a single cell is a sensitive method as there is the danger of the amplification of foreign DNA (DNA of the cumulus cells or sperm DNA). In order to avoid this problem, the procedure is carried out in a special PCR room with positive air pressure, and the method of fertilization is exclusively ICSI [2]. The solution to this problem would be multiplex PCR which would identify all 4 parental alleles, which would, in turn, ensure that the amplified DNA is exclusively of embryonic origin [2].

The second problem is DNA allele dropout or preferential amplification, whereby one of the two alleles is preferentially amplified in relation to the other, which, in dominant heterozygotes, may produce a falsely negative or falsely positive result [10].

FLUORESCENCE *IN SITU* HYBRIDIZATION – FISH

Fluorescence *in situ* hybridization (FISH) was first used in PGT in 1991 for the purpose of analyzing embryonic chromosomes in order to determine the sex of the embryo and chromosome aberrations, primarily aneuploidies [2].

As opposed to the PCR method, in FISH, there is no risk of sample contamination and we are therefore not limited only to the ICSI method in oocyte fertilization. The chromosomes most commonly analyzed are X, Y, 13, 16, 18, 21, and 22 [7]. By repeating the cycles and including a higher number of analyses, the efficiency of the procedure is diminished, and the probability of false positive and false negative results is increased. As FISH can be used to analyze only certain chromosomes, and since the number of reported false positive and false negative results is high, this method is no longer used in preimplantation genetic testing [14].

SNP i NGS

Metoda *SNP* otkriva ne samo aneuploidije, već i duplikacije i delecije, a može dati informaciju o poreklu hromozoma od oca i od majke kod uniparentalne dizomije (UPD) [2,16].

Danas se u kliničkoj praksi kao standard koristi *NGS*. Ova metoda uključuje prethodnu amplifikaciju celog genoma (engl. *whole genome amplification*), što omogućava da se uradi veći broj analiza u isto vreme, na samo jednoj ćeliji. *NGS*-om se detektuju mutacije, visoko polimorfne sekvence, aneuploidije, kao i epigenetički profil [17]. Ciljana *NGS* strategija, koja se fokusira na amplifikaciju i analizu specifičnih sekvenci, pokazala je mnogo veću moć detektovanja mozaicizma u odnosu na sve prethodne metode [8].

PRIMENA

Tri grupe naslednih bolesti mogu biti dijagnostikovane uz pomoć PGT-a [3] :

- monogenske bolesti (engl. *single gene defects*),
- bolesti trinukleotidnih ponovaka,
- hromozomske aberacije.

MONOGENSKE BOLESTI

Monogenske bolesti se mogu nasleđivati autozomno dominantno, autozomno recesivno, kao i X vezano.

Prve autozomno dominantne bolesti koje su dijagnostikovane primenom PGT-M metode su: Marfanov sindrom, familijarna adenomatoza, Hantigtonova bolest, miotonična distrofija i bolest krhkih kostiju (lat. *Osteogenesis imperfecta)*. Danas se PGT-M primenjuje za većinu autozomno dominantnih bolesti [3].

Neke od autozomno recesivnih bolesti koje se mogu dijagnostikovati pomoću PGT-M metode su: cistična fibroza, srpasta anemija, *Tay Sachs*-ova bolest, spinalna mišićna distrofija, ß talasemija, adrenogenitalni sindrom, i hipofosfatemija.

Beta talasemija je izazvana mutacijom u beta globinskom genu. Međutim, postoji veliki broj različitih mutacija u okviru beta globinskog gena, pogotovo između različitih etničkih grupa, što dodatno komplikuje PGT-M [3].

Imajući u vidu da je cistična fibroza najučestalije monogensko autozomno recesivno oboljenje kod ljudi bele rase, opravdana je najčešća upotreba PGT-M, u slučajevima kada se sumnja na ovu bolest. Ono što ovu, gotovo rutinsku proceduru, može otežati jeste postojanje 800 mutacija koje se dovode u vezu sa razvojem ovog patološkog stanja [3].

Zahvaljujući primeni PGT-M, prilikom sumnje na X vezana oboljenja moguće je izvršiti selekciju embriona za embriotransfer, koji nisu nosioci mutacije, bez obzira na pol – zdravi muški i ženski embrioni sa normalnim kariotipom [3].

COMPARATIVE GENOMIC HYBRIDIZATION AND MICROARRAY- CGH

In the meantime, new genetic methods have been developed, enabling simultaneous analysis of all chromosomes with far greater precision. One of these methods is metaphase comparative genomic hybridization - mCGH [2]. Although this molecular cytogenetic method could reliably detect aneuploids, its weakness lay in the time necessary for analysis (3 to 5 days) which is why embryo transfer could not be performed within the required time interval. Abandoning the previous freezing method (slow freezing) and employing the more sophisticated vitrification method, which is still in use nowadays (survival of blastocysts upon thawing is over 96%) [15], enabled the application of mCGH. Vitrification upon biopsy provides enough time for genetic analysis and interpretation of results and allows for embryo transfer to be performed at a time of optimal endometrial receptivity [14].

Subsequently, mCGH was substituted with the microarray-CGH method. The advantage of this technique over other methods lies in the shortening of the analysis time to one day, in increasing the number of chromosomes analyzed, as well as in a more precise detection of aneuploidies [2].

SNP and *NGS*

The SNP method discovers, not only aneuploidies, but also duplications and deletions, and it can also provide information on the origin of the chromosomes from the father and the mother in uniparental disomy (UPD) [2,16].

Today, NGS is used as the standard in clinical practice. This method includes previous whole genome amplification, which enables performing multiple analyses, at the same time, on only one cell. NGS detects mutations, highly polymorphic sequences, aneuploidies, as well as the epigenetic profile [17]. Targeted NGS strategy, focused on the amplification and analysis of specific sequences, has shown a much greater power of detecting mosaicism than all the previous methods [8].

APPLICATION

Three groups of hereditary diseases can be diagnosed with the use of PGT [3]:

- monogenic disorders, i.e., single gene defects,
- trinucleotide repeat disorders,
- chromosome aberrations.

MONOGENIC DISORDERS

Monogenic disorders (single gene defects) can have autosomal dominant inheritance, autosomal recessive inheritance and X-linked inheritance.

BOLESTI TRINUKLEOTIDNIH PONOVAKA

Bolesti trinukleodinih ponovaka koje nastaju prisustvom dinamičkih mutacija, moguće je dijagnostikovati primenom PGT-M metode.

Broj ponavljanih tripleta se povećava iz generacije u generaciju, što za posledicu ima težu kliničku sliku i raniju pojavu bolesti u narednoj generaciji [14].

Hantingtonova bolest i sindrom fragilnog X hromozoma su prve u grupi bolesti trinukleotidnih ponovaka, koje su dijagnostikovane zahvaljujući PGT-M metodi. [3].

Za sve bolesti trinukleotidnih ponovaka postoje definisane granične vrednosti (enlg. *cut-off*). PGT-M omogućava razlikovanje embriona koji će razviti bolest izazvanu mutacijom od onih koji su zdravi.

HROMOZOMSKE ABERACIJE

Treća grupa bolesti koje se dijagnostikuju PGT-om su hromozomske aberacije. Hromozomske aberacije mogu biti numeričke i strukturne.

Numeričke hromozomske aberacije autozoma su uglavnom letalne, sa izuzetkom trizomija 13, 18 i 21, dok kod polnih hromozoma neke od aneuploidija su kompatibilne sa životom (Tarnerov sindrom - 45, X0; Klinerfelterov sindrom - 47, XXY; trizomija X hromozoma - 47, XXX; Jakobsov sindrom - 47, XYY) i moguće ih je detektovati primenom PGT-A metode.

Strukturne hromozomske aberacije mogu biti balansirane i nebalansirane. PGT-SR dijagnostika otkriva nosioce balansiranih hromozomskih rearanžmana od kojih su najčešće u populaciji balansirane translokacije. Nosioce karakteriše normalan fenotip, međutim često se javlja problem infertiliteta, rekurentnih pobačaja i rođenja deteta sa hromozomskim anomalijama [6].

ETIČKA RAZMATRANJA PGT-a

Kada govorimo o PGT-u, neophodno je detaljno razmotriti etičke i moralne aspekte. Prilikom sprovođenja procesa VTO može se dobiti veći broj embriona, ali embrioni sa genetičkim opterećenjem ostaće neiskorišćeni. Nameće se pitanje: šta se dešava sa tim preostalim embrionima? [18]. Stav koji je trenutno zastupljen u američkom pravosuđu i zdravstvenoj politici je da je odbacivanje embriona na ovom stadijumu daleko više etički opravdan postupak u odnosu na uništavanje fetusa prilikom abortusa [18].

Sa druge strane, postoje izvesna protivljenja PGT-u koja proističu iz istih etičkih razloga kao i protivljenja genskoj terapiji i genetičkom inženjeringu. Selektivna implantacija nedvosmisleno vodi ka sprečavanju postojanja određenih genotipova, čime se ugrožava genetička raznovrsnost i razvija izvesni oblik diskriminacije invaliditeta. Zagovornici ovog stava pozivaju se

The first autosomal dominant diseases to be diagnosed with the PGT-M method were: Marfan syndrome, familial adenomatosis, myotonic dystrophy, and brittle bone disease (lat. *Osteogenesis imperfecta)*. Nowadays, PGT-M is used for diagnosing most autosomal dominant diseases [3].

Some of the autosomal recessive diseases that can be diagnosed with the PGT-M method are the following: cystic fibrosis, sickle cell anemia, Tay-Sachs disease, spinal muscular atrophy, beta thalassemia, adrenogenital syndrome, and hypophosphatemia.

Beta thalassemia is caused by mutation in the beta-globin gene. However, there is a large number of different mutations within the beta-globin gene, especially among different ethnic groups, which additionally complicates PGT-M [3].

Bearing in mind the fact that cystic fibrosis is the most common single gene autosomal recessive disorder in Caucasians, the predominant use of PGT-M is justified, in cases where this disease is suspected. What may impede this practically routine procedure is the existence of 800 different mutations which are linked to the development of this pathological state [3].

Thanks to the application of PGT-M, when there is suspicion of X-linked disorders, it is possible to perform the selection of embryos that are not carriers of the mutation for embryo transfer, regardless of sex – healthy male and female embryos with a normal karyotype [3].

TRINUCLEOTIDE REPEAT DISORDERS

It is possible to diagnose trinucleotide repeat disorders occurring in the presence of dynamic mutations with the application of the PGT-M method.

The number of triplet repeats increases from generation to generation, which results in a more severe clinical presentation and an earlier onset of the disease in the next generation [14].

Huntington's disease and the fragile X syndrome were the first diseases in the group of trinucleotide repeat disorders to be diagnosed with the PGT-M method [3].

There are cut-off values defined for all trinucleotide repeat disorders. PGT-M enables the differentiation of the embryos that will develop disease caused by mutation from the healthy embryos.

CHROMOSOME ABERRATIONS

The third group of diseases diagnosed by PGT are chromosome aberrations. Chromosome aberrations can be numerical and structural.

Numerical autosomal chromosome aberrations are mostly lethal, with the exception of trisomy 13, 18, and 21, while in sex chromosomes some of the aneuploidies are compatible with life (Turner syndrome - 45, XO;

na ismevanje pravog značenja roditeljstva, lišavanja mogućnosti roditelja i dece za lični i moralni rast koji se ostvaruje iskorišćavanjem maksimalnih potencijala onoga što im je priroda podarila [18].

Umanjivanje genetske raznolikosti, kao problem od izuzetnog značaja, izneli su predstavnici osoba sa invaliditetom. Kao argument, navode da složeni skupi i neprirodni postupci za odabir embriona bez nepravilnosti u genomu prenose poruku o postojanju diskriminacije prema osobama sa invaliditetom. Iako se smislenost ove tvrdnje ne može dovesti u pitanje, ne postoji način da se ograniče reproduktivne slobode parova koji žele da smanje rizik od rađanja deteta sa invaliditetom [18].

Sve tehnike, koje su trenutno u upotrebi, razvijene su sa ciljem da favorizuju zdravlje u odnosu na bolest. Mogućnost zloupotrebe ovih tehnika za odabir embriona prema polu ili na osnovu drugih osobina, koje nisu u vezi sa zdravljem, nedvosmisleno postoji. Upravo to je jedan od razloga zabrinutosti bioetičara [18].

Opravdan i prihvatljiv razlog za izbor pola deteta jeste postojanje visokog rizika za razvoj poremećaja koja se nasleđuju X-vezano ili Y-vezano. Selekcija u odnosu na pol, radi uravnoteženja porodice, u smislu broja muške ili ženske dece, ne nailazi na odobravanje [19].

U porodicama u kojima postoji već rođeno dete sa teškim monogenskim oboljenjem ili ako postoji visok rizik za nastanak aneuploidija, korišćenje PGT, u etičkom smislu, nailazi na odobravanje, jer se izbegava abortus ili rana smrt novorođenčeta i omogućava dobijanje zdravog potomstva. Takođe, korišćenje PGT u svrhu donacije stem ćelija ili tkiva bolesnom bratu/sestri, tzv. preimplantaciono tipiziranje tkiva, u većini zemalja Evropske Unije nailazi na odobravanje [20].

Izbor osobina koje se povezuju sa razvojem nekog talenta, određenih fizičkih atributa ili bilo kakvih osobina koje nisu u direktnoj vezi sa zdravljem nailazi na oštre osude [19]. Dejvid King izražava zabrinutost u vezi sa takvim načinom odabira potomstva jer bi on značio determinizam koji je daleko snažniji od samih gena. On smatra da bi se na taj način ugrozio genofond uticajem privremenih kulturoloških koncepata koji imaju za cilj stvaranje savršene jedinke [19].

ZAKLJUČAK

PGT omogućava precizniju selekciju najkvalitetnijih embriona, sa jasnim, moralnim ciljem – rođenje zdravog euploidnog deteta.

Ako imamo u vidu senzitivnost i rezoluciju metode *NGS,* koja se danas koristi kao standard u dijagnostici, jasno je zašto primena *NGS*-a u PGT-u, u odnosu na ranije korišćene metode, značajno povećava stopu uspešnosti začeća u VTO.

Klinefelter syndrome - 47, XXY; trisomy X - 47, XXX; Jacob's syndrome - 47, XYY), and they can all be detected with PGT methods.

Structural chromosome aberrations may be balanced and unbalanced. PGT-SR diagnostics discovers the carriers of balanced chromosomal rearrangements, of which the most common ones in the population are balanced translocations. The carriers are characterized by a normal phenotype, however, the problem of infertility, recurrent miscarriages, and birth of children with chromosomal anomalies often occur [6].

ETHICAL CONSIDERATIONS OF PGT

When speaking of PGT, it is necessary to consider the ethical and moral aspects in detail. In the process of IVF, multiple embryos may be obtained, however, embryos with a genetic load will remain unused. The question remains as to what happens with the residual embryos [18]. The current prevailing attitude within the American judiciary and health policy systems is that discarding embryos at this stadium is far more ethically acceptable than the destruction of the fetus during abortion [18].

On the other hand, there are certain oppositions to PGT, stemming from the same ethical reasons as the opposition to gene therapy and genetic engineering. Selective implantation unequivocally leads to the prevention of the existence of certain genotypes, whereby genetic diversity is jeopardized and a certain type of disability discrimination is developed. The proponents of this attitude suggest that this is a mockery of the true meaning of parenthood, depriving the parents and the children of an opportunity for personal and moral growth, which is achieved through maximal utilization of the potentials provided by nature [18].

Decreasing genetic diversity, as a problem of utmost importance, has been brought forward by representatives of persons with disability. They state, as a key argument, the fact that expensive and unnatural procedures of selecting embryos without abnormalities in the genome, send a message of discrimination towards persons with disabilities. Although the reasonableness of this claim is not to be disputed, there is no way to limit the reproductive liberties of couples who seek to reduce the risk of giving birth to a child with disability [18].

All the techniques currently in use have been developed with the aim of favoring health over disease. The possibility of abusing these techniques for the purpose of choosing the sex of the embryo, or favoring any other trait, which is not connected with health issues, is undoubtedly present. This is the very reason for concern expressed by bioethicians [18].

Dosadašnji podaci iz literature daju nam nedvosmislenu informaciju o napretku metoda koje se koriste u preimplantacionom genetičkom testiranju, ali otvaraju mogućnost za razvoj novih, manje invazivnih i neinvazivnih metoda analize i procene kvaliteta embriona, a sve u cilju dobijanja zdravog potomstva.

Značaj i korisnost razvoja PGT-a nije moguće osporiti. Ipak, u svakom trenutku, treba sagledati što širu sliku, imajući u vidu potencijalnu zloupotrebu do sada razvijenih metoda i svest o etičkim i moralnim ciljevima PGT-a.

SPISAK SKRAĆENICA

CGH – komparativna genomska hibridizacija (engl. *comparative genomic hybridization*)

DNK – dezoksiribonukleinska kiselina

ESHRE – Evropsko udruženje za humanu reprodukciju i embriologiju (engl. *European Society for Human Reproduction and Embryology*)

FISH – fluorescentna *in situ* hibridizacija (engl. *fluorescence in situ hybridization*)

HLA tipizacija – humana leukocitarna antigen tipizacija (engl. *human leukocyte antigen typing*)

ICM – unutrašnja ćelijska masa (engl. *inner cell mass*)

ICSI – intracitoplazmatično injektiranje spermatozoida (engl. *intracytoplasmic sperm injection*)

IVF – *in vitro* fertilizacija

mCGH – metafazna komparativna genomska hibridizacija (engl. *metaphase comparative genomic hybridization*)

mtDNK – mitohondrijalna DNK

NGS – sekvenciranje nove generacije (engl. *next generation sequencing*)

PCR – lančana rekacija polimeraze (engl. *polymerase chain reaction*)

PGD – preimplantaciona genetička dijagnostika

PGS – preimplantacioni genetički skrining

PGT – preimplantaciono genetičko testiranje

PGT-A – preimplantaciono genetičko testiranje na aneuploidije

PGT-SR – preimplantaciono genetičko testiranje na prisustvo strukturnih hromozomskih rearanžmana

PGT-M – preimplantaciono genetičko testiranje na prisustvo monogenskih oboljenja

SNP – polimorfizmi pojedinačnih nukleotida (engl. *single nucleotide polymorphism*)

TE – trofoektoderm

TESE – testikularna ekstrakcija spermatozoida

UPD – uniparentalna dizomija

VTO – vantelesna oplodnja

Sukob interesa: Nije prijavljen.

A justified and acceptable reason for selecting the sex of a child is the existence of a high risk of developing a disorder that is X-linked or Y-linked. Selection of the baby's sex for the purpose of balancing the family ratio of boys and girls has not met with approval [19].

In families where there is already a child with a severe monogenic disorder or in couples at high risk of the occurrence of aneuploidies, the use of PGT, ethically speaking, is being met with approval, as it enables avoiding abortion or early death of the newborn and enables couples to have healthy children. Additionally, the use of PGT for the purpose of donating stem cells or tissue to a sick brother or sister, the so-called preimplantation tissue typing, is also being met with approval in most countries of the European Union [20].

The selection of traits linked to the development of certain talents, certain physical properties, or any other traits that are not directly linked to health, is being met with strong disapproval [19]. David King expresses concern regarding this type of offspring selection, as it would imply a determinism that would be far stronger than the genes themselves. He feels that the gene pool would thereby be jeopardized, since it would be influenced by temporary culturological concepts aimed at producing the perfect human being [19].

CONCLUSION

PGT enables a more precise selection of embryos of the best quality, with a clear moral goal – the birth of a healthy euploid child.

Bearing in mind the sensitivity and the resolution of the NGS method, which is used today as the diagnostic standard, it is clear why the application of NGS in PGT, when compared to methods employed earlier, significantly increases the success rate of conception in IVF.

The data currently available in literature provide us with clear information on the advancement of the methods applied in preimplantation genetic testing, however, they also open the possibility for the development of new, less invasive, and noninvasive methods of analyzing and assessing the quality of embryos, all for the purpose of producing healthy offspring.

The importance and benefit of developing PGT is undeniable. However, we must always bear in mind the broader picture, in order to preempt potential abuse of the methods developed so far, and not lose sight of the ethical and moral goals of PGT.

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LIST OF ACRONYMS AND ABBREVIATIONS

CGH – comparative genomic hybridization
DNA – deoxyribonucleic acid
ESHRE – European Society for Human Reproduction and Embryology
FISH – fluorescence *in situ* hybridization
HLA typing – human leukocyte antigen typing
ICM – inner cell mass
ICSI – intracytoplasmic sperm injection
IVF – in vitro fertilization
mCGH – metaphase comparative genomic hybridization
mtDNA – mitochondrial DNA
NGS – next generation sequencing
PCR – polymerase chain reaction
PGD – preimplantation genetic diagnostics
PGS – preimplantation genetic screening
PGT – preimplantation genetic testing
PGT-A – preimplantation genetic testing for aneuploidies
PGT-SR – preimplantation genetic testing for chromosomal structural rearrangements
PGT-M – preimplantation genetic testing for monogenic disorders (single gene defects)
SNP – single nucleotide polymorphism
TE – trophectoderm
TESE – testicular sperm extraction
UPD – uniparental disomy

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