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IMPACT OF BIOPSY ON HUMAN EMBRYO CONCERN WITH PREIMPLANTATION GENETIC DIAGNOSIS (PGD): A REVIEW

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Abstract:
Pre-implantation genetic diagnosis (PGD) is generally defined as the testing of pre- implantation stage embryos or oocytes for genetic defects. PGD involves embryos which are examined prior to their transfer into uterus. Embryos are obtained by in vitro fertilization with intracytoplasmic sperm injection (ICSI), and are biopsied mostly on day 3; blastocyst biopsy is mentioned as a possible alternative. The genetic analysis is performed on one or two blastomeres, by fluorescent in situ hybridization (FISH) for cytogenetic diagnosis, or polymerase chain reaction (PCR) for molecular diagnosis PGD involves identification of sex selection, antigen compatible embryo also some extent to cancer. In this paper, techniques for the embryo biopsy and application of PGD is discussed. And new concepts for reproductive health and analysis of embryo at different stages for detecting genetic disorders is being discussed.
Keywords: PGD, IVF, FISH, PCR, SNP's, TE, PGT, ESHRE

Introduction:

Preimplantation genetic diagnosis (PGD) is a tool with which we can find whether an embryo is having any abnormalities and with the help of this technique the foetus can be implanted with correct chromosomes, without any genetic disorders. Embryo biopsy detects aneuploidy, Down's syndrome and many genetic diseases. Autopsy accounts the cause of death of foetus. Attempting conception/fertilization through IVF technique, single nucleotide polymorphism (SNPs) and microarray technique analysis can be done after biopsy to understand the chromosomal abbreviations {1}. PGD has an objective for the problems frequently occurring in prenatal diagnosis. For prenatal testing, commonly used technique is amniocentesis and

chorionic villus sampling. The evaluation of embryo is done on the basis of blastocyst degree of expansion and the quality of the inner cell mass and of the trophectoderm cells (TE). Normally

developing embryos' reach the 6- to 10-cell stage on the morning of Day-3 post insemination, allowing a reproducible biopsy approach of a single cell, the timing of blastocyst expansion can vary by over 24 h and occur on Day 5 or 6, or even Day 7 in ~5% of embryos {2}. Fertilized human embryos can be biopsied 3 days after in- vitro fertilization and is examined for effects on viability and development in vitro/ after removal of one or two cells at the 8-cell stage. After inseminating on any of the following 2nd, 3rd, or 5th day for biopsy, transvaginal puncture and micromanipulation of the cleavage by removing one or two cells is done.

The embryo on which the biopsy is done, does not have any adverse effect on developing blastocyst. Micro focus computed tomography (micro-CT) is quite successful technique for the future as it shows 3D structure with the help of micro focus X-ray for detection of disease. Intracytoplasmic sperm injection (ICSI) technique helps in analysing whether the microinjection of spermatozoa from an infertile man with impaired sperm parameters into an oocyte can be experimented to check whether there are any genetic disorders and any risk in termination of foetus can be signified by embryo biopsy. For preimplantation genetic diagnosis, different type of biopsies is accounted such as cleavage stage biopsy which is performed on day 3 where there is removal of one or two cells. Blastocyst biopsy occurs between 5-7 days which is dependent upon zona pellucida disintegration. It has been discussed that impact of embryo biopsy at the blastocyst stage is the elegant prospective non-selection study. Third one is trophectoderm biopsy. Trophectoderm (TE) biopsy involves removing a small segment of trophectoderm where the range of cells obtained is not precisely defined. Also, the TE layer may differ in elasticity between blastocysts of different quality. Cleavage stage biopsy being easier to perform and standardize when compared with blastocyst biopsy, where the rate of embryo development is asynchronous and often different morphological qualities and degrees of expansion that may impact on the time and quality of the biopsy. Biopsy technique can be categorised on the basis of targeting of the blastomere during the cleavage stage and targeting of the Trophectoderm (TE) during the blastocyst stage. This also aims to the main cause of failing of cell division of sister chromatids. Thus, failure of homologous chromosomes leads to nondisjunction. This nondisjunction can be known with the help of preimplantation genetic diagnosis (PGD). Preimplantation genetic testing (PGT) is used to sex embryos for family balancing and to select for specific genetic traits {2}. Common Mendelian disorders that utilize PGD in many centres include cystic fibrosis, beta-thalassemia, sickle cell disease, myotonic dystrophy, Huntington's disease, Fragile X syndrome, and spinal muscular atrophy among others. Another sign for PGD is in numerical structural chromosomal abnormalities which are found after miscarriages via autopsy and how the foetus is affected. Structural alterations include translocations, inversions, deletions, and other rearrangements in the chromosomes {3}. Embryo biopsy thus serve as a diagnostic tool for studying and detecting genetic disorders and genetic analysis of embryo. In this paper, types of embryo biopsy, techniques for the embryo biopsy and detection of genetic disorders via these autopsies is discussed. And new concepts for reproductive health and analysis of embryo at different stages for detecting genetic disorders is being discussed.

Types of biopsy: There are several types of biopsy which are done on embryo at different stages.

Cleavage stage biopsy:

Cleavage stage biopsy is normally performed on day 3 embryos with at least 6 blastomeres. It is generally considered that the best moment to remove one or two cells from human embryos is between the six- and ten-cell stage, because at that moment all the cells are considered as totipotent, compaction has in most embryos not yet occurred, and sufficient time is left for diagnostic procedures before transfer on Days 4 or 5 {4}. The zona pellucida is opened and Ca++, /Mg++ free medium is used in order to loosen cell-cell adhesion and facilitate selected blastomere removal. The biopsy is mainly conducted following three methods of zona breaching, namely, laser-assisted, mechanical, and Tyrode's drilling. The embryo is kept in the media surrounding the temperature about 60-80°C which depends upon laser beam intensity. Zona pellucida breaching itself can impact subsequent processes along preimplantation development up to the blastocyst stage. In particular, several studies highlighted the impairment of the blastocyst hatching process, whose seriousness depends on number and size of holes produced {5}. Using good to excellent quality eight-cell embryos on Day 3 gives a higher chance of reaching top quality, good quality and early blastocysts on Day 5 {4}. According to the ESHRE PGD Consortium data collections, usually aspiration of one or two blastomeres, is common approach for genetic material for PGD analysis in cleavage stage biopsy (Harper et al., 2006). Precompaction eight-cell embryos are usually biopsied early on day 3, and following genetic diagnosis, the embryo transfer may be performed on the same day (Boada et al., 1998) or delayed to day 4 or until the embryo has reached the blastocyst stage. {6}

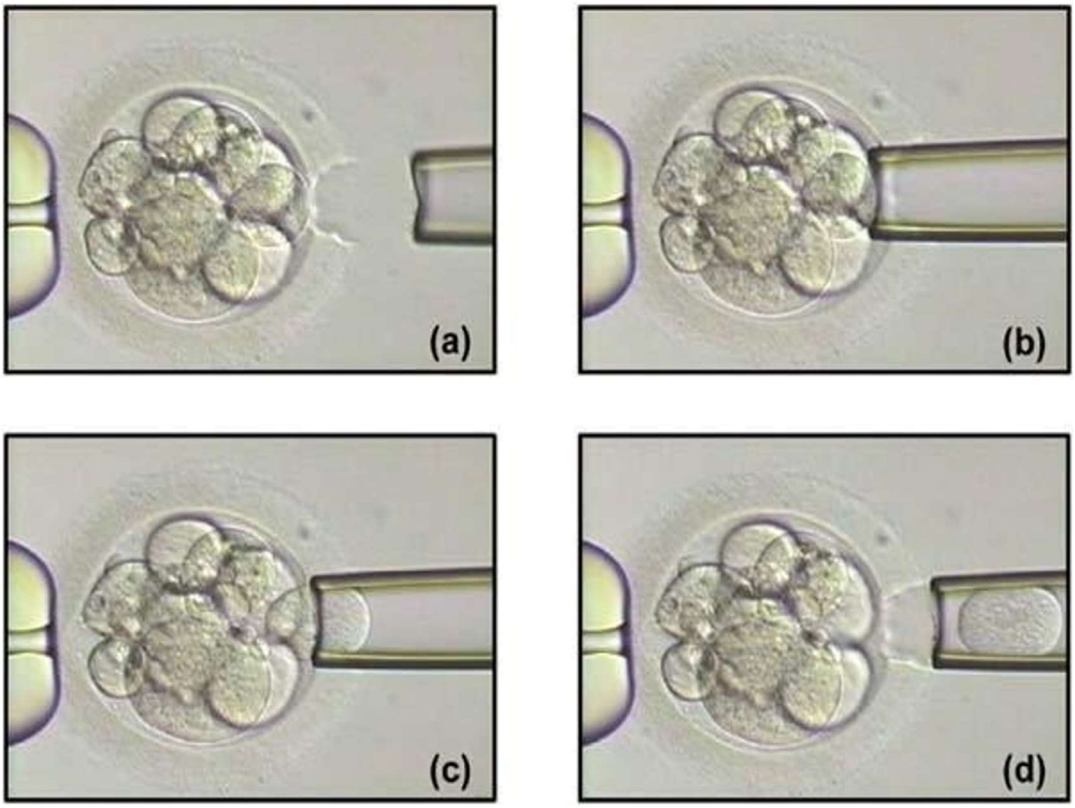

Figure 1: Cleavage stage embryo biopsy in the present study: (a) the embryo is stabilized by a holding pipette (left) with negative pressure, (b) a hole is created in the zona pellucida (zona drilling) using

laser, (c) and (d) a blastomere is gently pulled away from the embryo using a biopsy pipette (right) with negative pressure through the hole in the zona pellucida
{8}.

Blastocyst stage biopsy:

In blastocyst stage biopsy, embryos are cultured to the blastocyst stage, and on day 5 after fertilization, they are assessed for blastocyst formation. Embryos developed to blastocyst stage when it contains three to five (TE) trophectoderm cells biopsied (Kokkali et al., 2005)
{5, 9}. Biopsy of multiple trophectoderm cells from blastocyst is preferred rather than a single cell from cleavage stage because it potentially lead to improved PGD outcome for patients. Blastocyst trophectoderm biopsy using micromanipulation methods was first reported by Dorkas et al. (1990) {5}. Biopsy at the blastocyst stage provides the advantage that more cells can be removed for genetic analysis and, with respect to PGD for monogenic disorders. {5} In blastocyst biopsy, relatively high implantation rates observed in the present study are in accordance with 41% per biopsied fresh blastocyst reported by McArthur et al. (2005) {5, 10} In blastocyst stage biopsy, embryo viability and implantation potential is not affected {11}. Blastocyst stage biopsy investigates blastocyst morphology with the help of sequencing techniques.

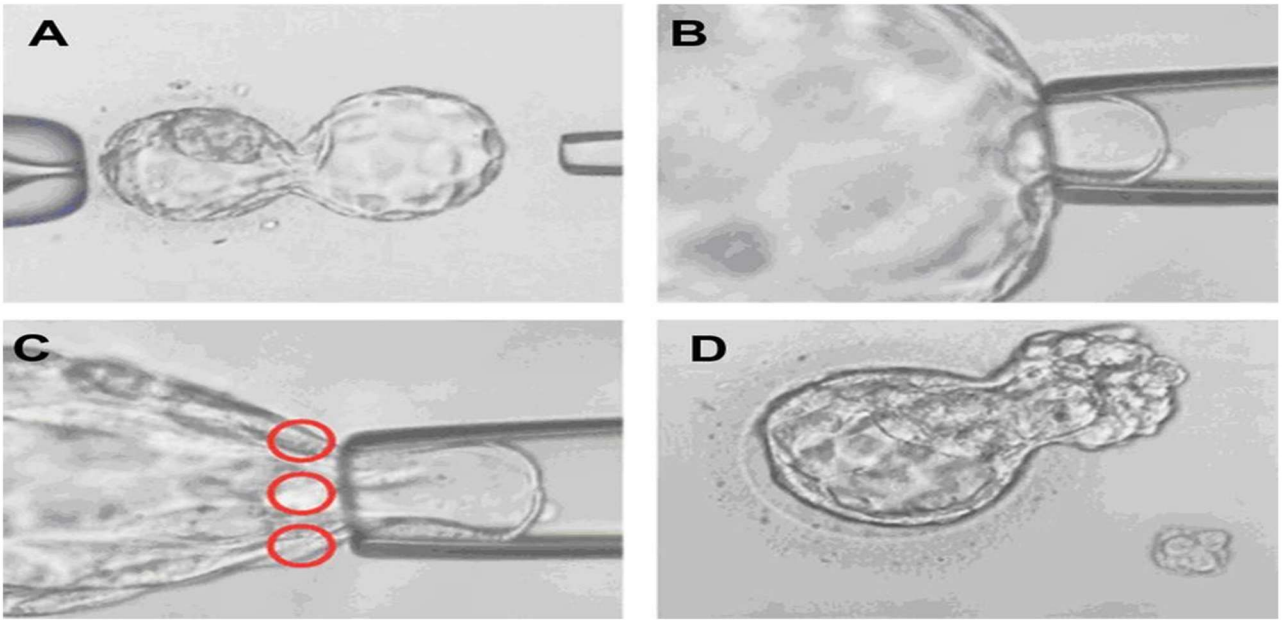

Figure 2: Day 5 blastocyst-stage embryo biopsy: Expanding blastocyst with the trophectoderm protruding through an opening in the zona pellucida made on day 3 or 4 with a micro laser (a). Aspiration of the protruding trophectoderm cells through the zona pellucida (b). Laser shots, represented by red circles, are applied to break down the tight junctions between trophectoderm cells (c). Aspirated trophectoderm cells (range: 2–9 cells) (d) {12}

Trophectoderm Biopsy

TE biopsy for PGT was first reported 15 years ago, with the development of comprehensive chromosomal screening (CCS) technique, it is becoming a major technique for PGT-A. The number

of biopsied cells is one of the most critical factors affecting TE biopsy, the general consensus among researchers is that this number may be 5 cells. {13} Cimadomo et al showed that 8 trophectoderm cells were required for successful DNA amplification and conclusive diagnostic results; thus, the most suitable cell number for biopsy may be 5-10 cells rather than 1-5 cells. {13,14} Goossens et al57 reported that the removal of one or two cells of blastomere in cleavage stage had a significant influence on embryo development on

Day 5, that is; removing a smaller number of cells by embryo biopsy is less invasive for embryo development. Similarly removing 5 to 10 TE cells by biopsy may be less invasive for expanded blastocyst, which have a larger number of total cells than early-stage blastocyst, which have fewer cells. So the chances of pregnancy gradually increases with TE biopsy
{13,15}. Most blastocysts are vitrified within 30 minutes after TE biopsy, enabling high rates of clinical pregnancy per transfer. Recent studies have indicated that excess laser pulses may induce a higher frequency of mosaicism. Smaller zona holes produce thinner TE cells at the hatching point, which may facilitate biopsy. Five to 10 TE cells are recommended for biopsy to decrease amplification failure and also more number of TE cells induce reduce in overlapping of cells {13}.

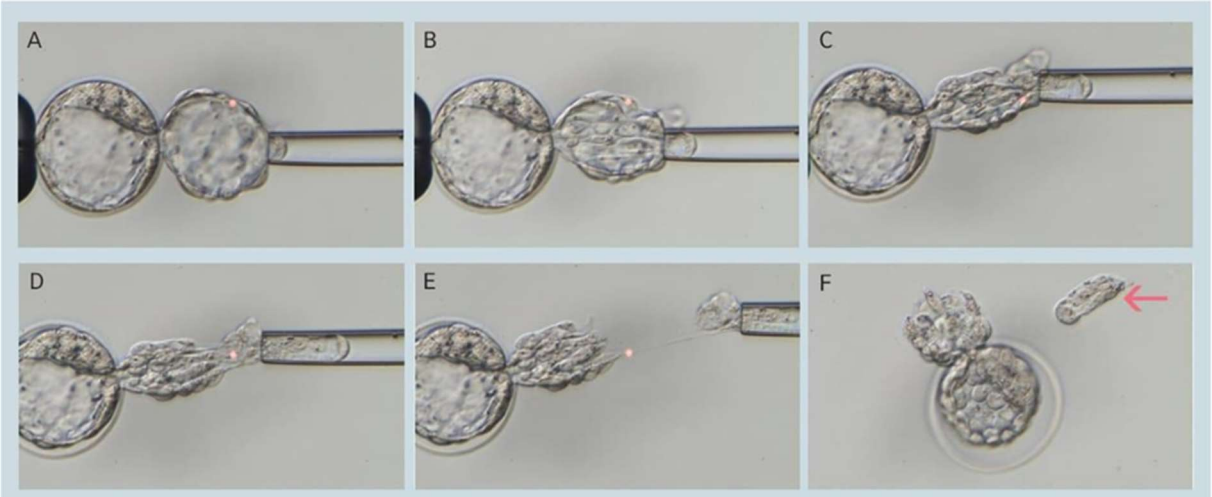

Figure 3: Trophectoderm biopsy using the "pulling" technique, indicated for an early hatching blastocyst. A-B) A blastocyst is firmly immobilized with the holding

Figure 3: Trophectoderm biopsy using the "pulling" technique, indicated for an early hatching blastocyst. (a- b) A blastocyst is firmly immobilized with the holding capillary. Note the ICM positioned away from the biopsy side. Gentle suction is applied to aspirate the trophectoderm cells facing the biopsy needle. (c- e) While applying laser pulses, the biopsy pipette is slowly pulled away from the embryo. Note the importance of the correct internal diameter of the biopsy pipette, so that, after embryo collapse, trophectoderm cells are well controlled inside the pipette and are not lost after detaching from the blastocyst. (f) The blastocyst together with the excised trophectoderm cells (arrow) after the biopsy procedure. Note the importance of having a good microscope optics and electronic condenser to be able to have an optimal image of the nuclei and the cell junctions {16}.

Techniques for different type of biopsies:

Preimplantation Genetic Diagnosis (PGD) requires multiple steps and manipulations of the gametes and embryos in order to select unaffected embryos for transfer and subsequent potential pregnancy. {3} Assisted reproductive technology (ART) has enabled the possibility of allowing couples with a family history of a genetic disease, or a disease-carrying gene, to have preimplantation genetic diagnosis (PGD) to reduce the chance of them having a child with the genetic disorder (Handyside 1989; Cimadomo 2016) {17, 18}. With the updated International Glossary on Infertility and Fertility Care, PGD was renamed preimplantation genetic testing (PGT) for monogenic/single gene defects (PGT-M) (Zegers-Hochschild 2017). {17, 19} The PGT-M and PGT-A are techniques for detecting abbreviations and defects in genes further used for different purposes. PGT-M is used for patients with genetic disease or a disease-carrying gene, and PGT-A is used for patients with repeated implantation failure, recurrent miscarriage, or advanced maternal age {17}.

PGT-M has been used for a range of single gene disorders including cystic fibrosis, Tay- Sachs disease, haemophilia A, sickle cell disease, spinal muscular atrophy, Duchenne muscular dystrophy, thalassemia, reciprocal or Robertsonian translocations, Down syndrome (trisomy 21) and Edwards syndrome (trisomy 13) (Demko 2010 {20}; De Rycke 2017{21}; Lee 2017{22}) {17}. Biopsy for PGT-M is usually performed at day 3 of cleavage-stage embryo development, when the embryo is at the six- to eight-cell stage (Harper 2010). {17, 23} Two common PGT-M tests are polymerase chain reaction and fluorescent in situ hybridisation for comparison of day 3 and day 5 embryo biopsy (Demko 2010; De Rycke 2017) {17, 20, 21} An IVF procedure is necessary to have access to the oocytes and create multiple embryos for testing. A transvaginal needle aspiration of multiple ovarian follicles is then performed to retrieve the oocytes when they are appropriately developed. The oocytes are then inseminated in vitro and the resulting embryos followed in culture several days if viability continues. Fertilization of the oocytes can occur within hours of oocyte retrieval by one of two mechanisms: 1) conventional insemination, where several hundred thousand sperm are placed around the oocyte and the two gametes are allowed to spontaneously fertilize, or 2) intracytoplasmic sperm injection (ICSI), where one sperm is mechanically inserted into the oocyte.

Amplification of a very small amount of DNA (using PCR) is required to analyse and identify a particular genetic mutation. Once IVF has been performed, a biopsy procedure is required to remove cells for preimplantation genetic testing. The DNA is obtained from either the first and/or second polar bodies given off from the oocyte or the embryonic blastomeres.

Polar body biopsy may be most useful for FISH techniques utilized in aneuploidy testing assuming that most cases result from abnormal divisions in the oocyte during meiosis {3}. It seems that for the FISH PGD cycles, there is no need to biopsy two cells, since it does not increase the chance to obtain a diagnosis. Michiels et al. (2006), revealed a significant difference in proportion of embryos without diagnosis when one or two nuclei were available for FISH analysis {24, 25}. Non-transferable (poor or abnormal) embryos derived from clinical IVF/ICSI are useful for evaluation of optimal procedures for cryopreservation of biopsied embryos {26}.

Detection of genetic disorders via embryo biopsy:

PGD determines sex linked diseases, chromosomal disorders as well as single gene defects for broad study of genetic variations in embryology and developmental biology {7}. The first clinical application of PGD used a generic PCR protocol for gender determination to avoid the transfer of male embryos which have a 50% probability of being affected by an X-linked recessive disorder. Gender was determined in a single blastomere by a single round of PCR using primers for Y-chromosome specific repetitive DNA sequences {27} Monogenic diseases or chromosomal abnormalities and aneuploidies can be detected with a single TE biopsy. For monogenic disorders, the appropriate PCR-based assay was applied (Sermon et al., 2004). In case of X-linked recessive disorders, sexing of the embryos by FISH was offered if no specific PCR assay was available. For chromosomal aberrations, a specifically designed FISH procedure was used (Sermon et al., 2004) {28, 29}. PGD was primarily used for Mendelian disorders. Mendelian disorders are single-gene defects often defined by describing their basic pedigree patterns: autosomal dominant, autosomal recessive, X-linked (recessive or dominant) and Y-linked (rare) {3}.

The majority of embryo sexing is now accomplished using fluorescent in situ hybridization (FISH) which is less prone to contamination and can also provide the copy number for each chromosome tested thereby potentially avoiding the transfer of common chromosome abnormalities such as triploidy or X-monosomy. 8, 9. {27}. Aneuploidy is one of the greatest causes of failed implantation for pregnancy and miscarriage, as well as a major cause of birth defects in children. Microarray-based SNP genotyping and karyomap analysis of embryo biopsies has the advantage that both paternal and maternal meiotic trisomy's and monosomies of either origin can be identified accurately {30}. PGT - A is an analysis of embryo cells to determine if there is the normal amount of chromosomes. Preimplantation genetic testing for a monogenic disease (PGT-M) A disorder involving a single specific gene is due to a mutation in the DNA sequence. This results in diseases such as cystic fibrosis and sickle cell anaemia.

Conclusion:

Preimplantation genetic testing has helped many individuals prevent the birth of children with severe genetic diseases. The biopsy of only one cell significantly lowers the efficiency of a PCR-based diagnosis, whereas the efficiency of the FISH PGD procedure remains similar whether one or two cells are removed. For better results and amplification for sexing of embryos TE biopsy is preferable. Efficiency of PCR diagnosis is significantly lower in case of one-cell biopsy. Because non-amplification is the major cause of diagnosis failure, If one blastomere is not compatible with accurate diagnosis and if no multiplex PCR is available then in that case two cell biopsy is restricted. Using different biopsy techniques, one can use Preimplantation Genetic Diagnosis (PGD) as a great tool in reproductive techniques when it concerns with human embryo study. PGD techniques has shown increase in the number of pregnancies after Trophectoderm biopsy and PGT of human blastocysts. These biopsies which are accomplished successfully shows growth in future for embryonic and developmental studies. Using techniques like PCR, FISH, IVF, ICSI diagnosis of certain genetic diseases and prenatal deaths can be examined and the risk factors after performing embryo biopsy can

be reduced with the help of Preimplantation Genetic Diagnosis.

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