Original Contribution

METHODS OF EMBRYO SELECTION: POSITIVE AND NEGATIVE STATE OF SELECTED METHODOLOGIES

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ABSTRACT

Methods of embryo selection are based on invasive or noninvasive procedures which have a significant individual benefits and limitation that affect to transfer healthy embryo into uterus. Invasive techniques of preimplantation genetic diagnosis (such as FISH, CGH, PCR) increase implantation rates and reduce frequency of anomalies that are typical for developmental embryos. Using and improvement these techniques represent an effective strategy to reduce multiple gestation because of possibility to transfer singleton embryo during one IVF cycle. Non-invasive techniques such time-lapse microscopy represent new methodology that occur to analyse embryo in stable culture conditions without a risk of sample contamination or sample error. The aim idea of this review was to mention on variable techniques of selecting embryo, their positive and negative statement, and bring a future direction to eventual research.

Key words: embryo transfer, preimplantation genetic diagnosis, time-lapse monitoring

INTRODUCTION

Infertile couples face many physical, emotional and financial burdens while they are undergoing IVF cycles. In most cases they have problem with infertility for many years. The aim idea of IVF cycle is to maximize their chance of delivery in a given cycle to reduce a burden they must shoulder (1). Live and healthy newborn represents optimal outcome after IVF cycle (2). Vice-versa, aneuploidy is the most common abnormality in human embryos derived from IVF(3), also determine poor IVF outcomes (4). Live and healthy newborn can be achieved by transferring a single embryo (2). Single embryo transfer (SET) represents an effective strategy to reduce multiple gestation risk in IVF. Multiple gestation is the most significant complication of assisted reproductive treatment (ART) (4) because perinatal mortality rates are 4-fold higher for twins and 6-fold higher for triplets than for single embryo transfer. The aim of infertility therapy is a healthy child, and multiple gestation puts that goal at risk, multiple pregnancy must be regarded as a

acute complication of ART cycles (5). Elective single embryo transfer (eSET) reflecting the inadequacy of current methods of embryo selection, because of failure risk of implantation or the reluctance of body to accept selected embryo. The temporal and morphological grading criteria are significant to chosen embryo for transfer. Extending embryo culture to the blastocyst stage was shown to improve outcomes from eSET, but morphologically normal blastocysts retain a significant risk of aneuploidy (1) because acceptable morphology cannot negate the potential for chromosomal error in the selected embryo (4).

AIM AND METHODS

The aim idea of this review was to mention on variable techniques of selecting embryo, their positive and negative statement, and bring a future direction to eventual research

Methods of embryo selection

Methods of embryo selection are based on potential of embryos to be implant. These methodologies are based on invasive or noninvasive procedures which are used at various stages of development from the oocyte to cleavage-stage embryos and up to the blastocyst stage. Removal of a compartment of the oocyte or the embryo for another analysis

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is specific for invasive procedures (6). The most common invasive methods represents preimplantation genetic diagnosis (PGD) (6), which is used to analyze embryos genetically before transfer into the uterus (7). Using of noninvasive selection methods is based on identification of potential embryos without the risk of possible impacts due to the investigation itself (6). Time-lapse microscopy

(TLM) is an ideal noninvasive tool to study the dynamic biological processes of early embryo development which are significant for selection embryo into uterus (8). **Table 1** shows the aim invasive and non-invasive techniques of analyse of embryos.

Table 1. Invasive and non-invasive techniques of monitoring embryo

TECHNIQUES	INVASIVE	NON-INVASIVE
PGD	✓	
FISH	✓	
CGH	✓	
PCR	✓	
TLM		✓
METABOLOMICS		✓

PGD – *preimplantation genetic diagnosis*;
FISH - *fluorescence in situ hybridization*;
CGH - *comparative genomic hybridization*;
PCR - *polymerase chain reaction*; **TLM** - *time-lapse monitoring*; *metabolomics*.

Invasive technique – PGD, FISH, CGH, PCR

At least 50% of human embryos are abnormal. These abnormalities reduce implantation rates in embryos transferred during in vitro fertilization procedures, from 30% in women less than 35 years, to 6% in women 40 years or older (9). To analyse embryos (embryos from IVF cycle) for well-defined genetic defects is an early form of prenatal diagnosis that represents preimplantation genetic diagnosis (PGD) (10). Vice versa, preimplantation genetic screening (PGS) is screening method which allows to analyse embryos for aneuploidies. Only euploid embryos can be transfer into uterus. Indications such as advanced maternal age, repeated IVF failure, repeated miscarriage and testicular sperm extraction are used to improve pregnancy rates by PGS (11). PGD traditionally uses fluorescence in situ hybridization technique (FISH) for identification of structural chromosome abnormalities (12). FISH is the technique of choice to detect chromosome imbalance associated with chromosome rearrangements, and to select female embryos in families with X-linked disease for which there is no mutation-specific test. FISH is also

use to screen embryos for chromosome aneuploidy (13). Aneuploidy as alterations in the number of chromosomes (14) is the most commonly identified chromosome abnormality in humans, occurring in at least 5% of all clinically recognized pregnancies (15) and also it is the most common known cause of mental retardation and the principal cause of pregnancy loss (16). FISH analysis involves hybridization of chromosome-specific DNA probes labelled with fluorochromes to complementary DNA sequences on target chromosomes, followed by detection of the bound probes under a fluorescence microscope. Study of thousands of spermatozoa in relatively short period allows FISH technique. This is especially important for the accurate evaluation (17). Whether for X-linked disorders are used specific centromeric probes for X and Y chromosomes. One probe per chromosome is needed in Robertsonian translocations and for reciprocal translocation, three probes can be used: two probes on either side of the first chromosome implicated in the translocation, and the third probe proximal or distal to the other gap. Screening is usually used to analyse chromosome 13, 18, 21, X and Y for chromosomal aberration and chromosome 15, 16, 22 for errors which are usually occur in aborted children. Probability of hybridization failure and the possibility of overlap fluorescent signals of two chromosomes are the limits of this technique (7) Fixation of a

single cell on a microscope slide is a procedure that requires skill and experience and this fact represents another limitation of FISH approach which can be usually associated with any diagnostic problems (18). Another invasive technique for studying human embryo is comparative genomic hybridization (CGH). CGH is a molecular cytogenetic technique that can be used with single cells in interphase to allow simultaneous enumeration of every chromosome (19). CGH has been successfully applied to the PGS of polar bodies, cleavage-stage embryos and blastocysts with promising clinical outcomes. CGH analyse chromosomal aberrations by comparing intensities of fluorescence of two different fluorescently labeled DNA probes – reference sample of DNA, control sample of DNA. CGH is also competent to identify losses and gains of chromosome segments and should be capable of detecting imbalances caused by variety of chromosome rearrangements (18).

Mostly cases, the disease is inherited as a mendelian recessive, and both members of the couple must be carriers of the abnormal gene to be transmitted. Cystic fibrosis is a typical example which affects 1 child in every 2500 births. To identify disease such as cystic fibrosis is carried out by amplification of the gene involved using polymerase chain reaction (PCR). Mutations that can be detected by PCR are limited. Analyse embryo by PCR diagnose mutation of genetic information of holder monogenetic diseases such as cystic fibrosis, Huntington disease or disease linked to chromosome X (20). Diagnosis of single-gene disorders is the aim of PCR technique. In order to facilitate analysis, PCR amplifies DNA, extracted from a single cell into thousands of copies. Recent study introduce new technique called multiple displacement amplification which is used to increase the product of DNA available for testing from micrograms to milligrams. This reduces the risk of failure to amplify a particular allele of a certain gene, which is known as allele drop-out. To avoid contamination with extraneous DNA and subsequent misdiagnosis, the laboratory environment must be strictly controlled (21).

Noninvasive technique – TLM, Metabolomics

For embryo development such as complex process in which the exact timing and sequence of events are as essential as the accurate execution of the events themselves is time-lapse monitoring (TLM) an ideal tool to study the dynamic biological processes of early embryo development, as it provides

morphological, dynamic and quantitative timing data in a non-invasive manner (8). Time-lapse monitoring is using cameras incorporated in the incubation chamber (22). This process is able to capture images at defined intervals over a specific period of time. Captured images are processed into a time-lapse sequence and from the video sequence, morphological, dynamic and quantitative data can be extracted. For example TLM enable images to be captured at 20-min, 5-min or even 10-s frequencies. TLM is integrated with image analysis software which allows image capture and storage image, optical alignment and focusing. Ability to maintain samples in an optimal culture environment, reduce chances of sample error and improve sample tracking and data analysis are the aim benefits of TLM (8). Using traditional incubators represents a conflict between the need to capture a detailed picture of embryo development and the risk of compromising stable culture conditions (22). Normal embryo growth and development depend on stable culture such as culture pH and temperature. Samples such as embryos are sensitive to variations in culture pH and temperature and it can have adverse effects on biological samples (8). Metabolom as attitude of intra and extracellular micromolecules in culture media may have a correlation with implantation of human embryo into uterus after IVF cycle. In recent study, pyruvate, glucose or amino acids were analyse and it was found that pyruvate correlate with developmental competence of mouse, bovine and human embryos. Using changes in amino acid profiles in culture media from embryos shows similar results. Glucose consumption of embryos after compaction does allow prediction of the sex of an embryo and even live birth. For identification of aneuploidy has been used amino acid profiling (6). Applicability of these procedures in the clinical routine have proven prognostic value, but in present it is not allowed due to the technical complexity of the diagnosis and due to the need of adequate equipment (23).

DISCUSSION AND FUTURE DIRECTIONS

Less than 1000 children worldwide have been born as a result of PGD in the past decade (24). The proportion of newborns from ART varies by state and territory (25). Process of PGD involves in vitro fertilization, followed by the extraction of a single cell from the IVF embryo and genetic testing of DNA to determine which embryos to implant and which to store for future use or discard (24). Effective technique of ART such preimplantation genetic diagnosis

have many positive applications. Before routinely accepting PGD as a tool in the reproductive medicine, care should be taken to the ethical implications of its use (26). PGD offer huge perspectives but at the same time gives rise to serious ethical and practical questions (7). Using PGD to select genetically normal embryos and to transfer them into uterus generate controversial debate. Debate is occur over the implications of PGD, safety of embryonic manipulation, sex selection and questions about eugenics (27). Sex selection is frequently subject of debates, because of ethical and social implications of it (28). However, PGD needs intensive effort and expensive techniques for the medical team, and for the couples PGD present psychological and physical difficulties. It is an invasive procedure for the embryo, and the risk of mosaicism is not excluded (7). Development of ART technologies allows observation of morphological events during human embryonic development and allows reduce the risk of mosaicism (29). PGD is a hybrid procedure, bringing together the technological advances of ART and genomic medicine. Techniques of PGD have their own set of risks and benefits for the woman and the children. It is important for couple to understand the risks, benefits and implications of these procedures. These informations help couple to reduce physical and emotional burdens while they are undergoing PGD (30).

CONCLUSION

Using and improvment these techniques represent an effective strategy to reduce multiple gestation because of possibility to transfer singleton embryo during one IVF cycle. Non-invasive techniques such time-lapse microscopy represent new methodology that occur to analyse embryo in stable culture conditions without a risk of sample contamination or sample error.

Using and improving these methodologies (such PGD, TLM) bring new challeges for infertile couples to have healthy children and knowledges from these methods form strongly core for future reasearch.

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