PGDIS

19th INTERNATIONAL CONFERENCE ON PREIMPLANTATION GENETICS

PGDIS BERLIN

APRIL /10-13/ 2022

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FINAL PROGRAM

INDEX

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WELCOME MESSAGE

Dear Colleagues,

It is our pleasre to welcome you to 19th International Conference on Preimplantation Genetics, organized by PGDIS. As you see from Scientific Program, the Conference will address the most recent progress in preimplantaton genetic testing (PGT) for improving standards of ART and genetic practices, with critical analyses of the key PGT developments, with emphasis on emerging novel technologies to facilitate its application to a wider spectrum of conditions of health importance.

Due to the increasing importance of an appropriate interpretation of PGT-A results, PGDIS has recently published an updated PGDIS position statement on mosaic embryo transfer to assist clinics in the decision making for mosaic embryo transfer (https://www.readcube.com/articles/10.1007/s43032-022-00899-x). It is evident that there is a need for further understanding of the origin, clinical and biological significance of mosaicism and segmental variations, which will be explored in a special session.

It also remains important to discuss the additional adjunct methods to identify developmentally competent euploid embryos, as it is evident that chromosomal status of the embryo is not the only selection criterion necessary to improve pregnancy outcomes. While the utility of some of the previously described embryonic profiles, such as mitochondrial DNA, epigenetic and genetic expression, time-lapse imaging and endometrial receptivity, are still being explored for their clinical utility, the emerging methods of metabolomics and transcriptomics will be the other specific approaches to be addressed in more detail. Current progress in whole embryo sequencing will be also addressed.

Among other emerging technologies, will be the progress in automation required for improvement of both IVF and PGT, including the demonstration of an automated sampling procedures, examples of robotic application to NGS-based PGT-A and the experiences of artificial intelligence application for pre-selection of embryos in PGT-A.

Considerable progress in the increased application of PGT-M will be addressed in relation to the recent developments of expanded carrier screening (ECS). This is not only changing the spectrum of indications for PGT-M and impacting significantly on the uptake of this technology but is also demonstrating that PGT is a genuine approach to the primary prevention of monogenic disorders.

While the laws regarding PGT in neighboring countries are advancing, it still remains restricted in Germany, the venue of this Conference. Addressing ethical and legal challenges for modern PGT advances will therefore be of special relevance for future health policy development.

Although there has been an increasing interest in the development of non-invasive PGT (NIPGT) in the last few years, many sources of contaminating DNA have not been completely overcome. Thus, a special session will be devoted to this topic, with the emphasis on detection and avoiding DNA contamination, as the key for the further development of NIPGT as a diagnostic method. Alternatively, its restricted role as a possible screening test for the prioritization of transfer of euploid embryos will be considered.

We do hope that you will enjoy not only scientific discussions but also the city of Berlin, which is the second largest city in Europe and a popular tourist destination.

Don Leigh, PhD
President of PGDIS

Anver Kuliev, MD, PhD
Executive Director of PGDIS

GENERAL INFORMATION

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More information: https://www.langenbeck-virchow-haus.com/en.html

The official language of the congress is English. No simultaneous translation will be provided.

EACCME Accreditation

PGDIS Conference program was approved by Berlin Chamber of Physicians for 21 continuing medical education (CME) credits.

Poster Session

- Abstracts accepted for poster presentation will be presented as **E-Poster** (electronic poster).
- **Poster size:** Landscape 16:9; approximately 80,01cm HIGH x 142,24cm WIDE or 31,5inch HIGH x 56inch WIDE.
- In order to engage interactivity during the congress the Scientific Committee ask the authors to include in the bottom margin of the poster the email of the presenting author or corresponding author. Participants will be able to send questions to this email and the authors should answer these emails during the Congress days.

Congress badges

All participants, accompanying persons and exhibitors must wear the Congress identification badges. Entrance to meeting rooms and exhibition area will not be allowed to any person without badge.

Certificate of attendance

A certificate of attendance will be sent by email to each delegate at the end of the congress sessions. For special requirements, please contact the Technical Secretariat.

Liability

Upon registration, participants agree that neither the Organizing Committee nor the Technical Secretariat assume any liability. Participants should, therefore, organize their own health and travel insurance.

PRE-CONGRESS PROGRAM

Pre-Congress Session, Sunday, 10th April (2:00 pm-5:00 pm)

PGT in Population Settings with Restricted PGT Regulations: From Preconception PGT to NIPGT

Chairs: Luca Gianaroli & Andreas Schmutzler

Experience of Preconception Genetic Testing with Restricted Number of Allowed Oocytes for IVF – **Luca Gianaroli**

Preconception Genetic Testing with Restricted Embryo Manipulation – **Andreas Schmutzler**

Preconception Genetic Testing for Monogenic Disorders – **Svetlana Rechitsky**

Preconception Testing for Aneuploidy - **Alan Handyside**

Prospect with Application of NIPGT – **Don Leigh**

Panel Discussion

Pre-Congress Session, Monday, 11th April (9:00 am -12:00 am)

Current Progress in PGT Technology

Chairs: Joe Leigh Simpson & Richard Scott

Present Accuracy and Reliability of PGT-M – **Svetlana Rechitsky**

RCTs on PGT-A: Critical Evaluation - **Richard Scott**

Evaluation of Mosaic Embryo Origin - **Alan Handyside**

PGT-M for Common Late-Onset Conditions - **Joe Leigh Simpson**

Universal PGT and PGT Future - **Mark Hughes**

Panel Discussion

MAIN SCIENTIFIC PROGRAMME

MONDAY APRIL, 11TH

14:00-14:15 **OPENING AND WELCOME – A Kuliev**

14:15-14:45 **PLENARY LECTURE:** Current Status of Whole Genome Sequencing - Prospect for Expanding PGT Application
S Antonarakis

14:45-16:15 **SESSION 1. Beyond aneuploidy - adjunct methods to assist identification of developmentally competent euploid embryos**
Chair: D Wells and J Vermeesch

- 14:45-15:15 Emerging technologies to improve embryo developmental competence
  D Wells
- 15:15-15:45 Transcriptomics: Mapping transcriptional profile and its correlation to embryo developmental competence
  S Madjunkova
- 15:45-16:15 Metabolomics: Prospect of Application to PGT
  S Munne

16:15-16:45 **COFFEE BREAK**

16:45-17:15 **PLENARY LECTURE: Origins and mechanisms of aneuploidy, sub-chromosomal variations and mosaicism**
U Eichenlaub- Ritter

17:15-18:45 **SESSION 2. Identification and transfer of embryos with abnormal copy number variations**
Chairs JL Simpson & U Eichenlaub- Ritter

- 17:15-17:45 Follow-up of Embryos with Mosaicism and Segmental Aneuploidy
  M Viotti
- 17:45-18:15 Sub-chromosomal Variations- Clinical and Biological Significance
  M Katz-Jaffe
- 18:15-18:45 PGDIS Policy on Mosaic Embryo Transfer
  D Leigh

TUESDAY, APRIL, 12TH

8:30-9:00 **Summary of highlights of previous day and introduction to the second day**
D Leigh

09:00-09:30 **PLENARY LECTURE:** PGT-A as Standard IVF practices
R Scott

09:30-11:00 **SESSION 3. Free communications (selected abstracts related to Clinical PGT-A)** –
Chairs: S Kahraman & K Xu

11:00-11:30 **COFFEE BREAK**

11:30-12:00 **PLENARY LECTURE:** Expanded Carrier Screening: Impact on PGT-M Uptake
J Goldberg

12:00-13:30 **SESSION 4. Towards robotization to standardize PGT procedures**
Chairs: T Gordon and T Strowitzki

12:00-12:30 NGS
P Echave

12:30-13:00 Sampling
S Munne

13:00-13:30 Artificial Intelligence
J Blazek

13:30-14:30 **LUNCH BREAK**

14:30-16:00 **Session 5. New approaches for universal PGT**
Chairs: G Harton & A Thornhill

14:30-15:00 Practical application of universal PGT
J Vermeesch

15:00-15:30 Third generation sequencing
D Cram

15:30-16:00 Whole Embryo Sequencing
C Gawad

16:00-16:30 **COFFEE BREAK**

16:30-18:00 **Session 6. Free communications (selected abstracts related to session 5)**
Chairs: M Viotti & A Kuliev

WEDNESDAY, APRIL, 13TH

08:30-09:00 **Summary of highlights of previous day and Introduction to the third day**
JL Simpson

09:00-09:30 **PLENARY LECTURE:** Expanding PGT-M Application
S Rechitsky

09:30-11:00 **Session 7. Debate: Genetic Counseling and Ethical Challenges for Application of Modern PGT Advances**
A Schmutzler (Moderator)
Panel: D Wells (Genetics); **A Schmutzler** (Medicine); **L Gianaroli** (Counseling); **B Imthurn** (Ethics); **J Taupitz** (Law); **J Müller-Jung** (Press)

11:00-11:30 **COFFEE BREAK**

11:30-13:00 **Session 8. Free communications (selected abstracts related to NIPGT)**
Chairs: C Rubio & S Madjunkova

13:00-14:30 **Session 9. Controversy in Application of Non-invasive PGT: Screening or Diagnosis**
Chairs: R Scott & L Gianaroli
Panel Discussion: L Gianaroli; C Rubio; D Leigh

14:30 **CLOSING REMARKS: A Kuliev**

BOOK OF ABSTRACTS

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

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SPEAKER'S ABSTRACTS

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MORE GENOMIC KNOWLEDGE, MORE OPPORTUNITIES AND MORE CHALLENGES

Stylianos E. Antonarakis[1,2]

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Preimplantation genetic testing could now diagnose a considerable number of high impact variants in the genome of the embryo, that cause severe disorders. The detection methods have greatly improved in the last years for more accurate diagnosis. However, there are thousands of additional (near)-Mendelian severe phenotypes for which diagnosis could not be provided because the causative gene/variants are unknown, or the functional consequences of variants are unknown. Below I list some challenges of PGT; this list is certainly biased coming from a geneticist that is not considered an insider in this field. Challenges and opportunities from the use of other omics methods and epigenetic modifications of the genome (methylation and histone modifications) are beyond the scope of this presentation.

1. There is a growing list of genes that cause mendelian disorders (approximately 1 per day). All of these new genes could be used in PGT even though it takes time to decipher the full spectrum of the resulting phenotypes.
2. The major issues of penetrance and phenotype modifiers complicate the diagnostic predictions and the genetic counseling. As the molecular basis of these challenging phenomena is elucidated, PGT will enjoy more accuracy regarding the severity of the outcome. The recent example of the FOXI3-related dysmorphic syndrome will be presented.
3. Every embryo has *de novo* variants in the protein-coding fraction of the genome, and their frequency increases with advanced paternal age. Therefore, the search for *de novo* variants could theoretically be done in all cases of assisted reproduction. The interpretation of these *de novo* variants either loss-of-function or gain-of-function or dominant-negative provide a diagnostic and genetic counseling challenge.
4. Widespread use of carrier screening for autosomal recessive and/or X-linked disorders of parents will provide additional candidate PGT cases. The functional combination of likely pathogenic alleles will be a further challenge in the interpretation of results.
5. The complete sequence of the human genome using long-read technology will clarify the pathogenicity of Structural Variants (SV). Since SV are likely to be frequent causes of severe phenotypes, their accurate detection will further enhance the PGT diagnostic possibilities. Long read sequences could also provide haplotype information for diagnostic purposes.
6. The use of Polygenic Risk Scores (PRS) for embryo selection is very controversial. These scores are based on data from genomewide association studies and provide some indication of the risk for adults regarding a number of late onset phenotypes including cancers. There are many factors that lower the predictive power of PRS and the current knowledge is rather preliminary. In addition, there is a call for a society-wide conversation regarding the place of PRS in PGT (see also PMID 34192436). Recommendations from professional societies are necessary for setting up standards of practice.

TRANSCRIPTOMICS: MAPPING TRANSCRIPTIONAL PROFILE AND ITS CORRELATION TO EMBRYO DEVELOPMENTAL COMPETENCE

Madjunkova, S.

Reproductive Genetics at CReATe Fertility Centre, Toronto, Canada

Despite the advances of assisted reproduction and improved success rate of in vitro fertilization (IVF), the take home baby rate remains lower than 50%. Single embryo transfers in assisted reproduction has reduced complications associated with multiple gestations and shifted the focus to enhanced selection of embryos for transfer. Current process of selecting an embryo for uterine transfer uses an impromptu combination of morphological criteria, kinetics of development, and/or preimplantation genetic testing for aneuploidy (PGT-A) by whole genome low pass sequencing of trophectoderm (TE) biopsy. Selection of an embryo for transfer based on ploidy determined by PGT-A is the most significant stand-alone parameter to increase the implantation and ongoing pregnancy rate per transfer. Regardless of these advances in embryo selection by PGT-A, only half of euploid embryos implant. In addition, the molecular underpinnings of the initial stages of embryo development and implantation are not fully understood. It seems plausible that a better understanding of the molecular signature of embryo competence would hold the key to improve embryo selection and implantation.

One such avenue to assess the functional state of an embryo and provide additional insight into the role of the blastocyst in the initial stages of implantation is through the use of whole transcriptome RNA sequencing (RNASeq). Recent technological advances have enabled the full transcriptomic profile from single cells and have facilitated the application of RNAseq to study the transcriptional events in early human development. Our group pioneered a method for parallel genomic (gDNA) and transcriptomic (mRNA) sequencing (DoubleSeq) from a single trophectoderm biopsy (TE 4-5 cells), which is compatible with clinical PGT-A, as currently offered worldwide to prioritize embryos for transfer. Our pilot study showed that our method is comprehensive and sensitive enough to provide full transcriptomic profiles using the RNA from a single TE biopsy. The present talk will give the first insight into the results from current prospective clinical non-selection study to evaluate the transcriptomic signature of embryos with successful implantation compared to non-implanted and establish a novel genome-transcriptome based embryo selection tool to be further assessed in a randomized controlled study. In a clinical setting we were able to obtain high quality genomic and transcriptomic data with unchanged specificity and sensitivity of the standard PGT-A testing of more than 850 embryos and confirm the clinical performance of the new parallel DNA/RNA approach is comparable to standard PGT-A with a TE re-biopsy rate and non-informative results <2%. We have identified critical pathways enriched in developmentally competent euploid embryos including the upregulation of genes involved in oxidative phosphorylation and MYC pathways which are critical for the maintenance of pluripotency and in cell reprograming and have identified a transcriptomic signature of competent embryos. Furthermore, aneuploidy dramatically alters the transcriptome compared to euploid embryos and have overall increased expression of genes (~1000) involved in cell division, fatty acid synthesis and mitochondrial respiration and downregulate genes (~500) involved in angiogenesis and placentation. Applying an innovative bioinformatic approach using RNAseq that integrates gene expression and allelic imbalance not only are we able to assess the transcriptomic profile for gene expression changes associated with implantation and embryo competence, we are also able to infer ploidy which produces an RNA based PGT-A result, that affirms the utility of the transcriptomic profiling of TE biopsy in ploidy assessment. Transcriptomic profiling emerges as a powerful tool and a novel approach in assessment of embryo competence enhancing the current embryo selection approaches with the potential to improve outcomes of assisted reproduction.

ORIGINS AND MECHANISMS OF ANEUPLOIDY, SUB-CHROMOSOMAL VARIATIONS AND MOSAICISM

Ursula Eichenlaub-Ritter

Universität Bielefeld, Fakultät für Biologie, 33501 Bielefeld, Germany

It is long known from clinically recognized pregnancies that aneuploidy is the most common chromosomal aberration in the human, and that maternal age is a main determinant of aneuploidy in oocytes, pre-implantation embryos, spontaneous abortion, still births and human trisomic conceptuses (Bond and Chandley, Oxford Monographs on Medical Genetics, 1983). Hendersen and Edwards (Nature, 1968) using a mouse model were among the first to detect univalents in spreads of oocytes of aged females which at the time led to proposing the existence of a production line. According to this there is an order in progression through prophase I of meiosis in oogenesis and that oocytes with different recombinational histories are recruited sequentially for maturation with advancing maternal age- the last oocytes having low numbers and unusual sites of recombination predisposing them to meiotic errors. This hypothesis has been challenged by more recent observations (Nagaoka et al., 2012), for instance by analysis of spread oocytes of the fetal ovary (Rowsey et al., Am. J. Hum Genet., 2014). Subtle differences appear to exist but do not explain the huge increase with aging (Hassold et al. Am J Hum Genet 2021) while age may impact recombination in the next generation. While it is clear that maternal age is the most important etiological factor in human aneuploidy, more efficient and detailed methods of chromosomal analysis and studies on the impact of the genetic background, age and environmental factors such as used in preimplantation genetic testing for aneuploidy (PGT-A) and PGT – SM (PGT for structural chromosomal mutations), genome-wide association studies (GWAS) and such in individual oocytes, sperm, embryos and patient cohorts have shed new lights on highly conserved mechanisms in the genesis and sex-specific origin of aneuploidy and sub-chromosomal aberrations and mosacism in the embryo. Furthermore, studies on morphology, in particular cytoskeleton, organelles and mitochondria, and on cell cycle progression and chromosome behavior of in vitro maturing human oocytes and such in experimental animal models, including wild type, knockouts and meiotic mutants and their transcriptome, proteome, metabolome and epigenome have contributed to a much more detailed view on the contributing factors in male-, female- and embryo-derived aneuploidy. Life imaging provided more information on the origins of mitotic errors and mechanisms in human aneuploidy, generation of mosaic embryos and embryonic plasticity protecting from chromosomal instability.

I will provide an overview of these findings showing that:

i. there is sexual dimorphism and oocytes are at higher risk for nondisjunction compared to spermatocytes due to the extended meiosis, less stringent checkpoint control sensing pairing failures in prophase I and a chromosome dominated formation of an acentriolar bi-polar spindle with a relaxed spindle checkpoint in the large ooplasm that does not sense few unaligned chromosomes.

ii. there is a U-shaped correlation between maternal age where young maternal age involves chromosomes differently from old age, and aneuploidy in the young appears mainly based on nondisjunction of homologs at meiosis I, particularly of large chromosomes.

iii. in contrast aneuploidy in aged oocytes involves frequently untimely loss of attachment between homologs, chromatids and their centromeres that predisposes to premature sister chromatid segregation (PSSC) and reverse segregation (RS) at meiosis I followed by risks for second meiotic errors, particularly of small acrocentrics.

iv. loss of cohesion and progression into anaphase I in presence of multipolar spindle and unaligned chromosomes predisposes particularly aged oocytes to chromosome lagging and induction of whole chromosome and segmental aneuploidy.

v. increases in adducts and ROS by advanced glycation end products (AGEs) and mitochondrial dysfunction can affect cellular homeostasis in aged oocytes that together with an “aged environment” results in altered patterns of gene expression, chromatin conformation and lack of control over spindle attachment and actin cytoskeleton.

vi. rare mutations affecting DNA repair, recombination, chromosome cohesion or cell cycle control can cause meiotic errors or arrests, POI and sub- or infertility in both sexes.

vii. recent meta-analysis suggests that paternal age does not increase whole chromosome or segmental chromosome aneuploidy but may decrease fertilization rate and increase incidence of segmental aneuploidies in pre-implantation embryos. Mutagenic repair by microhomology mediated end joining in embryos is suspected to mediate mutagenic deletions and insertions.

viii. failures in pronuclear apposition and juxtaposition of maternal and paternal chromatin facilitating alignment of maternal and paternal chromosomes at the spindle equator upon transformation from an acentriolar spindle to a centriolar spindle in the one-cell zygote may be one factor predisposing to mitotic errors in the first mitotic divisions, unaligned chromosomes, and lagging and to mitotic aneuploidy, micronuclear formation, chromothrypsis and mosaicism in preimplantation embryos. This is unrelated to maternal particularly after age and involves preferentially paternal chromosomes. The active spindle checkpoint but absence of apoptosis in first mitotic divisions can contribute to chromosomal instability and mosaicism in few or many cells in the pre-implantation embryo.

ix. Altered gene expression and activation of checkpoints in presence of monosomies, trisomies and chromosomal imbalance in blastomeres upon zygotic gene activation can delay or arrest cell proliferation and elimination or restrict numbers of aneuploid cells in the pre-implantation embryo.

x. self-correction by allocation of aneuploid cells to extra-embryonal tissues appears to contribute to the formation of a euploid embryo that is originally derived from a mosaic blastocyst.

In conclusion, keeping in mind the amazing plasticity in early human development and the still lacking convincing evidence for improving outcomes by routinely performed PGT-A in embryo selection, PGT-A should be restricted and only used with appropriate counseling and informed consent by patients (PGDIS position statement, 2019, Gleicher et al., Reprod Biol Endocrinol.), and PGT-SM in certain patient groups, e.g. in translocation carriers. However, studies in animal models and human have identified a number of predisposing factors, for instance by life style, smoking, exposures such as to endocrine disrupting agents and chemotherapeutic agents and ROS. Together with data obtained in human PGT, concepts for prevention and reduction become now feasible, and the improvements in analysis including PTG-A and non-invasive technologies to detect aneuploidies and mosacism promise to advance treatment and prevention of aneuploidy in spontaneous and assisted reproduction.

SUB-CHROMOSOMAL VARIATIONS- CLINICAL AND BIOLOGICAL SIGNIFICANCE

Mandy Katz-Jaffe

Colorado Center for Reproductive Medicine, USA

PGT with TE biopsy has been repeatedly shown to be reliable in the detection of whole-chromosome aneuploidies. However, the true ability to diagnose sub-chromosomal gains and losses either full or mosaic is uncertain. Concern has been raised about the possibility that the incidence of all types of chromosomal mosaicism, whole and segmental, could be overestimated and represent false-positive errors. Indeed, reanalysis studies of embryos with complete segmental losses or gains have revealed a significantly higher concordance rate than for mosaic sub-chromosomal aneuploidies. Furthermore, this distinction could also reflect different biological etiologies between complete segmental aneuploidies and sub-chromosomal mosaicism. Further investigations to unravel the clinical and biological significance of these sub-chromosomal variations is critical for the ongoing success of preimplantation genetic testing.

PGT-A AS A STANDARD IN IVF PRACTICES

Richard T. Scott, Jr., MD, HCLD, ALD
IVIRMA Global, USA

Few things in the field of ART generate more passion than PGT-A. It has been mired in controversy since its inception and that has not diminished as the field has evolved. It is a favorite target for the press and sometimes regulatory agencies as they talk about how important it is to prevent patients from being exploited. In reality, they do enormous harm but not critically reviewing the state of PGT-A in contemporary clinical practice.

PGT-A seems to be maturing as a technology. That does not mean that it will not evolve, but it has certainly developed sufficiently to be considered safe and effective. Those are the standards which the FDA, EA, and other regulatory bodies evaluate medical technologies.

The big contemporary is where does PGT-A fit in contemporary clinical practice? To answer that question, we must first decide what we expect the test to do. The fundamental issue is “how much is enough”. Stated more specifically, what magnitude of improvement in what specific aspects of clinical care are sufficient to make a valuable adjunct to clinical care.

PGT-A is about reducing failure. Seemingly obvious, the reality is that it is a concept which is lost on many as they critically evaluate the field. It begins with why patients who come to a clinic for help with conceiving ultimately go home without a baby. Today, the greatest reason for that ultimate failure is giving up too soon. Patients get discouraged by the physical and emotional burden of care and just quit. They run out of money. They abandon their current clinical team and go elsewhere – and commonly are asked to start at the beginning. We do not know all the reasons couples give up, but there is no question that they do and it is the greatest reason that they fail to achieve their dream of having a family.

Our goal as providers of clinical care is to get the best possible outcomes. This includes the highest delivery rate per transfer, but also per cycle, or cumulatively over the entire cohort of available embryos. There should be fewer clinical losses. The burden losses bring to patients is enormous and is frequently neglected when considering the benefits of PGT-A. In contact groups with patients, it is considered extremely important. It is important to do 100% eSET’s without fail. Class 1 data are available which show the elimination of polyzygotic multiple gestations has provided an enormous increase in safety and prevented countless short- and long-term sequelae and the children which result from ART. There is no question that PGT-A is safer.

One of the great frustrations in discussing PGT-A with those who criticize the technology is the issue of cost. There is no question that the cost per baby born is lower when using PGT-A. Even when considering a single retrieval cycle. There are fewer futile cycles, lower cryopreservation fees as there are fewer embryos, and less health care provided during clinical losses. There is no question that PGT-A REDUCES the financial burden of care and those that do not use PGT-A make more money from a given patient than those who employ it. PGT-A also allows effective embryo banking for patients who want to bank sufficient embryos while they are young enough to have quality oocytes and who plan to build their family progressive over several years.

We have brought much of this on ourselves. FISH was inadequate. The STAR trial and the recent NEJM paper by Yan are methodologic disasters. Analytical platforms are put into clinical use with insufficient validation. The introduction of qPCR, by my own lab, is a great example of this. It was a disappointment at best.

We have overfocused on Mosaicism – by a very great amount. Much of what was initially was analytical error. Look at how the prevalence has gone down in many labs over the last couple of years. From sixty percent to less than 10% for whole chromosomal mosaicism and less than 20% even after adding in the segmentals. When considering embryos selected for transfer, it is a few percent of embryos at the most as shown by Tiegs in a prospective non-selection study where mosaics had completely normal sustained implantation rates. We were never trying to screen for mosaicism and continues to not be a priority. The story with segmental abnormalities requires more work. Outcomes are diminished but by how much and the risk to the neonates requires more work.

You need a good IVF lab. Well, we all need good labs anyway.

The magnitude of the benefit varies for different groups of patients. There is less benefit where the intrinsic sustained implantation rates are higher, but recent data shows improved outcomes even when using donor

oocytes. The field needs more information on extreme low responders, but remember these patients pay the biggest penalties for lost time during futile cycles or clinical losses.

PGT-A is a powerful adjunct and should be in common use in every facility where there is no regulatory burden preventing its application.

EXPANDED CARRIER SCREENING: IMPACT ON PGT-M UPTAKE

James D. Goldberg, M.D., FACMG, FACOG

Carrier screening for serious autosomal recessive and X-linked disorders has been a part of reproductive care since the early 1960s. Initially, screening was ethnically guided and available for a limited number of disorders including Tay-Sachs disease, sickle cell disease, and later cystic fibrosis, spinal muscular atrophy and other hemoglobinopathies. In 2009 the concept of pan-ethnic expanded carrier screening was introduced into clinical care. This provided screening for potentially hundreds of disorders in an efficient, cost effective way. Several studies have demonstrated that ECS results in expanded identification of at-risk carrier couples (ARCs) in the screened population. These studies will be summarized.

Professional guidelines have lagged behind the advances in ECS. Because of this, until recently, there has been no consistent guidance regarding ECS. This includes who should be offered screening, what disorders should be screened for, and the timing of screening. This has resulted in laboratories offering different size screening panels and screening for different disorders. This will be discussed along with the implications of screening for an increasing number of disorders.

In 2021, the American College of Medical Genetics and Genomics (ACMG) published a practice resource which gave recommendations for a standardized screening panel of 113 genes that should be offered to all pregnant couples or those considering pregnancy. These recommendations will be discussed in detail.

These recommendations will impact PGT-M testing in several ways. First, if widely accepted and implemented, there will be a standard basic panel offered to all couples. This will identify an increased number of ARCs leading to an expanded need for PGT-M in those patients electing to undergo IVF. In addition, ACMG has recommended that insurers cover the cost of ECS testing which will allow more couples to be tested.

CHOOSING THE RIGHT AUTOMATION SOLUTION FOR YOUR NEEDS

Echaves, P.

Perking Elmer, USA

Automation allows labs to do more with less. Throughput can be increased and sample handling errors reduced all while reducing hands-on-time and the expertise needed to run applications. As genetic testing becomes more embedded into assisted reproduction with applications including preimplantation genetic testing for aneuploidy (PGT-A), structural chromosome rearrangements (PGT-SR), and monogenic diseases (PGT-M) of embryos, there is a growing need for automation.

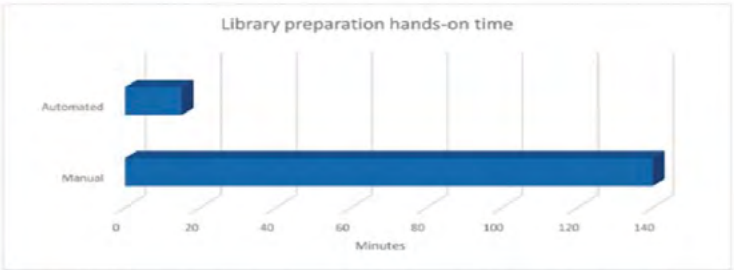
To determine the suitability of automating PGT applications, library yield, fragment size, % mapped reads, and PG-Find Quality scores were analyzed from samples prepared manually and using automation. Two automated runs of 48 samples were performed according to the standard PG-Seq™ 2.0 automation protocol using Whole Genome Amplified (WGA) products. These automated runs were compared to two manual runs.

Euploid male genomic DNA (Promega) and aneuploid cell line DNA GM04965, karyotype 48,XXY,+21 (Coriell Institute) were used as input material. WGA was performed using the standard PG-Seq kit 2.0 protocol. The NGS results were analyzed using the PG-Find™ software.

To assess inter-preparation variability of the libraries prepared using automation, twenty WGA reactions for each sample type were pooled so that they could be spread across multiple library preparation reactions. Two separate sample preparations were analyzed.

Automation in the PGT-A Lab

QC	Automated	Manual
Library Yield	50 ng/µL ± 2.8	35 ng/µL ± 16
Fragment Size	445 bp ± 53	436 bp ± 29
% Mapped Reads	98.9% ± 0.4	97.8% ± 1.8
PG-Find Quality Score	0.029 ± 0.0004	0.017 ± 0.016



High quality Libraries with minimal hands-on time

A comparison of automated and manual NGS library preparation QC metrics for cfDNA will be presented, demonstrating the universal utility of lab automation. In this presentation you will learn:

1. Why you should automate your workflows.
2. What you need to consider before you chose an automation solution.
3. What PGT workflows are amenable to automation.

LEVERAGING COMPUTATIONAL TOOLS TO IMPROVE EMBRYO PRIORITIZATION AND IMPACT CLINICAL RESULTS

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The tremendous global growth in assisted reproductive treatment (ART) has led to an influx of valuable information pertaining to all aspects of an IVF cycle. Concurrent with this data accumulation, advanced computational methods including artificial intelligence (AI) and its subtype, machine learning (ML), have evolved to allow for effective application on large data sets, priming the field of IVF for rapid technological advancement. Today, AI/ML tools are being developed with the aim to cover all aspects of IVF from personalized drug treatment regimens for stimulation and endometrial preparation to gamete selection and embryo prioritization.

Embryo transfer prioritization is one of the most widely studied but non-standardized components of an IVF cycle. Today one of the key prioritization measures is the morphological assessment of embryos however this determination is subjective and requires a training and experience to gain the highest skills leading to varying levels of efficacy in this practice. The introduction of pre-implantation genetic testing for aneuploidy (PGT-A) has significantly improved the embryo screening process and been shown to result in a significant reduction in the number of miscarriages caused by chromosomal aneuploidy by allowing the prioritization of embryos for transfer based on their diploid status. Paired with morphological assessment it is not uncommon for IVF clinics to achieve >70% clinical pregnancy rate per euploid embryo transferred.

AI/ML tools continue to enhance the way in which embryos are prioritized both at the morphological level and through advancements in PGT-A bioinformatics algorithms and analysis tools. CooperGenomics launched the first PGT-A solution leveraging AI tools in 2019 and have now increased our sequencing capacity by 100-fold over previous off-the-shelf methods allowing us to leverage our data to improve capabilities when screening for aneuploidy in embryos. The utilization of AI has allowed us to implement fully automated karyotype reporting, improved our ability to determine signal from noise, and added significant capabilities that were previously out of our reach including the detection of female haploidy and triploidy, the characterization of the parental origin of meiotic aneuploidy, and now the ability to assess for expected inheritance patterns in a trophectoderm biopsy.

This talk will focus on these developments in AI/ML and how their application in PGT-A and morphological assessment is continuously evolving to allow for better prioritization of embryos for transfer.

PRACTICAL APPLICATION OF UNIVERSAL PGT

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Large scale whole genome and exome sequencing is uncovering a plethora of novel mutations that cause highly penetrant, early-onset, severe, or later-onset life-threatening dominant and recessive disorders. For couples who are known carriers of mutant alleles, preimplantation genetic testing (PGT) enables the detection of genetic disorders in embryos that have been fertilized *in vitro*, thereby avoiding their transmission to offspring. Traditional PGT methods require a mutation and family specific work-up. We and others have developed generic methods that can be readily applied for all transmitted genetic disorders. The methods reconstruct genome-wide haplotype architectures as well as the copy-number and segregational origin of those haplotypes by employing phased parental genotypes and deciphering WGA-distorted SNP B-allele fractions. I will present the principles, the use and the clinical and biological outcomes since clinical implementation. The introduction of genome- wide screening of embryo's raised novel ethical questions. The principles guiding embryo selection and prioritization that are applied according to the chromosomal content and mutational load of the embryos, will be presented. I will present novel technical developments broadening the scope of use.

APPLICATION OF THIRD GENERATION SEQUENCING TO PGT

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Introduction: Third generation sequencing (TGS) is a relatively new technology that opens the way to obtaining contiguous allelic sequence information from long DNA molecules. Are there opportunities for improving PGT using TGS?

Methods: Long molecules (~5 kb) from embryonic DNA were generated by multiple displacement amplification. For parental DNA samples, long molecules (~10-20 kb) were obtained by fragmentation and size selection from agarose plugs. TGS was performed on the Nanopore and PacBio sequencing platforms.

Results: In initial proof of concept PGT studies, we compared the results of affected embryos with TGS results from the original biopsied embryos. Two affected embryos diagnosed with the homozygous SEA deletion associated with alpha thalassemia were confirmed by TGS. For 4 embryos with a unbalanced t(1;18) translocation, we were able to map the precise breakpoints. Using the long sequencing reads, copy number analysis correctly revealed euploid embryos, whole chromosome aneuploid embryos and embryos with segmental imbalances. In other studies, from parental workups, we were able to derive high definition SNP maps around the breakpoints for 15 balanced translocation carriers requesting PGT-SR and the causative mutations in the *BCKDHB* and *SETX* genes for two couples requesting PGT-M for autosomal recessive maple syrup urine disease (MSUD) type 1b and autosomal dominant amyotrophic lateral sclerosis 4 (ALS4), respectively. Using the linked SNP information, we have successfully performed PGT-SR for three translocation couples resulting in babies with a normal karyotype and PGT-M for MSUD resulting in a non-affected carrier baby.

Conclusions: TGS is a good option for PGT-SR cases where the patient prefers non-carrier balanced embryos for transfer. In complex PGT-M cases where there is limited STR or SNP information around the gene of interest, TGS on parental samples may provide a better option to design a more accurate embryo genotyping test.

GENOME-WIDE DISEASE SCREENING IN EARLY HUMAN EMBRYOS WITH PRIMARY TEMPLATEDIRECTED AMPLIFICATION

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Current preimplantation genetic testing (PGT) strategies enable the selection of embryos based on fetal aneuploidy or the presence a small number of preselected disease-associated variants. Here we present a new approach that takes advantage of the improved genome coverage and uniformity of primary template-directed amplification (PTA) to call most early embryo genetic variants accurately and reproducibly from a preimplantation biopsy. With this approach, we identified clonal and mosaic chromosomal aneuploidy, *de novo* mitochondrial variants, and variants predicted to cause Mendelian and non-mendelian diseases. In addition, we utilized the genome-wide information to compute polygenic risk scores for common diseases. Although numerous computational, interpretive, and ethical challenges remain, this approach establishes the technical feasibility of screening for and preventing numerous debilitating inherited diseases.

EXPANDING PGT-M APPLICATION

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Current PGT-M referrals and indication profile have changed significantly, as seen from our ongoing PGT-M experience, which represents the world's largest series in one center. Initially almost all PGT-M cases were ascertained following an affected proband in a family, which in the last 5 years has changed dramatically towards ascertained through expanded carrier screening, which impacted also the PGT-M indication profile. Such dynamics of increase of at-risk couples presenting for PGT-M was analyzed for 58 different conditions, compared to the baseline referrals through the traditional approach, showing the increase of prospective referral not only for common genetic disorders, such as cystic fibrosis (CFTR), deafness (GLB2), Fragile X (FMR1), and sickle cell disease (HBB), but also for many rare conditions. Thus, the overall number of prospective PGT-M cases more than doubled, with a similar dynamics for almost each condition tested. Thus, expanded carrier screening is currently becoming the major source for performing PGT-M, allowing to offer PGT-M prospectively before the birth of an affected child.

Our data show significant increase of the PGT-M uptake with expanding gene panels for carrier screening, demonstrating the utility for offering PGT-M prospectively to the couples at risk for various adult-onset heritable disorders. The greatest applicability of PGT-M for adult-onset heritable disorders lies in heritable cancers (close to one 20% of PGT-M in our experience), 39 of which represent a familial cancer susceptibility syndromes of adult-onset autosomal dominant inheritance. Interest in PGT-M is often initiated when a family member is found to have a heritable cancer and the mutation is identified. Unaffected relatives of reproductive age naturally seek to learn if they themselves have the same cancer-susceptible mutation. If this proves to be the case, avoiding transmission of their mutant alleles to offspring is sought through PGT-M. Heritable cancers most often subjected to PGT included breast cancer, familial adenomatous polyposis 1 (FAP1), Fanconi anemia, neurofibromatosis, tuberous sclerosis and hereditary nonpolyposis colon cancer (HNPCC).

Thus, PGT-M is longer a retrospective in its practical application, but is currently applied prospectively as primary preventive approach, with also significant changes in indication profile towards increasing proportion of late-onset common conditions with genetic predisposition.

CONTROVERSY IN APPLICATION OF NON-INVASIVE PGT: SCREENING OR DIAGNOSIS

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Pre-implantation Genetic Testing (PGT) includes all techniques aimed at detecting chromosomal abnormalities, structural rearrangements and/or specific genetic diseases in pre-implantation embryos. Up to know, the gold standard procedure to obtain DNA for this kind of analysis has been trophectoderm cell biopsy. However, this procedure involves further embryo manipulation, it requires specific skills and equipment (thus increasing costs for the Center and for the patients) and it raises concerns about potential impact on subsequent embryo development.

For these reason, in recent years, scientist have been trying to develop less invasive techniques to obtain DNA, while guaranteeing reliability of analysis performed on it. These techniques are identified as NIPGT (Non Invasive PGT).

Nowadays, the most common NIPGT procedures are blastocelic fluid (BF) analysis and spent culture media (SCM) analysis. Both these techniques initially showed promising results, but they also showed potentially critical issues related to the quantity and quality of DNA available, as well as to diagnostic reliability. (Leaver and Wells, 2020)

In order to perform an accurate analysis it is necessary to isolate a sufficient amount of DNA, which must also be representative of the chromosomal and genetic status of the embryo.

With specific reference to BF, it is well-acknowledged in the current scientific literature that it harbours genomic DNA suitable for amplification and potential PGT applications, and even though the extent to which these DNA molecules (BF-DNA) are representative of the corresponding embryo ploidy is still a matter of debate, this alternative non-invasive source of embryonic DNA remains attractive and fascinating.

In a first pilot study, BFs and TE cells were retrieved from 51 blastocysts derived from 17 couples undergoing PGT-A on polar bodies (PBs) or blastomeres. DNA was detected in 76.5% of BFs. In 97.4% of the cases the ploidy condition of BFs was confirmed in TE cells, and the rate of concordance per single chromosome was 96.6%. In relation to the whole embryo, the ploidy condition corresponded in all cases with a per-chromosome concordance of 98.1%. The testing of PBs and blastomeres had 93.3% and 100% prediction of BF ploidy condition with a concordance per chromosome of 93.5% and 94%, respectively. (Gianaroli et al., 2014)

We further deepened these findings in another report discussing the data obtained in BFs and TE cells retrieved from 116 blastocysts, derived from 51 couples undergoing PGT-A or PGT-SR on PBs or blastomeres. Separate chromosome analysis was performed in 70 BFs. DNA detection rate was 82%. In 97.1% of BFs, the ploidy condition corresponded to that found in TE cells. The rate of concordance per single chromosome was 98.4%. Ploidy and chromosome concordance with PBs were 94% and 97.9%, respectively; with blastomeres, the concordances were 95% and 97.7%, respectively. Lastly, segmental abnormalities which were detected in PBs or blastomeres, were also identified in the corresponding BFs. (Magli et al., 2016)

The reproducibility of this method was recently shown once again in an analysis of paired BF and trophectoderm biopsies from 256 blastocysts. Successful DNA amplification occurred in 71% of BF samples; of which 87% provided an informative chromosome copy number result. As previously reported, rates of ploidy concordance and concordance per single chromosome were 93.6% and 96%, respectively, whilst full chromosome concordance rates were slightly lower (66.3%). Intriguingly, the incidence of DNA amplification was significantly lower in BFs from TE-euploid blastocysts (45%) when compared with the aneuploid ones (81%). After the transfer of 53 TE-euploid blastocysts, the clinical pregnancy rate was 77% in the group with BF-failed amplification, and 37% after BF-successful amplification. The same trend was found for the ongoing pregnancy rate (68% vs. 31.5%, respectively). In this scenario, these findings could open new perspectives for the selection of the most viable embryo for transfer because, after submitting BFs to DNA amplification, priority would be given to TE-euploid blastocysts with BF-failed amplification. Similarly, BF-failed amplification could be an additional selection criterion to prioritize embryos for transfer even in conventional IVF cycles with blastocysts that were vitrified after BF aspiration. (Magli et al., 2018)

On the other hand, a number of authors investigated the possibility to exploit the combination of blastocoelic fluid and spent culture media as a starting point for PGT applications. Technically speaking, laser-artificial shrinkage was employed to allow for the release of blastocoelic fluid in the culture media which was subsequently retrieved and submitted to the analysis.

In a first report, 47 embryos from 35 patients were tested (28 frozen-thawed and 19 fresh culture samples). Embryonic DNA was successfully amplified in all the samples; for frozen-thawed embryos the concordance rate between BF-SCM vs. TE biopsy, BF-SCM vs. whole embryo and TE vs. whole embryo was 87.5%, 96.4% and 91.7%, respectively, and the rate of concordance per single chromosome was 99.3%, 99.7% and 99.7%, respectively. (Kuznyetsov et al., 2018)

Subsequently, the same authors confirmed these data in a study comparing the results from BF-SCM and TE cells biopsy in 145 blastocysts undergoing PGT-A. The overall concordance rate per sample for euploidy/aneuploidy status between BF-SCM and TE cells biopsy samples was 97.8%. (Kuznyetsov et al., 2020)

In another setting, 41 vitrified blastocysts donated by 22 couples known to carry a chromosome rearrangement, and 21 vitrified blastocysts donated from 8 couples with normal karyotypes were used. For PGT-SR, both BF-SCM and TE biopsy samples showed 100% clinical concordance with the corresponding whole embryo samples in detecting chromosomal rearrangements. Additionally, BF-SCM and TE biopsy samples showed 90% and 100% karyotype concordance with the corresponding whole embryo, respectively. For PGT-A, BF-SCM and TE biopsy samples displayed 90% and 86% clinical concordance with the corresponding whole embryo, respectively, while both BF-SCM and TE biopsy samples showed 76% karyotype concordance with the corresponding whole embryo samples. (Jiao et al., 2019)

Nevertheless, different results were described by other investigators. In this scenario, a total of 40 day 5 donated embryos were tested for BF-SCM producing results not properly concordant with TE cells biopsy, and the whole remaining embryo samples. More in detail, in 15 cases the chromosomal patterns derived from all three types of samples corresponded. In 2 cases BF-SCM and TE biopsy were concordant but differed from the remaining embryo. In 12 cases TE biopsy method and the remaining embryo gave the same results but differed from BF-SCM. In 4 BF-SCM and the remaining embryo gave the same results but diverged from TE biopsy. In 7 cases the three types of samples were in disagreement with one another. Overall, the BF-SCM method had a sensitivity of 89.47% and specificity of 68.42%, while TE biopsy method 89.47% and 73.68%, respectively. At the individual chromosome level, the BF-SCM method showed a sensitivity of 59.38% and specificity of 95.84%, while TE biopsy method 83.33% and 97.63%, respectively. (Li et al., 2018)

Other authors recorded similar data from a total of 32 blastocysts using BF-SCM and the whole corresponding embryo as a control. Briefly, the amplification rate for BF-SCM and the whole corresponding blastocyst were 87.5% and 96.9%, respectively. Concerning the chromosomal status, the concordance rate was 66.7%. (Zhang et al. 2019)

In conclusion, NIPGT is certainly appealing because it might simplify technical procedures and reduce costs. Encompassing extremely novel procedures, the amount of studies available on these techniques is still limited and data are significantly inhomogeneous in terms of amount and quality of DNA available, amplification rates and concordance rates. For these reason, further studies are required to assess the diagnostic value of NIPGT procedures before they can reliably be routinely used in clinical practice. Technological advancements in the field must be also kept in due consideration.

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NON-INVASIVE PGT-A: SCREENING OR DIAGNOSIS

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Emerging evidence indicates that, during in vitro development, embryos secrete cell-free DNA (cfDNA) into their culture medium; this phenomenon suggests the potential for an alternative, non-invasive assay for aneuploidy. Embryonic cfDNA-based assays exhibit high concordance with trophectoderm biopsies, inner cell mass and the whole blastocyst. Yet, informativity and concordance rates may be influenced by several factors: culture day when medium is collected, contamination with external and/or cumulus cell DNA, and previous manipulation of the embryos. Non-invasive aneuploidy testing using spent blastocyst medium (SBM) for embryonic cfDNA analysis, in combination with morphology evaluation, would significantly improve clinical outcomes in IVF programs. This approach could be used to inform prioritization, whereby blastocysts are ranked for their likelihood to result in a healthy baby.

Our group conducted a study on 46 couples with PGT-A indication (mainly advanced maternal age) whereby we used TE biopsy results to guide single embryo transfers (SETs) and retrospectively calculated clinical outcomes in two different scenarios: when euploid trophectoderm (TE) was concordant with euploid SBM and when euploid TE was discordant with aneuploid SBM. Ongoing implantation rates were three-fold higher in euploid TE/euploid SBM compared to euploid TE/aneuploid SBM (52.9% vs. 16.7%, respectively), though without reaching statistical significance due to the small sample size. Interestingly, SETs did not end in miscarriage when TE biopsy and SBM were euploid concordant (Rubio et al., 2019).

Several groups have evaluated non-invasive PGT-A (niPGT-A) with transfer of euploid embryos, based only on SBM diagnosis, showing good results. In a pilot study with only 7 SETs in couples with PGT-A or PGT-SR indication, 5 pregnancies were achieved resulting in 5 livebirths (Xu et al., 2016). In a second study, in couples with recurrent spontaneous abortion or repeated implantation failure, 50 transfers of euploid media were performed resulting in a 58% clinical pregnancy rate, with 27 healthy babies born (Fang et al., 2019). Similar results were described in good prognosis patients <38 years of age with ongoing pregnancy rates comparable to PGT-A (61.5%), and higher than conventional IVF or ICSI (48.5%) (Franco et al., 2021).

Non-invasive PGT-A with the analysis of the embryonic cfDNA in the SBM is a powerful biomarker, but nowadays, should not be considered a substitute of TE biopsy, since has not reached the diagnostic test category yet. However, it might help to prioritize for transfer the embryos with higher reproductive potential. In this scenario, all embryos could be considered for transfer according to their priority order. This could be an attractive option for those patients that want to increase their clinical outcomes without the need of an invasive TE biopsy, and for those patients that do not want to discard embryos.

CONTROVERSY IN APPLICATIONS OF NON-INVASIVE PGT

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Since the first reports that a small piece from an embryo could be used to characterize the remaining embryo, researchers have sought to improve embryo transfer outcomes by identifying the most genetically suitable embryo. Early efforts involved removing cells (or polar bodies) and either using FISH to identify selected chromosomes or amplifying the DNA to a level suitable for some sort of genetic analysis. The advent of microarrays, followed quickly by massively parallel sequencing (NGS) changed how comprehensive this analysis could now be. It was realized very quickly that not only was comprehensive chromosome typing required to properly characterize the embryo but procedures for getting the embryo sample needed to have as small an impact on subsequent embryo vitality as possible. Despite original concerns that growing embryos to advanced developmental stages was beyond some groups, the general practice of PGT settled on blastocyst stage as the most appropriate and least disrupted point for acquiring a suitable embryo specimen. Some recently published trials however, have raised the issue as to whether removing part of the embryo was as innocuous as it first appeared. With doubts about how big an impact this might actually be on the subsequent potential for implantation and successful pregnancy outcome, the search for an alternative continued.

It is widely accepted that the environment of a living cell system will often contain cellular discard components from that system, especially DNA- a virtual fingerprint of the cell. The first reports of cell free DNA (cfDNA) analysis using embryo spent medium laid the basis for the opportunity (and hope) that analysis of these discard remnants would be sufficient and accurate enough to characterize the original embryo. The technologies, (whole genome amplification, microarrays and NGS) to enable this to be approached were already in place and in routine use in many facilities. Early results though, were mixed in outcome with non-concordant chromosome profiles raising concerns about the suitability of this approach.

What was underlying the apparent failures for cfDNA analysis being a suitable alternative to biopsy approaches? The efforts to implement this approach have raised many more questions than what have been answered. Many groups appear to have not fully appreciated requirements for accurate trace, degraded DNA investigations and just treated the process as one identical to biopsy-based testing. The source(s) of any background contaminating DNA must be understood and has a different implication for accuracy of any answers. Is extraneous, non-embryo associated DNA present? How much, if any, DNA from extra-embryonic structures is present? Does embryonic cfDNA, if present, actually represent the remaining embryo? Or was it genuine discard because of biological error and clean up? How appropriate are the technical processes for analyzing this DNA? Has biological reality been sidelined by a mix of user hope and commercial hype?

ORAL COMMUNICACTIONS

OC-01

AN AI-BASED TOOL THAT PREDICTS EUPLOIDY DISCRIMINATING BETWEEN MORPHOKINETIC BEHAVIORS OF EUPLOID AND ANEUPLOID BLASTOCYSTS

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Artificial intelligence, time lapse monitoring, PGD-A, morphokinetic

Introduction: Previous studies using manual annotation of images from Time Lapse Monitoring (TLM) incubators indicated that euploid embryos show blastulation earlier than aneuploid embryos. A deep learning-based Artificial Intelligence (AI) tool, CHLOE (Fairtility), has been demonstrated to simultaneously evaluate large numbers of embryos using TLM images. AI tools, such as CHLOE (Fairtility), eliminate inter-operator variation by automatically extracting standardized time-lapse annotations and allowing more robust and efficient embryo evaluation. The aim of the study was to compare morphokinetic behaviors of euploid and aneuploid blastocysts automatically annotated using AI.

Material&Methods: Single centre study analyzing a retrospective cohort that took place between 2019-2020, at Istanbul Memorial Hospital, ART and Reproductive Genetics Center. Time-lapse videos of 1231 blastocysts (486 euploid, 745 aneuploid) which underwent PGT-A analysis, with 301 euploid single embryo transfers (SETs). CHLOE's morphokinetic assessments (tPNa,tPNf,t2,t3,t4,t5,t6,t7,t8,t9,tM,tSB,tB,tEB), blastocyst score (calculated at 30hpi) and implantation score were compared using Student's t-test (SPSS). CHLOE blastocyst and implantation score efficacy of prediction of ploidy and clinical outcomes was quantified using the metric AUC.

Results: When annotated using AI, the average time (Mean hours post insemination ±Standard deviation (SD)) for t4 (39.4±6.5 vs 38.7±4.7,p=0.02), t5 (50.1±7.7 vs 49.0±6.7,p=0.012), t6 (53.0±7.3 vs 52.0±6.1,p=0.018), tSB (98.2±7.7 vs 96.3±7.1,p<0.001), tB (106.1±7.6 vs 103.8±7.2, p<0.001) and tEB (113.1±6.5 vs 109.6±7.6, p<0.001) were significantly later in aneuploid embryos compared to euploids.

Implantation score was significantly higher in euploid embryos compared to aneuploids (0.76±0.25 vs 0.67±0.27, p<0.001), and was predictive of euploidy with an AUC (95% CI) of 0.621 (0.589-0.651,p<0.001). Blastulation score was similar (0.96±0.18 vs 0.94±0.21, p>0.05) and was not predictive of euploidy (AUC (95% CI) = 0.529 (0.497-0.562), p>0.05).

Following euploid SETs (n=301), blastulation and implantation scores were not predictive of pregnancy (blastulation score; AUC(95% CI)=0.541 (0.463-0.619), p>0.05 and implantation score; AUC(95% CI)=0.532 (0.450-0.613), p>0.05), clinical pregnancy (blastulation score; AUC(95% CI)=0.564 (0.484-0.644), p>0.05 and implantation score; AUC(95% CI)=0.535 (0.452-0.617), p>0.05) and live births (blastulation score; AUC(95% CI)=0.479 (0.391-0.567), p>0.05 and implantation score; AUC(95% CI)=0.472 (0.382-0.562), p>0.05).

Conclusion: AI-based tools have the potential of increasing consistency, efficiency and efficacy of embryo evaluation. Although there is a need to validate AI tools for ploidy detection before their incorporation into clinical practice, the information on quantitative and qualitative morphokinetics and predictors such as blastulation, implantation scores that AI tools such as CHLOE provide, seem very promising for embryo selection and ploidy prediction for PGT-A analysis.

OC-02

MORPHOKINETIC PARAMETERS TO PREDICT EUPLOIDY: AN ASSESSMENT USING DONOR CYCLES

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Morphokinetic parameters, ploidy, implantation, donor oocytes

Introduction: Several research groups have proposed analysis and evidence of using morphokinetic parameters to predict ploidy and implantation status. However, some papers had carried out assessments on the aneuploidy and implantation prediction models proposed by these groups but they failed to replicate similar results. The cause of the discrepancy is still unknown. This study aims to investigate the morphokinetics parameters in embryos derived from donor cases to determine their associations with ploidy and implantation outcomes. The hypothesis is that embryos derived from donor oocytes will be less likely to be affected by other variations, and the predictive value of the morphokinetic parameters for ploidy and implantation can be sufficiently detected.

Materials and Methods: This retrospective analysis involved donor oocytes cycles from January 2017 to December 2019 that fulfilled the below criteria: (1) the donor had donated at least three or more cycles, (2) the embryos were cultured in Embryoscope® (Vitrolife), (3) with or without preimplantation genetic testing-aneuploidy (PGT-A), (4) pregnancy outcomes. Based on these criteria, a total of 345 embryos derived from 16 donors were included in this observational study. The time is defined as the starting time after ICSI. The morphokinetic development (i.e. time when both pronuclei had fade (tPnF), time from insemination to completion of division to 2, 3, 4 5, and 8 (t2, t3, t4, t5 and t8, respectively) and time from insemination to formation of a full blastocyst (tB)) of embryos was examined. Trophectoderm biopsy was performed and followed by chromosomal evaluation (for those cycles with PGT-A) using Next Generation Sequencing (NGS) (VeriSeq Protocol, Illumina). Morphokinetics parameters was assessed using Receiver Operator Characteristics (ROC) curve (GraphPad Prism version 8.0, CA).

Results: Area under the curve (by comparing aneuploid and euploid embryos) for tPnF, t2, t3, t4, t5, t8 and tB were 0.53, 0.55, 0.53, 0.53, 0.55, 0.56 and 0.55, respectively. In addition, when comparing aneuploid and whole chromosomal mosaicism embryos, the results were 0.55, 0.54, 0.59, 0.56, 0.50, 0.55 and 0.55, respectively. Area under the curve (by comparing implanted and non-implanted embryos) for tPnF, t2, t3, t4, t5, t8 and tB were 0.54, 0.55, 0.51, 0.52, 0.56, 0.56 and 0.57, respectively. All the area under the curve were near 0.5 thus showing that the tests could not find any significant difference between the morphokinetic parameters of aneuploid and euploid embryos, implanted and non-implanted embryos.

Conclusions: Morphokinetic parameters tPnF, t2, t3, t4, t5, t8 and tB tested in this study failed to discriminate embryos with aneuploid and euploid status. These parameters were also unable to discriminate aneuploid and whole chromosomal mosaicism embryos, implanted and non-implanted embryos. This study can be further extended to includes other morphokinetic parameters and to increase more samples.

OC-03

PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY, A RETROSPECTIVE MULTICENTRE STUDY TO DETERMINE EFFICACY AND INTER-CLINIC VARIATION

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PGT-A, multi-centre, live birth outcomes

Introduction: Preimplantation genetic testing for aneuploidy (PGTA) is used with the aim of improving live birth rates from invitro fertilisation (IVF) cycles by reducing implantation failure and miscarriage associated with aneuploidy. Previous research including randomised controlled trials (RCTs) have shown mixed success of PGTA over conventional IVF to improve live birth outcome. However, to our knowledge, no studies have compared the extent to which individual clinics vary in their success following PGTA. The purpose of this study is to determine if there is an improvement in live birth with PGTA over conventional IVF without genetic screening and how this varies between individual clinics.

Methods: We retrospectively analysed live birth outcomes of 2,961 PGTA (both aCGH and NGS) cases at four UK clinics (A-D) between 2011 and 2017. These outcomes were compared to 14,292 cases which did not use PGTA during the same period. Subsequent, frozen embryo transfers up to December 2019 were included.

Results: Percentage live birth per embryo transferred was higher in the PGTA group vs the Non PGTA group for all age groups when all four clinics were combined.

Table 1: Live birth per embryo transferred by maternal age

	<35	35-37	38-39	40-42	43-44	>44
PGTA	36%	42%	41%	41%	11%	40%
Non PGTA	35%	27%	19%	10%	5%	1%

When broken down by clinic, the only clinic which saw a significantly higher live birth rate following PGTA for <35 was clinic D. For patients over 35 the PGTA group saw a significantly higher live birth rate in all clinics (except clinic A for 35-37 age group and clinic B for 43-44 age group), although the degree of improvement did vary, with clinics B and D performing the best. We will provide further breakdown by aCGH and NGS and outcomes by treatment started.

Table 2: Live birth per embryo transferred by maternal age, broken down by clinic						
Clinic		**A**	**B**	**C**	**D**	
Age		% (N. Live birth)	% (N. Live birth)	% (N. Live birth)	% (N. Live birth)	**Total transfers**
<35	PGTA	38%(35)	35%(38)	34%(61)	42%(21)	**433**
	Non PGTA	39%(1556)	31%(1383)	37%(503)	27%(70)	**10,094**
	P value	0.753	0.418	0.426	0.006	
Clinic		**A**	**B**	**C**	**D**	
35-37	PGTA	41%(21)	38%(39)	44%(71)	44%(40)	**408**
	Non PGTA	29%(632)	26%(572)	27%(330)	20%(58)	**5867**
	P value	0.680	0.008	<0.001	<0.001	
38-39	PGTA	41%(24)	46%(28)	36%(36)	42%(41)	**315**
	Non PGTA	19%(264)	19%(256)	18%(146)	13%(17)	**3686**
	P value	<0.001	<0.001	<0.001	<0.001	
40-42	PGTA	42%(32)	51%(37)	38%(57)	39%(36)	**393**
	Non PGTA	11%(141)	9%(104)	11%(146)	11%(19)	**3928**
	P value	<0.001	<0.001	<0.001	<0.001	
43-44	PGTA	33%(25)	17%(1)	36%(8)	29%(5)	**340**
	Non PGTA	7%(20)	6%(11)	4%(19)	7%(3)	**1029**
	P value	<0.001	0.315	<0.001	0.035	

Conclusions: The work described in this study, suggest that PGTA improves live birth rates for patients over 35 on an embryo transfer basis, however this same benefit is not seen for patients under 35. The degree of improvement seen with PGTA varies between clinics.

OC-04

HOW CRUCIAL IS TUBING PROTOCOL FOR EFFICIENT PGT-A?

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PGT-A, WGA, Tubing Protocol, Trophectoderm Biopsy

Introduction: Trophectoderm biopsy is widely performed for PGT-A, yet the efficiency of PGT-A results of different IVF centers show substantial variability. Most of the PGT-A samples are outsourced, commonly referred as transport PGT. In conjunction with the reported findings claiming that the potential discrepancies in the efficiency of Whole Genome Amplification (WGA) quality parameters of biopsy cells obtained from different centers might originate from the diversity in the techniques of biopsy practitioners, in this study we investigated the results obtained from the application of experienced embryologists on trophectoderm biopsy from 6 different IVF centers in order to reveal the effect of inappropriate tubing protocols on PGT-A results.

Material & methods: Total of 790 embryos from 207 patients were collected from 6 different IVF centers. WGA of collected trophectoderm biopsy samples was performed using Ion SingleSeq Kit (Thermo Fisher Scientific). WGA samples were analysed with gel electrophoresis for amplification quality control and samples that have passed the quality check were further processed with ReproSeq PGS Kit (Thermo Fisher Scientific) for aneuploidy screening and additional quality parameters, Median of all absolute values of pairwise differences (MAPD) and total read count, were analysed.

Results & conclusions: In order to highlight the crucial role of tubing protocols in PGT-A, we compared the quality parameters of trophectoderm biopsy samples. Initial quality check was performed after WGA with gel electrophoresis and average percentage of low to well amplified samples was calculated; 96.1% for center 1, 96.3% for center 2, 78.8% for center 3, 75.4% for center 4, 99.4% for center 5, 95.7% for center 6. Afterwards, WGA products were further processed and additional quality parameters, MAPD value and total read count, were compared. Samples with MAPD value above 0.3 and total read count below 100000 were assumed low quality. The highest quality score was for clinic 5 (99.4%) and the lowest score was for clinic 3 (71.2%). Moreover, for other clinics we also obtained differences; 96.9% for clinic 1, 98.8% for clinic 2, 82.6% for clinic 4, 95.7% for clinic 6.

Mosaic aneuploidy ratio diversity of different laboratories have been linked to stress in culturing conditions that affect the mitotic division and biopsy techniques in some studies. In this study, we investigated the causes of significant difference in WGA efficacy between some IVF centers, in which highly trained and experienced embryologists are efficiently performing biopsy for routine PGT-A. We found that inappropriate washing and tubing protocols are also detrimental beyond factors like the number of biopsied cells, biopsy localization and biopsied cell transport conditions.

Since we use three different commercial platforms for PGT-A of 10000 embryos annually, it is a requirement for us to determine the low mosaicism ratio limits uniquely for the laboratories and evaluate the results in this sense. In addition to the recent guideline of ESHRE on the transport traffic of PGT, a declaration of an international standardization, in which the KPI and benchmarks particular to the specifications for biopsy and tubing protocols are recommended, will have a significant contribution in this context.

OC-05

ASSOCIATION OF TYPE OF ANEUPLOIDY AND EMBRYO MORPHOKINETICS DEVELOPMENT EVALUATED BY TIME-LAPSE MONITORING

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aneuploidy, embryo, PGT-A, mitosis, meiosis

Introduction: Human embryos are affected by a high rate of aneuploidy, around 35% in natural conceptions, but this rate in pre-implantation embryos derived from assisted reproductive technics (ART) is substantially higher. Aneuploidy of meiotic origin, derived from the oocyte, has long been recognized as the main factor of fertility decline related to female age. However, mitotic errors during early embryogenesis are also common, giving rise to mosaic embryos. These types of embryos are more frequent in young women and generally affect several chromosomes simultaneously. The aim of this study was to analyze the association of embryonic development morphokinetics with the type of aneuploidy in ART-derived embryos.

Material and Methods: This retrospective cohort study used prospectively collected data from 509 embryos from 109 patients undergoing assisted reproductive treatment between April/2018 and September/2019 in a private reproductive medicine center. Mature (MII) oocytes were fertilized by intracytoplasmic sperm injection (ICSI) and cultured in a time-lapse monitoring system (Embryoscope®). The morphokinetics of embryonic development was evaluated from fertilization to the blastocyst formation. A total of 509 blastocysts were biopsied for preimplantation genetic test for aneuploidies (PGT-A) and analyzed at Igenomix Brasil through NGS. Blastocysts were diagnosed as euploid or aneuploid. Aneuploid blastocysts were yet classified according to the type of alteration as monosomy, trisomy, partial or complex alteration (two or more chromosome alterations).

Results: The mean age of women was 38.2±3.4 years and euploid blastocyst rate was 36.3% (n=185). The complex alterations were the most prevalent (n=150, 29.7%) followed by monosomy (n=81, 15.9%) and trisomy (n=76, 14.9%). Partial alterations were observed in only 16 blastocysts (3.1%). The 9 cells stage marks the beginning of embryonic genome activation and the time between 9 cells and blastocyst formation (t9-blast) was compared between euploid blastocysts and each type of aneuploidy.

The t9-blast of euploid blastocysts was 35.6 ± 9.9 hours, similar to monosomy (37.4 ± 8.5 hours; p = 0.172), trisomy (35.9 ± 8.7 hours, p = 0.855) and partial alterations (36.2 ± 12.1 hours, p = 0.839). However, the t9-blast of blastocysts with complex alterations was significantly higher (38.6 ± 9.9, p = 0.006).

Conclusion: Our findings suggest that embryos exhibiting single chromosome alterations (monosomy or trisomy) do not show disturbances in the blastocyst morphokinetics. On the other hand, two or more alterations can cause a delay in blastocyst formation. According to our data and the available literature, we can speculate that single chromosomal alterations originate predominantly from oocytes (meiotic origin) and, thus, can affect all blastomers since the formation of the embryo, although not hindering embryonic development. However, complex alterations probably resulting from mitotic errors cause the formation of mosaic embryos during embryogenesis, which, in turn, can hinders morphokinetics development. These hypotheses must be confirmed with deeper studies on the origin of aneuploidy and its effect on embryo morphokinetics.

OC-06

WHAT TO ADVISE TO PATIENTS WITH ONLY ONE GOOD QUALITY BLASTOCYST? PGT-A OR NOT?

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PGT-A, single blastocyst, pregnancy outcomes, diminished ovarian reserve

Introduction: Preimplantation Genetic Testing for Aneuploidy (PGT-A) shortens the time to reach to a healthy pregnancy by selecting chromosomally normal embryo and reduces risk of miscarriage, particularly in the presence of a sufficient number of blastocysts suitable for biopsy. However, there is a lack of data in the literature regarding the potential advantages and disadvantages needed for effective decision and patient counselling of whether or not to undergo PGT-A. In the presence of a single blastosist makes difficult this decision of whether or not to undergo PGT-A. The aim of our study is to investigate whether PGT-A is beneficial for patients who have only one blastocyst available for biopsy.

Material & methods: This retrospective study was based on 2064 cycles performed at Istanbul Memorial Hospital in women between the 20 and 45 years old from August 2011 to March 2021 which resulted in single blastocyst. The single blastocyst was tested as euploid in only 208 out of 1126 PGT-A cycles (18.5%). The control group consisted of cases (n=938) with only single frozen (n=93) or fresh (n=845) blastocysts available for transfer.

PGT-A was done by aCGH (array comparative genomic hybridization) in 21.7% of the cases using 24Sure kit (Illumina, USA) between 2011-2016 and NGS ReproSeq on Ion Torrent S5 (ThermoFisher) in 78.3% between 2017-2021 following trophectoderm biopsy.

Demographic parameters, cycle characteristics and pregnancy outcomes were analyzed between PGT-A and non-PGT-A cycles. Univariable and multivariable evaluations of patient demographics and cycle characteristics in relation to clinical pregnancy, total pregnancy loss and live birth were done by using generalized linear mix models (GLMM).

Results: Although the mean age of females, history of recurrent spontaneous abortion (RSA) and the diagnosis of diminished ovarian reserve (DOR) were significantly higher in PGT-A group, biochemical and clinical pregnancy rates per embryo transfer were found to be significantly higher in single blastocyst euploid embryo transfer group 70.1 % and 63% than non-PGT-A group 44.8% and 38.6% respectively ($p < 0.05$)). In the GLMM, female age and PGT-A variables were found to be significant variables on clinical pregnancy, total pregnancy loss and live birth. PGT-A increases the probability of clinical pregnancy and live birth by 3.907 and 3.448 fold, respectively and decreases the probability of total pregnancy loss, independent of other risk factors in patients with a single usable blastocyst and diminished ovarian reserve. The likelyhood of a total pregnancy loss was found to be 1.943 fold higher ın non PGT-A cases compared to PGT-A group.

Conclusions: In the presence of a single blastocyst, PGT-A increases clinical pregnancy and live birth, decreases total pregnancy loss regardless of age. On the other side, embryo transfer was canceled in 81.5% of PGT-A cycles due to aneuploid embryos. Therefore, it would be appropriate to decide whether to perform PGT-A after providing detailed information to the patients in the presence of a single blastocyst.

OC-07

PREIMPLANTATION GENETIC TESTING FOR WISHKOTT-ALDRICH SYNDROME REVEALS SOMATIC AND GERMLINE MOSAICISM IN THE CARRIER MOTHER

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germline mosaicism, PGT-M

Introduction: Wiskott-Aldrich Syndrome (WAS) is an X-linked recessive immunodeficiency characterized by thrombocytopenia, eczema and recurrent infections. The index patient was diagnosed to have WAS at 1-month of age and was confirmed to be hemizygous for a nonsense mutation (WASc.97C>T). He received gene therapy at the age of 1 year. Due to the uncertain long term safety of gene therapy, the couple decided to have PGT, hopefully to have another child that is HLA match to their sick boy. The carrier mother (aged, 33 years) was found to have mosaic mutation.

Material & methods: Genetic status of the trios was confirmed by Sanger sequencing on their peripheral blood genomic DNA. Minisequencing primer targeted the mutation was tested on 2-lymphocyte samples. Microsatellite markers were designed within 1.1 Mb flanking the targeted gene. Mutant haplotype was deduced from the trios. PGT-M for WAS performed by mutation analysis and haplotyping. HLA-typing was performed by linkage analysis according to ESHRE best practice guideline. PGT-A was performed by next-generation sequencing (NGS).

Results: During the pre-PGT workup, Sanger sequencing on maternal DNA sample showed a minor mutation allele. In order to reduce allelic dropout (ADO), 2-lymphocyte samples of mother were used for validation of minisequencing. Mutation was detected in 30% (3/10) of the lymphocyte samples, further reconfirmed maternal somatic mosaicism.

During the PGT cycle, trophectoderm biopsy was performed on 12 blastocysts. PGT-M via minisequencing and haplotyping revealed maternal germline mosaicism with 3 haplotypes: haplotype A (mutant, high-risk haplotype containing c.97C>T allele), haplotype B (normal, high-risk haplotype without c.97C>T allele) and haplotype C (normal, low-risk haplotype without c.97C>T allele). Among 12 blastocyts, 2 of them inherited with haplotype A, 2 with haplotype B while 8 with haplotype C. Four HLA-matched blastyocysts were identified, among which 1 of them was male inherited with haplotype B while 3 were female with either haplotype B and C. After extensive counselling, patient decided to replace the euploid normal male HLA-matched embryo. The patient is pregnant and prenatal testing by amniocentesis confirmed a normal male fetus.

Conclusions: It is suggested that germline mosaicism is a pitfall in PGT-M for X-linked disorders (1,2), especially in the case of a female embryo displaying an ADO of the causative mutation. This report further exemplifies the importance of both mutation analysis and haplotyping in PGT-M. In case when germline mosaicism is suspected on an X-linked recessive disorder, selection of normal male embryos shall be of the first priority.

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OC-08

LONG-READ-AMPLICON GUIDED HAPLOTYPING ENABLES COMPREHENSIVE PREIMPLANTATION GENETIC TESTING IN FAMILIES WITH *DE NOVO* PATHOGENIC VARIANTS

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long-read sequencing, pre-PGT workup, PGT haplotyping, ONT, oxford nanopore

Introduction: Preimplantation genetic testing (PGT) aims to select embryos devoid of inherited pathogenic variants. Current comprehensive genome-wide haplotyping PGT methods cannot be applied for couples where one of the partners carries a de novo mutation, as absence of affected close relatives restricts variant phasing to establish the disease-associated haplotype. To overcome this limitation, we implemented a long-read amplicon guided haplotype imputation method that allows to determine parent-of-origin of mutant allele during the pre-PGT workup.

Material & methods: Thirty-two couples with de novo mutation in one of the partners were enrolled into the PGT program at UZ Leuven. Genomic DNA from the mutation carrier (proband) and his/her parents was used for trio analysis via long-range PCR and long-read amplicon sequencing using PacBio RSII and/or Oxford Nanopore platforms. Targeted haplotype phasing was then performed to impute the disease-associated allele.

Results: The parental origin of the mutant allele was identified in 20 patients, resulting in the current 62.5% success rate. In 14/20 (70%) couples, the mutation occurred on the paternal allele. In the remaining families, the region of interest either had no SNPs or had insufficient number of informative SNPs linked to mutation. From the successfully analyzed couples, eight have proceeded to PGT cycle via genome-wide haplotyping and so far, three disease-free children have been born.

Conclusions: Targeted amplicon long-read sequencing represents a valuable approach for PGT workup that leverages comprehensive PGT application in families with *de novo* mutations.

OC-09

ONEGENE PGT - COMPREHENSIVE PREIMPLANTATION GENETIC TESTING PLATFORM UTILIZING NEXT-GENERATION SEQUENCING

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PGT-M, aneuploidy, monogenic disease

Introduction: The goal of our work was to develop and clinically validate a robust yet cost-effective PGT-M platform that allows linkage analysis, direct pathogenic variant(s) testing, and also aneuploidy detection to make the most informative embryo transfer decision possible. For this task we have introduced OneGene PGT. The technique also incorporates key quality control (QC) data to provide accurate and reliable results. Direct mutation testing is performed for known disease-causing pathogenic variant(s). To avoid misdiagnosis caused by ADO, OneGene PGT provides haplotyping using 60-100 highly heterozygous SNP markers present inside and around the gene of interest. Using the parental and reference DNA samples, the OneGene PGT tool automatically identifies haplotypes associated with the pathogenic variant(s) or normal allele.

Material and methods: OneGene PGT requests DNA samples from male, female partner, a reference from a family, and individual embryos. The platform utilizes whole genome amplification (REPLI-g Single cell kit, Qiagen, Germany) with a co-amplification of highly heterozygous SNPs inside and around the gene of interest. A direct pathogenic variant detection is performed as a simple add-on in the assay. An aliquote of WGA product enriched for SNP markers undergo library preparation using PG-Seq™ kit (PerkinElmer, USA) according to the manufacturer's protocol. Final libraries were sequenced with single-end 1x150 bp reads using the MiSeq or NextSeq system (Illumina, USA). Pathogenic variants detection and haplotype analysis is performed by in house developed software tool. Chromosome abnormalities are detected by CNV analysis using whole genome sequencing reads using dedicated software.

Results and conclusions: At our IVF center, we use OneGene PGT platform for genes that are common indications for PGT-M (such as *CFTR*, *HBB*, *HTT*, *FMR1*, *BRCA1*, *BRCA2* and *GJB2).* The OneGene PGT platform underwent thorough validation which included direct pathogenic variant testing, haplotype identification and aneuploidy calling. The validation was performed on the basis of the re-analysis of the original MDA sample. Direct testing of pathogenic variants in 55 trophectoderm samples resulted in 100% agreement between the OneGene PGT platform and Sanger sequencing. For linkage analysis, 45 embryos derived from specific PGT-M cases was re-analyzed by OneGene PGT using DNA samples from a male, female partner, reference, and amplified embryos. We observed a full correlation between OneGene PGT and karyomapping analysis. The final part of the validation was the assessment of chromosomal abnormalities. In total, 39 MDA samples originally analysed by karyomapping were reanalysed using OneGene PGT platform. We found 100% agreement for full aneuploidy detection, however, some samples showed partial discordance mainly due to mosaic findings.

OneGene PGT is a universal, comprehensive, robust and cost-effective PGT-M platform that can be set up and validated for any gene of interest. We introduced the technique into clinical practice in 2020 and have performed more than 70 PGT-M cycles using this new approach to date.

OC-10

DOES WHOLE GENOME AMPLIFICATION WITH TARGET SEQUENCE ENRICHMENT IMPROVE PGT-M RESULTS?

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PGT-M, WGA, Target Sequence Enrichment, ADO, Combined PGT

Introduction: PGT-M for genetic diseases that can be diagnosed has been applied for many years. Simultaneous monogenic disease testing and euploid embryo selection (combined PGT) has become possible with the development of Whole Genome Amplification (WGA) technologies. As a result of widespread use of whole exome/genome sequencing technologies, variety of single gene diseases referred for PGT-M has started to increase thus leading to a considerable elevation in the number of setup studies conducted for rare diseases. Therefore, uniformity in WGA and overcoming the Alel Drop Out (ADO) trap in addition to a steady test setup is inevitable. This study demonstrates that for a wide range of PGT-M cases, ADO ratio is reduced using Target Sequence Enrichment (TSE), which is a novel technology that also allows sequential aneuploidy screening.

Material & methods: 1357 biopsy samples from 215 PGT-M patients were classified in three groups: PGT-M only (group 1; 697 biopsy samples/101 patients), combined PGT (group 2; 151 biopsy samples/32 patients), combined PGT with TSE (group 3; 509 biopsy samples/82 patients). For WGA, Doplify Kit (PerkinElmer) was used together with in house designed target sequence specific primers. First, routine STR based PGT-M protocol using biopsies directly (group 1), WGA products without TSE (group 2) or with TSE (group 3) was performed. Following the selection of eligible embryos, WGA samples were further processed using PG-Seq Kit (PerkinElmer), with (10 samples) or without further mutation specific enrichment for direct mutation analysis and aneuploidy screening.

Results & conclusions: Our findings show that ADO ratio is 1.9% for group 1 and 6.9% for group 2. On the other hand, ADO ratio for group 3 was 2.8% showing that TSE protocol dramatically reduces ADO ratio compared to similar patient groups without TSE. Blastomere biopsies were also included in this study eventhough WGA on blastomere is a contradictory approach. ADO ratio in group 1 and 2 was relatively higher, 8.1% and 13.3% respectively. ADO was not observed in group 3, in which only 7 blastomere biopsies from 2 patients were included. Further studies using blastomere biopsies are required in order to support the effect of TSE on blastomere biopsy. Additionally, 36 WGA products from group 3 were further processed for mutation confirmation and aneuploidy screening. Simultaneous aneuploidy screening for 24 chromosomes and the detection of mutation regions with high read depth (500X-4000X) were possible with this methodology. WGA is the most critical stage in combine PGT applications and TSE increases the accuracy of the test. The number of new designs for novel conditions is still increasing annually eventhough a potential saturation in STR markers is expected. Most patients referred to our center carries a rare disease, thus STR markers are designed almost solely for that patient. This creates an extensive STR bank, which is seldomly used for a couple of cases due to this high variability in PGT demand. TSE technology is a valuable approach since it allows combination of PGT-M and PGT-A using existing STR markers and primers enabling their efficient use for rare diseases.

OC-11

PANDA CARRIER – AN EXTENDED CARRIER SCREENING PANEL AND ITS ROLE IN PGT-M INDICATIONS

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ECS, extended carrier screening, diagnostic panel, PGT-M

Introduction: Nowadays, extended carrier screening (ECS) in infertile couples is on the rise and plays an essential part of the initial examinations in couples undergoing fertility treatment. A robust test with high sensitivity is crucial to maximize the accuracy and sensitivity of the test, and therefore significantly and reliably reveal the increased risk of having an offspring with a genetic disease. Currently, it is possible to offer these at-risk patients preimplantation genetic testing for monogenic diseases (PGT-M), thereby significantly reducing the risk of having a baby with a genetic disease.

Material & methods: Custom extended carrier panel (PANDA Carrier™) based on molecular inversion probes technology (MIP) was developed and validated in cooperation with Perkin Elmer©. PANDA Carrier™ screens for 110 genes that are the major population contributors to severe autosomal recessive (AR) and X-linked (XL) monogenic diseases. The entire coding sequence of tested genes is covered with an overlap of 30 pb to intronic regions. Using a targeted bioinformatics pipe-line, pathogenic variants are also detected in genes whose analysis is challenging due to the presence of homologous DNA sequences (for example SMN1, CYP21A2, HBA1/2). Only pathogenic (P) and likely pathogenic (LP) variants according to Clinvar and ACMG rules respectively have been reported. Copy number variants were reported for three and more exons in relevant genes. Patients at risk of having an affected offspring decided on further management after consultation with a clinical geneticist.

Results: A total of 606 patients (302 couples) were screened by the PANDA Carrier™ test between May 2021 and January 2022 at REPROMEDA clinic. At least one P/LP variant was detected in 407/606 (67,2 %) patients. On average, 1.19 (maximum 6) P/LP variants were detected per patient. Of the 303 couples examined, 20 (6.6 %) were identified as both being carriers of a P/LP variant in the same gene with autosomal recessive inheritance pattern for monogenic disease. Additional 9 (3 %) female patients were carriers of a P/LP variant in genes with a known X-linked disease. In one case, both autosomal recessive variants in both partners and an X-linked variant in the female partner were identified. Thus, the total increased risk of having a child with autosomal recessive or X-linked disease was determined in 28/ 303 (9.2 %) of the couples examined. Based on these results, PGT-M was indicated by the clinical geneticist in 25 cases (8.25%). The remaining three cases were variants or combinations of variants with mild or no clinical manifestations of the disease.

Conclusion: Our results confirm that the introduction of the extended carrier panel into routine practice has a high added value for patients and can significantly reduce the risk of having a genetically diseased child when combined with PGT-M methods. A major challenge for the entire field of human genetics at a time when exome sequencing is on the horizon is to be able to correctly classify the detected variants in order to decide as accurately as possible whether it is appropriate to offer PGT-M to couples.

OC-12

IMPROVED CLINICAL UTILITY OF PREIMPLANTATION GENETIC TESTING BY THE INCORPORATION OF PLOIDY AND PATHOGENIC MICRODELETIONS

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Genotyping; Microdeletions; NGS; PGT; Ploidy

Introduction: Standard methodologies employed in Preimplantation Genetic Testing for Aneuploidy (PGT-A) allow to identify chromosomal aneuploidies but do not distinguish embryo ploidy status due to the normalization process during conventional comprehensive chromosome assessment. Transferring embryos with abnormal ploidy configurations can result in miscarriage or molar pregnancy. Moreover, minimal resolution limits of current methodologies prevent the detection of microdeletions smaller than 10 Mb. Microdeletions can involve in genomic disorders associated with neurodevelopmental disabilities and multiple congenital anomalies, multiple congenital anomalies, requiring later-stage invasive prenatal diagnosis. The development of this sequencing strategy can resolve these limitations and add valuable clinical utility in the refinement of the euploid embryo selection.

Material & methods: Ploidy determination was validated using 244 embryo samples of known ploidy status. While the dataset for microdeletions detection validation included 576 negative cases and 72 positive cases for eight common pathogenic microdeletion regions (-4p=Wolf-Hirschhorn, -8q=Langer-Giedion, -1p=1p36 deletion, - 22q=DiGeorge, -5p=Cri-du-Chat, -15q=Prader-Willi/Angelman, -11q=Jacobsen, -17p=Smith- Magenis).
PGT-A products were reamplified and sequenced using a custom AmpliSeq panel targeting 357 genomic regions harbouring high frequency SNPs across the genome and within selected microdeletion critical regions. Sequencing data were processed by a bioinformatic algorithm which accounts for sequencing noise through gaussian-mixture modelling of B-allelic ratios measured at each SNP locus and estimates the likelihood of different ploidy levels and of the presence of microdeletions.

Results: Ploidy was correctly determined in 233/234 cases (PPV=94.1%, NPV=100%), with only one diploid sample misclassified as triploid but requires further confirmation on re-biopsy by orthogonal technology.
Microdeletions could be consistently detected with high reliability (PPV=98.5%, NPV=99.5%) in 6 out of the 8 considered regions. Detection of microdeletions of 1p and 4p were less reliable due to the presence of recurrent haplotype blocks in the population, as confirmed by the analysis performed in One Thousand Genome Project database (1kGP). The only microdeletion apparent false positive case (-22q) requires further testing with the support of parental DNA use.
This analytical framework was blindly applied to: (i) a PGT-M case affected by DiGeorge syndrome (female partner was carrier of del22.q11.21(20754422-21440514), where all the nine embryos were classified coherently with the previous results by indirect linkage analysis; (ii) 99 transferred human euploid embryos resulting in pregnancy losses, where no ploidy alteration was found while an estimated microdeletion (-8q, -22q) prevalence of 2/99 was detected.

Conclusions: Here we showed the design and the validation of a next-generation-sequencing integrated approach from a single trophectoderm biopsy and we evaluated its diagnostic performance for simultaneous assessment of aneuploidy, ploidy, and common pathogenic microdeletions within PGT. This new assay allows an accurate characterization of these chromosomal abnormalities expanding PGT-A clinical informativity and diagnostic capabilities. Moreover, this strategy will also help elucidate fundamental biological and clinical questions related to the genetics of implantation failure and pregnancy loss of apparently euploid embryos at preimplantation stage, increasing our knowledge on these phenomena.

OC-13

CLINICAL EXPERIENCE OF NON-INVASIVE PRENATAL TESTING FOR MONOGENIC DISORDERS: A NEW TOOL FOR PGT-M PREGNANCIES

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NIPT, monogenic disorders, ccffDNA, maternal blood, PGT-M

Introduction: Prenatal diagnosis (PND) is recommended in pregnancies resulting from preimplantation genetic testing for monogenic disorders (PGT-M). However, PGT couples are unwilling to undergo invasive PND because of the associated risk of fetal loss. Circulating fetal DNA (ccffDNA) in maternal blood allows non invasive prenatal testing of monogenic disorders (NIPT-M) avoiding the PND risk. Since only around 10% of DNA present in maternal plasma is fetally derived (the remaining 90% is maternal DNA), NIPT-M was initially limited to the analysis of *de novo* or paternally inherited traits to ensure their fetal origin. Lately, new technologies such as NGS or Digital PCR (ddPCR) have helped in broadening the scope of NIPT to maternally derived fetal mutations. Our group started with NIPT-M of *de novo* or paternally inherited fetal mutations using a primer based-extension technology and short-tandem repeats (Bustamante-Aragones A. *et al*, 2014). Now, we have moved to the use of ddPCR and therefore the analysis of maternally inherited variants have also been incorporated (Perlado S. *et al*, 2016).

Here, we present our clinical experience in 17 NIPT-M cases using the ddPCR technology, including analysis of *de novo*, paternal and maternal single base fetal mutations.

Material and Methods: A total of 17 at-risk pregnancies were studied. One of them was a pregnancy conceived after PGT-M*:

- AD disease with maternal origin: Epidermolysis bullosa dystrophica (1).
- AD disease (*de novo* variant): Achondroplasia (6), Epileptic encephalopathy (1).
- AR disease: Congenital disorder of glycosylation, type Ia (4), Mucopolysaccharidosis type IIIB (2), Multiple mitochondrial dysfunctions syndrome (1).
- XL disease: Retinoschisis* (2).

General protocol included collection of 1 to 3 independent maternal blood samples at different gestational ages (8-31 weeks of gestation) following by plasma DNA extraction. Fetal genotyping was performed by ddPCR: analysis of paternal/*de novo* variants, was determined by a presence/absence criteria and determination of maternal variants was performed by relative mutation dosage (RMD) analysis.

When available (10/17), NIPT-M results were compared with PGT-M, conventional PND or at birth.

Results: NIPD-M by ddPCR showed:

- a diagnostic rate of 94% (16/17 cases) since in one case, diagnosis was not possible due to a low fetal fraction.
- 100% accuracy and positive/negative predictive values in those 10 out of 17 cases in which comparison with results at birth or PND was possible.

Conclusions: PGT-M patients are reluctant to undergo conventional PND due to the fetal loss risk associated to invasive obstetric procedures. NIPT-M is a more accepted alternative since it allows fetal genetic diagnosis without risk for the pregnancy (*Toft et al*, 2022). In these work, we present our results from clinical practice in 17 NIPT-M cases using ddPCR technology, showing a 100% diagnostic accuracy for single base variants with independence of parental origin. Although this strategy has shown to be an accurate alternative to conventional invasive PND, it usually requires family-specific design and optimization and therefore, an extensive work-up. Development of future comprehensive NIPT methods could offer a combined diagnosis for both NIPT-A & NIPT-M as they currently do for PGT.

OC-14

NON-INVASIVE PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDIES BY METABOLOMIC PROFILING OF CULTURE MEDIA: A PROSPECTIVE STUDY

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Non-invasive, PGT-A, Raman spectroscopy, metabolomic profiling, machine learning

Introduction: Studies on non-invasive preimplantation genetic testing for aneuploidies (ni PGT-A) using spent culture media (SCM) have been devoting effort to examining its concordance with invasive trophectoderm biopsies results. However, ploidy calling by sequencing embryonic cell-free DNA in the SCM is prone to maternal contamination from cumulus cells. Besides, the reagent expenses and turn-around time of sequencing increase the cost of each cycle. Alternatively, metabolomic profiling of SCM by Raman spectroscopy combined with machine learning algorithm has recently been reported as a rapid and cost-efficient method that could potentially serve as an alternative to a sequencing-based PGT-A approach. However, its applicability to different culture media and its prediction of reproductive outcomes are yet to be determined.

Material & Methods: A prospective study was structured in two phases: Phase I replicated the previously established methods to select machine learning algorithms with good best performance in the G2 medium. Four algorithms were evaluated, including extreme gradient boosting (XGB), Stacking, k-nearest neighbors (kNN), and Random Forests (RF). Phase II further applied the outperforming algorithms from Phase I to different types of frequently used SCMs in clinical practice. Established algorithms were trained and blindly tested on SCMs. In total, 319 SCMs from 120 patients from two reproductive medicine centers from Jan 2017 to Dec 2019 were included. Raman spectra of the SCM were classified into euploid or aneuploid. Sensitivity and specificity were assessed by using trophectoderm biopsy results as standard. Reproductive outcomes were followed up after elective single euploid embryo transfer, as determined by conventional trophectoderm biopsy-based PGT-A.

Results: In Phase I (99 G-2 medium), both XGB and Stacking algorithm outperformed the other two algorithms (kNN and RF) by having 100% specificity (43/43). In Phase II, after equal-ratio algorithm training, XGB and Stacking remained better than the others with 76.8% (43/56) and 80.4% (45/56) sensitivity, whilst 100% (43/43) and 81.4% (35/43) specificity respectively in G-2 medium. We further performed specific medium-based algorithm optimization and applied both algorithms to 165 G-2 Plus medium and 55 G-TL medium. In the G-2 Plus medium group, the sensitivity and specificity of both XGB and Stacking were higher than 90% (XGB: 90.4% sensitivity, 47/52; 92.9% specificity, 105/113; Stacking: 92.3% sensitivity, 48/52; 95.6% specificity, 108/113). In the G-TL medium group, XGB (88% sensitivity and 86.7% specificity) and Stacking (88% sensitivity and 90% specificity) had comparable performance to each other. Notably, the performance of Raman spectroscopy in predicting pregnancy outcomes after euploid transfers reach >91% for both sensitivity and specificity for implantation (93.8% sensitivity and 95.7% specificity), miscarriage (91.7% sensitivity and 96.0% specificity), and ongoing pregnancy/ live birth (96.0% sensitivity and 100% specificity) respectively.

Conclusions: Raman spectroscopy is a robust non-invasive approach applicable to different types of culture medium with decreased cost and good performance for predicting reproductive outcomes.

OC-15

NON-INVASIVE PGT-A APPLIED TO DAY-5 AND 6 FROZEN THAWED BLASTOCYSTS: OPTIMAL TIMING FOR MEDIA COLLECTION

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Aneuploidy, non-invasive PGT-A, culture medium, frozen blastocysts.

Introduction: Non-invasive PGT-A (niPGTA) is a new technique that allows the study of the chromosomal content of blastocysts. The informativity and concordance rates for fresh embryos have been related with the time in culture and the day of media collection (Rubio, 2020). However, there is a need to estimate the minimum time in culture required for frozen blastocysts on day-5 or day-6 of development.

Material and methods: Prospective study including 95 spent blastocyst media (SBM) samples and the corresponding blastocyst samples from thawed day-5 and day-6 blastocysts from PGT-A and non PGT-A cycles from patients aged 29-42 years, following approved IRB protocols. After thawing, each blastocyst was washed to remove remaining cumulus cells and individually transferred to a 10µL droplet of fresh media or to a time lapse plate with 20µL volume per well. Day-5 blastocysts were cultured 8 or 24 hours, and day-6 blastocysts were cultured 8 hours. The SBM and blastocysts samples were then collected and frozen until analysis using Next Generation Sequencing with proprietary algorithms. Informativity and concordance rates between cell-free DNA in SBM and blastocysts samples were compared between PGT-A (previously biopsied) and non PGT-A (non-biopsied zona intact) cycles, according to the different timing in culture. Ploidy concordance was considered as the overall agreement between the SBM and the blastocyst sample considering them euploid or aneuploid. Ploidy concordance included both full concordance (when the chromosomal status for all the chromosomes in both samples is the same) and partial concordance (the chromosomal status for some chromosomes might differ between samples, but they are both aneuploid).

Results: Table 1 shows the detailed results in each subgroup. No significant differences were observed for informativity according to the treatment type (PGT-A and non PGT-A), with significantly lower informativity rates for day-5 blastocysts with only 8 hours in culture, in both PGT-A (p=0.0031) and non PGT-A (p=0.0015). Regarding concordance rates, there were no significant differences among subgroups.

TABLE 1	PGT-A			Non PGT-A		
	Day-5/8hrs	Day-5/24hrs	Day-6/8hrs	Day-5/8hrs	Day-5/24hrs	Day-6/8hrs
No. of samples (%)	15	12	10	22	20	16
Informative SBM (%)	7/15 (46.7%)*	12/12 (100%)	8/10 (80%)	13/22 (59.1%)**	20/20 (100%)	15/16 (93.8%)
Ploidy concordance (%)	7/7 (100%)	12/12 (100%)	7/8 (87.5%)	11/13 (84.6%)	17/20 (85%)	14/15 (93.3%)
Full concordance (%)	5/7 (71.4%)	10/12 (83.3%)	6/8 (75%)	9/13 (69.2%)	15/20 (75%)	11/15 (73.3%)
Partial concordance (%)	2/7 (28.6%	2/12 (16.7%)	1/8 (12.5%)	2/13 (15.4%)	2/20 (10%)	3/15 (20%)

**p=0.0031; **p=0.0015 (Fisher´s exact rest)*

Conclusions: niPGT-A can be applied to frozen-thawed blastocyst with or without a previous biopsy, indicating that a previous hole in the zona pellucida does not add any additional benefit in terms of informativity or concordance of the SBM. The optimal time in culture for day-5 blastocyst is 24 hours, with similar results than day-6 blastocysts cultured only for 8 hours after thawing. Therefore, this niPGT-A approach can be offered to patients with previously frozen blastocysts. It could also be a good strategy to rescue cases with non-informative results after previous cfDNA analysis, and even for non-informative trophectoderm biopsies.

OC-16

CELL-BASED NONINVASIVE PRENATAL TESTING FOLLOWING PGT. A RISK-FREE ALTERNATIVE TO INVASIVE CHORIONIC VILLOUS SAMPLING

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Cell-based non-invasive prenatal testing, Preimplantation Genetic Testing for Monogenic disorders

Introduction: Prenatal testing is often recommended following preimplantation genetic testing (PGT) to verify that a pregnancy with an unaffected fetus has been achieved. Although the risk of misdiagnosis following PGT is small, the consequences associated with an undetected misdiagnosis are often severe. Based on a patient questionnaire study performed at our PGT center, the risk of miscarriage associated with invasive methods of prenatal testing, such as chorionic villus sampling (CVS), is a major factor discouraging patient from the procedure despite a desire to know the status of the fetus (Toft *et al.*, 2022). Therefore, non-invasive alternatives to prenatal testing are warranted. Furthermore, results from the questionnaire showed that patients deferring CVS would opt for a non-invasive procedure. Here we present data from cell-based non-invasive prenatal testing (cbNIPT) performed concurrently with CVS in 20 pregnancies following PGT.

Materials and methods: A blood sample (30 ml) was obtained in gestational week 10-14 from each woman who had achieved pregnancy following PGT for a monogenic disorder. Potential extravillous trophoblast cells were isolated from the blood within 48 hours in by ARCEDI Biotech. Potential extravillous trophoblast cells were analyzed by short tandem repeat (STR) marker analysis and direct mutation detection using the same markers as during PGT. Presence of at least one fully informative paternal marker was required for a cell to be identified as of fetal origin. Mutational status was assigned based on the STR-marker profile and the direct mutation analysis. Specificity for mutational status was assessed by concordance between the result from fetal cells to the CVS results. Sensitivity for mutational status was assessed by concordance between maternal cells in cases where the mother was known to be the carrier of the monogenic disorder. Test results can reliably be obtained within a week from blood sampling.

Results: A total of 20 blood samples were collected. Seventeen samples showed the fetus to be unaffected or a carrier (85 %), while no result was obtained from the remaining three samples. A total of 193 potential extravillous trophoblast cells were isolated (median of 6.5 per sample, range 2-26) of which 69 were shown to be of fetal origin (median of 3 fetal cells per sample, range 0-10), 55 to be of maternal origin (a median of 1 maternal cell per sample, range 0-14) and the remaining 69 were inconclusive following analysis (median 2.5, range 0-11). Complete concordance was observed for all successfully analyzed fetal cells and the CVS result, providing a sensitivity of 100 % (CI_{95} 95.7 % - 100 %). Complete concordance as also observed between successfully analyzed maternal cells and the mothers known genotype, providing a sensitivity of 100 % (CI_{95} 93.3 % - 100 %).

Conclusion: Our results show that cbNIPT can provide an informative test in the majority of pregnancies with high sensitivity and specificity, suggesting cbNIPT as a first-line risk-free alternative to CVS.

OC-17

NONINVASIVE PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY: CONCORDANCE WITH TROPHECTODERM BIOPSY AND CLINICAL OUTCOME EVALUATION

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non-invasive PGT-A, spent culture media

Introduction: A significant body of published work has shown the benefits of preimplantation genetic testing for aneuploidy (PGT-A). Currently, performing a trophectoderm biopsy at the blastocyst stage is the most reliable method to obtain DNA for PGT-A. Despite the increase in success rates that have been attributed to a trophectoderm (TE) biopsy, it's a time-consuming, invasive, and costly procedure. Since the discovery of cell-free DNA (cfDNA) in the spent culture medium (SCM), a growing number of studies have explored this noninvasive technique as a promising alternative to an embryo biopsy. In this study, we aim to investigate the accuracy of cfDNA for ploidy and sex compared to trophectoderm biopsies. In addition to the correlation between the two techniques, clinical outcomes with concordant and discordant SCM results were retrospectively evaluated.

Materials and Methods: Patients undergoing PGT-A as part of their IVF cycle from July 2020 to March 2021 were recruited for this study. Medium change and assisted hatching was performed on day 3 and the entire 20µl SCM droplet was collected and sent for analysis prior to the blastocyst biopsy. Whole genome amplification (WGA) was performed Picoplex (Rubicon), sequencing was done using the Ion S5 Sequencer (Life Technologies) and results were scored for aneuploidy using the Ion Reporter 5.0 software. Concordance rates for ploidy and sex between the SCM and TE biopsies were assessed. Embryo selection for transfer was based on the PGT-A results of the TE biopsy and clinical pregnancy was defined as the presence of a fetal heartbeat.

Results: Embryonic DNA from TE biopsies and SCM corresponding to a total of 110 blastocysts was analyzed and 20 of these embryos were transferred. Interpretable NGS results were generated in 98.1% of the TE biopsies and 91.8% of the SCM samples. Overall concordance for ploidy and sex was 73.1%. An increase in ploidy concordance was observed in the day 6 embryo cohort (85.7%) compared with the SCM collected on day 5 (64.8%, $p<0.05$). An increase in amplification failure was observed in spent media collected on day 5 (6.2%) compared with day 6 (2.0%). Eleven of the 13 discordant samples involving sex chromosomes were classified as female by the SCM, indicating maternal cell contamination. There was no difference in pregnancy rate when comparing euploid TE/euploid SCM (76.9%) and euploid TE/ aneuploid SCM (71.4%). Two successful deliveries occurred from the transfer of embryos that were classified as aneuploid based on the SCM.

Conclusions: Although this study demonstrates concordance between SCM and trophectoderm biopsy results, modifications in culture conditions and NGS protocols need to be made to minimize maternal cell contamination and improve the accuracy of the noninvasive diagnosis. An increased duration of exposure to the culture media appears to have an impact on the accuracy of the of the niPGT-A results. Additional concordance studies and trials based on clinical outcomes are required to determine if SCM could be a feasible, noninvasive approach for PGT-A.

OC-18

HOW CLOSE ARE WE TO EMBRYO REALITY WITH NON-INVASIVE PGT?

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NI-PGT, Cell Free DNA, Culture Media, PGT-A, Trophectoderm

Introduction: Use of trophectoderm cell source in NGS based aneuploidy screening is a general and widespread approach, but nevertheless potential effects of the biopsy procedure, possibilities of false positive/negative results due to mosaicism make PGT-A controversial. Recent achievements in the development of Non-Invasive PGT (NI-PGT) claim that the culture medium reflects the embryo reality more efficiently. Knowledge on 300-400bp size of cell free DNA (cfDNA) in the culture medium and related supportive study findings together with the information that cfDNA amount is higher in the culture medium than blastocoel fluid accelerated the improvement of these studies. Efficient Whole Genome Amplification (WGA) is well implemented in NI-PGT, but still factors like mosaicism, polar body and maternal cumulus contamination bring out the possibility of false positive/negative results. At this point, elaborate cumulus removal under varying culture conditions throughout the IVF procedure and optimization of the culture changing days, in addition to the development of efficient tubing protocols and avoiding these particular traps have become the fundamental aim. In this study, we investigated the effect of two protocols differing in the preparation of the oocyte before microinjection and culturing steps. In this context, we compared culture medium results with the trophectoderm findings of corresponding embryos.

Material & methods: Trophectoderm biopsy and culture media collection were performed on day 5 (n=29). Two different protocols with additional enzymatic cumulus removal and culture steps have been used before the collection of culture medium samples on day 5. NI-PGT application was performed using PG-Seq Rapid Non-Invasive PGT Kit (PerkinElmer).

Results & conclusions: WGA efficiency was 90% (26/29) and 3 samples with amplification failure were not included in the study. NI-PGT results obtained from the first group (n=7) showed higher concordance (86%) in terms of aneuploid chromosomes compared to their corresponding trophectoderm results. On the other hand, concordance ratio of the second group (n=19) was lower (53%). Furthermore, sex chromosome concordance was higher in first group (100% vs 84%) supporting our hypothesis that alterations in IVF procedures for the removal of cumulus are essential to exclude maternal contamination in NI-PGT application. Study showed that results obtained with first protocol, where enzymatic and chemical cumulus removal is performed in addition to routine oocyte denudation, which helps exclusion of maternal contamination is more satisfactory. This highlights the importance of culture step optimization and for NI-PGT-A, which will be an issue in providing global NI-PGT-A service.

Another encouraging highlight of the study was incresed amplification efficiency, which is a result of improved WGA technology. On the negative side, maternal contamination was observed for 3 cases (nonconordant gender) in addition to 5 euploid female embryos, where maternal contamination could not be excluded. SNP or STR based maternal contamination methods should be integrated into NI-PGT to resolve this issue and distinguish maternal contamination from genuine euploid results. Furthermore, we have observed reciprocal aneuploidy of same chromosome in 2 samples. Finally, eventough initial results are encouraging, standardisation of different culture protocols for NI-PGT-A and accumalation of data are obviously required.

LIST OF ACCEPTED POSTERS

P-01

THE IMPACT OF USING ARTIFICIAL INTELLIGENCE UPON PGT-A RESULTS USING HIGH RESOLUTION NGS

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PGT-ai, NGS, Artificial Intelligence

Introduction: The interpretation of high resolution NGS (hr-NGS) plots is dependent upon many factors such as the geneticist experience, cut-off points, chromosomal number, and the IVF center (4). These factors can affect the results and make the interpretation rather subjective. An obvious example is the difference of mosaicism rate in-between centers (4). Also, PGT-A has been criticized as it produces false positive results which might exclude euploidy embryos from being transferred and hence wasted. There is an urgent need for more objective and accurate way for calling the hr-NGS results. Artificial Intelligence (AI) looks to be a promising way to decrease subjectivity and improve accuracy of calling the results using machine learning, (2).

Material & Methods: This is a prospective ongoing study, where 3720 blastocysts from 983 patients (so far) have trophectodermal biopsy (either at D5/6). The samples are processed according to Illumina Miseq protocol. The results are manually called after reviewing Bluefuse software results (referred here within as subjective methodology). On the same time the raw data were uploaded up to PGTai^{SM} server (cooper surgical Fertility and Genomic solution), and the results were compared afterword with the “subjective methodology”. This study is carried out at Ganin Fertility Center, Cairo, Egypt.

Results: The average age of the female partners is 33.2 years. There was a difference in 415 results (11%) comparing between the “subjective methodology” and PGTai^{SM} interpretation.

The results are shown in the following table:

result	PGTai^{SM}	Subjective methodology	Relative increase/decrease
Euploid embryo no (%)	1703 embryos (45.7%)	1609 embryos (43.2%)	+5.84%
Aneuploid embryo no (%)	1350 embryos (36.3%)	1479 embryos (39.7%)	-8.72%
Low Mosaic embryo no (%)	221 embryos (5.9%)	284 embryos (7.6%)	- 22.18%
High Mosaic embryo no (%)	344 embryos (9.2%)	416 embryos (11.1%)	-17.3%
Total Mosaic embryo no (%)	565 embryos (15.2%)	700 embryos (18.8%)	-19.29%

Conclusion: PGTai^{SM} platform has a relative decrease of the mosaicism and increase euploid rates, that increases relatively the number of euploidy embryos available to be transferred back to the mother. That has significantly lowered the false positive rate and answer some of previous argument against doing PGTA, as some of the non-euploidy embryos that were transferred in previous studies have ended with normal prenatal outcome. The much lower rate of mosaicism noticed in the PGTai^{SM} group have several other advantages over the subjective method, such as it standardizes results in-between centers especially with regard to the cutoff points where mosaicism is counted 30-70% at some centers vs 20-80% in others. One can argue that low mosaicism in subjective calling can be consider as euploidy and should be transferred back to the uterus after counseling the patients, however 41.1% patients do not prefer to transfer mosaic embryo. Again, not all embryos of low mosaicism where recalled to be normal in PGTai^{SM}. The use of AI would standardize objectively the calling of hr-NGS results.

P-02

GENTYPE: PREIMPLANTATION GENETIC TESTING BY PEDIGREE HAPLOTYPING AND COPY NUMBER PROFILING SUITABLE FOR THIRD-PARTY REPRODUCTION

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Preimplantation genetic testing, monogenic disease; aneuploidy, third-party reproduction, reduced representation sequencing

Introduction: Preimplantation genetic testing (PGT) is performed in an assisted reproductive technology setting and was designed to prevent transfer of embryos affected by a genetic disorder. As of today, PGT is performed in three different contexts: testing for recognized heritable monogenic disorders (PGT-M), screening for aneuploidy (PGT-A), and screening for structural chromosomal rearrangements (PGT-SR). Given the varying natures of these investigations (e.g., targeted versus genome-wide, etc.), a cost-effective and automatable 'all-in-one' approach is necessary. We aimed to develop a workflow and user-friendly visualization platform for all-in-one PGT, suitable for parents-only haplotyping and, for the first time, third-party reproduction.

Material and Methods: 257 samples biopsied from cell lines and human blastocysts were whole genome amplified and processed by our newly developed reduced representation sequencing-based technology 'GENType'. Quality metrics, genome-wide haplotypes, b-allele frequencies and copy number profiles were generated by our novel visualization tool 'Hopla'. PGT-M results were deduced from relative haplotypes, while PGT-SR/PGT-A results were inferred from read-count analysis and BAF profiles. Suitability for parents-only haplotyping or third-party reproduction was assessed by excluding additional family members or one biological parent from analysis, respectively. Results were validated against reference PGT methods.

Results: Genome-wide haplotypes of single cells were highly accurate (mean>99%) compared to bulk DNA. Unbalanced chromosomal abnormalities (>5Mb) were detected by GENType. For both PGT-M as well as PGT-SR/PGT-A, our technology demonstrated 100% concordance with reference PGT methods for diverse WGA methods. Equally, for parents-only haplotyping and third-party reproduction, PGT-M results were 100% concordant. Furthermore, the origin of trisomies in PGT-M embryos was correctly deciphered by Hopla.

Conclusions: GENType together with Hopla offers an all-round PGT solution for diverse families without the need for personalized assays, microarray technology or whole genome sequencing.

P-03

EFFECTS OF RESVERATROL ON AUTOPHAGY AND EXPRESSION OF INFLAMMASOMES IN PLACENTAL TROPHOBLAST OXIDATIVE STRESS MODEL

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Oxidative stress,HTR-8/SVneo,autophagy,inflammasome

Introduction: The normal growth and development of placenta is the key to successful implantation and pregnancy, while the development and maturity of placenta is a complex process, which requires the regulation of trophoblast invasion and its differentiation and proliferation in decidua. The increase of fetal metabolic rate is related to the increase of oxidative stress in placenta. Unfortunately, at least 25% of women's pregnancies end in failure, which is mainly due to the failure of placenta formation. In addition, during pregnancy, placental dysfunction, preeclampsia and intrauterine growth restriction are responsible for a large proportion of perinatal mortality and morbidity. At present, there are no effective treatment for these diseases other than childbirth.

Based on the in vitro model of oxidative damage of HTR-8/SVneo in human placental trophoblasts, we aim to investigate whether there is activation of NLRP1 inflammasome and excessive autophagy in oxidative stress injury. Resveratrol (RES), an antioxidant and autophagy regulator, was taken as the research object to clarify its role in oxidative damage of human placental trophoblasts.

Materials and Methods: Based on our previously established model of oxidative stress in trophoblasts, the levels of IL-1 β and caspase-1 in the supernatant were detected by ELISA, and the levels of IL-1β, Caspase-1, NLRP1, LC3II and Beclin1 were measured by Western blot.

Resveratrol of different concentrations was added. After 8 hours induction, CCK-8 was used to measure the cell survival rate, flow cytometry was used to detect the intracellular ROS level and apoptosis rate; the content of LDH in the supernatant of each group of cells and the levels of SOD, MDA and CAT in the cell homogenate were measured by spectrophotometry, and the level of IL-1β and Caspase-1 in the supernatant of each group of cells was detected by ELISA. The expression levels of IL-1β, Caspase-1, NLRP1, LC3II and Beclin1 were detected by Western blot.

Results: The expression of NLRP1 and Beclin-1 in trophoblast oxidative stress model was significantly higher than that in the control group ($P<0.01$).

Compared with the model group, resveratrol (50μmol/L) treatment for 8 hours could significantly improve the morphological changes of cells caused by oxidative stress, significantly increase the survival rate of cells ($P<0.01$), significantly reduce the release of LDH, decrease the content of MDA, significantly increase the activity of SOD and CAT ($P<0.01$). At the same time, the expression of IL-1β, Caspase-1, NLRP1, LC3 and Beclin-1 decreased significantly ($P<0.01$).

Conclusions: In our trophoblast oxidative stress model, the expression of NLRP1, the activation of IL-1β and Caspase-1 inflammasomes, and the expression of LC3 and Beclin1 in trophoblasts were detected. Compared with the autophagy related proteins in normal trophoblasts, it was confirmed that trophoblasts in oxidative stress injury state had excessive autophagy.

As a potent antioxidant and autophagy regulator, resveratrol reduces apoptotic cells after oxidative stress injury by alleviation of H2O2-induced oxidative stress damage in HTR-8/SVneo cells., thus ensuring the normal biological functions of trophoblasts, which is paramount to the successful establishment and maintenance of normal pregnancy.

P-04

MONOMORPHIC TERATOSPERMIA MANAGMENT: FERTILITY PROGNOSIS AND PGD INDICATIONS

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Globozoospermia, Macrorephalic sperm head syndrome, AURKC, DPY19L2, SPATA16

Introduction: Monomorphic teratozoospermia has been better studied with the development of molecular exploration and morphological techniques that reveal numerous subtypes and mutations in the genes responsible for different infertility phenotypes.

Materials and methods: A retrospective study, about a serial of 24 Tunisian patients with monomorphic teratozoospermia and for who clinical, morphologic and genetic explorations have been done, was conducted.

Results: Macrocephalic sperm head syndrome (SMP) was noted in 14 cases and globozoospermia or round-headed sperm syndrome (STR) in 10 cases. The average age was 39 years old. The mean duration of primary infertility ranged from 5.6 years (SMP) to 7.6 years (STR). Oligo-astheno-teratozoospermia was noted in 80% of patients. 43% of SMP patients who were involved in assisted reproduction treatment (ART) failed to conceive in all cases, whereas among 70% of STR patients who were conducted into ART cycles, only one couple gave birth to a healthy twin using oocyte activation protocol. 71.4% of SMP patients were molecularly explored for the c.144delC microdeletion of exon 3 of the AURK C gene. Five SMP (type 1 form) patients were found to be positive and homozygous for the microdeletion whereas five patients (SMP type 2 form) were negative. 70% of STR patients were explored for the c.848G> A mutation of exon 4 of the SPATA 16 gene and only four STR patients for DPY19L2 gene rearrangements. But, no mutations were detected. The karyotype was normal for 23 patients with 46,XY formula whereas a reciprocal translocation involving chromosomes 4 and 16, was detected in one SMP patient (type 1 form).

Conclusion: Our study demonstrated the impact of the implementation of genetic and morphological techniques as well as new ART procedures in order to precise the diagnosis and the fertility prognosis of monomorphic teratospermia. Identification of the genetic causes should help refine diagnosis and treatment of monomorphic teratospermia. Fertilization and embryo development after oocyte activation may still be utilized in cases of partial globozoospermia, where a percentage of morphologically normal spermatozoa might still be present. Only in partial cases, a preimplantation genetic screening may be considered.

P-05

PROGNOSTIC VALUE OF TRIPLOID ZYGOTES ON PGTA CYCLES

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Aneuploidy, embryo, IVF, 3PN.

Introduction: The prevalence of triploidy among all pregnancies has been estimated to be approximately 1% to 3%. After ICSI, 3PN incidences are generally due to a retention of the second polar body. Although a higher proportion of triploid zygotes has been linked to worse IVF cycle outcomes, it is unknown whether the triploidy incidence may serve as a predictor of the aneuploidy rates of embryos derived from normally fertilized oocytes from the same cohort.

The two aims of our study were: i) to assess if the presence of at least one 3PN zygote was associated with higher aneuploidy rates in the rest of the cohort, ii) to investigate if cohorts with high 3PN incidence were more prone to have higher aneuploidy rates.

Material and methods: This is a retrospective cohort study including 1715 ICSI cycles requiring PGT-A, performed between June 2016 and December 2020 in the same fertility clinic. Embryo biopsies were carried out at the blastocyst stage and Next Generation Sequencing was used for chromosomal analysis. Only cycles with at least one blastocyst capable of being biopsied per cohort were included in the study.

Since maternal age impacts on euploidy rates, patients were divided into 5 groups. Group A: patients <35 yrs (n=137, 715 biopsied embryos); group B: 35-37 yrs (n=202, 866 biopsied embryos); group C: 38-40 yrs (n=674, 2051 biopsied embryos); group D: 41-42 yrs (n=453, 1210 biopsied embryos); group E: >42yrs (n=249, 502 biopsied embryos).

Results: The global aneuploidy rate was 63.21% and it was higher in the cycles that presented 3PN vs. in those that were absent (66.03% vs. 62.21%, p=0.049). When the analysis of the results was done after stratifying cycles according to maternal age, group C showed statistically significant differences (A=26.67% vs. 36.22% NS; B=47.05% vs. 46.24% NS; C=63.85% vs. 56.78% p= 0.03; D=77.02 % vs. 75.53% NS; E=78.70 % vs. 84.55% NS).

Overall, the 3PN incidence after ICSI was 4.15%. The aneuploidy rates were significantly higher in the cohort of patients who had ≥ 4.15% of embryos appearing triploid, (66.5% vs. 62.1%, p=0.025). After stratifying by maternal age, group C reached statistical differences again (A=27.40% vs. 35.73% NS; B=46.84% vs. 46.32% NS; C=64.43% vs. 56.69% p= 0.02, D=77.22% vs. 75.46% NS; E=78.17% vs. 84.73% NS).

Conclusions: Our results suggest that there could be a link between triploidy and an increasing rate of aneuploidy in the remaining normally fertilized siblings. Since the group including more patients reached statistical significance, further studies should verify whether the presence of 3PN could serve as a marker of embryonic aneuploidy and an indicator of the need for PGT-A in the rest of the cohort.

P-06

THE ROLE OF ANEUPLOIDY SCREENING IN PREIMPLANTATION GENETIC TESTING FOR MONOGENIC DISEASES

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PGT-A, PGT-M, Mosaic

Abstract: Preimplantation Genetic Testing for Chromosomal Aneuploidy (PGT-A; NGS) and Monogenic disorders (PGT-M; PCR) refers to procedures where embryos obtained through an IVF cycle are evaluated for genetic disorders prior to implantation. The purpose of these tests is very different from each other, with PGT-A identifying embryos affected by chromosomal abnormalities that will affect an embryo by chance, and PGT-M identifying embryos with certain genetic diseases or disorders and preventing those disorders being passed on to the child. In PGT-M, one or both partners have been genetically screened and identified to be carriers of a genetic disorder. Combining PGT-A with PGT-M techniques (PGT-M/A) enables genetic screening for aneuploidy and for gene-level disorders at the same time from a single biopsy, and potentially improving patient outcomes over only testing for PGT-M alone. Recently embryos with more than one cell line following PGT-A testing, mosaic embryos, are also being transferred, increasing the pool of embryos available to the patient, albeit at a significantly lowered pregnancy rate.

Superior A.R.T collected frozen-thaw embryo transfer (FET) cycle data from 2017-2019, for embryos cryo-stored after PGT-A, PGT-M, and PGT-M/A testing. The number of FETs (number of embryos transferred) was 1,294 (1,599), 24 (33) and 175 (211), the pregnancy rate (βhCG) was 68% (877/1,294), 67% (16/24) and 71% (125/175), and the implantation rate was 55% (877/1,599), 48% (16/33) and 59% (125/211) respectively.

Additionally, Superior A.R.T transferred a single mosaic embryo to each of 7 patients, using guidelines from the “PGDIS 2019 Position Statement on the Transfer of Mosaic Embryos in Preimplantation Genetic Testing for Aneuploidy (PGT-A)”. Pregnancy rate result was 43% (3/7), being 2 PGT-A patients and one PGT-M/A patient. Amniocentesis revealed no increased risk for all three patients.

The results, while not significant, indicate that use of the combined PGT-M/A can improve both pregnancy and implantation rates over use of PGT-M only. Furthermore results indicate, albeit on a very small number, that mosaic embryos can be transferred and have successful healthy pregnancies. This increases the pool of embryos available to patients for transfer.

P-07

THE IMPACT OF MALE FACTOR INFERTILITY ON THE CHROMOSOMAL STATUS OF THE PRE-IMPLANTATION EMBRYO

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Male factor infertility, PGT-A, paternal impact

Introduction: The vast majority of aneuploidies in pre-implantation embryos arise from failures in oocyte meiosis. The paternal impact on the chromosomal status of embryos is discussed controversially. Considering that even in oocyte donors the rate of aneuploid oocytes is around 20% and the rate of aneuploidy and mosaic embryos exceed the rate of euploid embryos, we are working with a strong bias when estimating a male impact. In comparison to oocytes, the rate of aneuploid spermatozoa is around 4.5%, however, with an increase of 2-6 fold in severe male factor infertility. To shed more light on the controversial topic whether reduced sperm parameter and male age would impact on the rate of aneuploid embryos in PGT-A cycles we performed a large retrospective multicenter study including more than 1000 tested blastocysts.

Patients & Methods: In this retrospective multi-center study (2016-2018) we included 298 PGT-A cycles (179 patients). Sperm parameters were evaluated according to the WHO 2010 criteria. Blastocyst culture was performed in single step culture medium cultured in an Embryoscope incubator. TE-biopsy was done on day 5-7. For WGA we used the PicoPlex (Rubicon Genomics) or the SurPlex (BlueGenome/ Illumina) platforms. NGS was processed using VeriSeq platform with data analysis by BlueFuse Multi (Illumina). To reduce for the bias of female age, we performed calculation on two groups including either women ≤ 35 years or ≥ 36 years.

Results: Comparing patients with normozoospermia to patients with reduced sperm parameters, we observed in the group of women ≤ 35 years a rate of 40.3% vs. 45.2% euploid blastocysts after TE biopsy. In the group of women ≥ 36 years the rate of euploid embryos was 19.3% vs. 23.1% in normozoospermia vs. non-normozoospermia men. Similarely, no difference in the amount of blastocysts with uniform or mosaic chromosomal abnormalities were found in these groups. Further, no impact on the number of sex chromosome aberrations was observed. In the same cohort the impact of male age on PGT-A results was analyzed. When comparing men ≤ 49 years and ≥ 50 years we found no difference in the rate of euploid blastcyosts. In the group of women ≤ 35 years a rate of 42.1% vs. 47.5% euploid blastocysts were found, respectively. In the group of women ≥ 36 years the rates for euploid embryos were 21.8% vs. 19.8%.

Conclusions: Summarizing the results, we found neither an impact of semen quality according to the WHO 2010 criteria, nor of male age on the rate of euploid blastocysts after PGT-A. This could be mainly due to huge gab in aneuploidy rates between oocytes and spermatozoa. Further, the chromosomal status of the pre-implantation human embryo could impede the analysis. In conclusion it does not seem to be advisable to perform PGT-A specifically due to male factor infertility or male age.

P-08

INCIDENTAL IDENTIFICATION OF CHROMOSOMAL STRUCTURAL REARRANGEMENTS POST-PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A)

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Preimplantation genetic testing, structural rearrangements, incidental findings

Introduction: Preimplantation genetic testing for aneuploidy (PGT-A) is routinely offered to patients undergoing in vitro fertilization treatment. Testing is commonly performed via next generation sequencing (NGS) on trophectoderm biopsy samples, providing a higher resolution than previous technologies. Euploid embryo(s) can then be selected for transfer, as those have the greatest probability of leading to a successful pregnancy. Occasionally, based on a pattern of abnormalities in a patient's PGT-A results, a chromosomal structural rearrangement (SR) or abnormality may be suspected. In those cases, karyotype or microarray is recommended for confirmation; if an abnormality is confirmed, PGT-SR can be performed in subsequent cycles. The goal of this study was to assess the frequency of incidental identification of parental SRs or abnormalities.

Materials and Methods: Analysis included a total of 28,974 PGT-A samples from 9,972 cycles received from May 2018 through December 2021. Whole-genome amplification, NGS, and data analysis were performed using the Ion ReproSeq™ PGS Kit and Ion Reporter™ software (Thermo Fisher Scientific). Results were reported as 1) no abnormal cells detected, 2) abnormal cells detected (with mosaicism percentage, if identified), or 3) did not pass quality control (QC) metrics. Cycles with suspected parental SRs or abnormalities were tallied and follow-ups were conducted with the ordering providers.

Results: Of the 9,972 PGT-A cycles, 32 cycles were suspected to have a parental SR or abnormality. Twenty-four of these suggested autosome-related abnormalities (mostly reciprocal translocations) and the remaining 8 suggested Y-chromosome microdeletions/duplications in the male partners; in certain cases, the suspicion arose after a pattern was noted across cycles for the same reproductive couple. Of the 32 cycles, 20 were confirmed by karyotype or microarray and one was refuted by normal karyotypes. Two were not confirmed due to the availability of euploid embryos. An additional 9 cases were pending karyotypes or pending reply regarding follow-up testing. In all of the cycles in which Y-chromosome abnormalities were confirmed, the findings were defined as variants of uncertain significance (VUS), likely benign, or "artifact". One additional couple without a suspected SR based on PGT-A results was later confirmed to have a Robertsonian translocation.

Conclusions: As previously known, PGT-A can result in incidental findings - mostly previously unidentified chromosomal SRs that can lead to recurrent pregnancy loss or unexplained infertility. Identifying these patients is important in order to provide them with more accurate expectations about euploid embryo yields for subsequent cycles as well as review implications for future generations and other family members. Our data shows that PGT-A can also identify chromosomal abnormalities of unknown clinical significance, particularly on the Y chromosome. These present an interesting clinical challenge, as 'aneuploid' embryos are typically not recommended for transfer but not all aneuploidy is created equal. For couples hoping to have male children, it would be difficult to argue that a predicted male embryo with a Y chromosome abnormality should not be transferred when the male partner presumably has the same finding and is healthy. In these cases, patients must weigh their preferences against the likely very low VUS-associated risks.

P-09

THE IMPACT OF TIMING OF BLASTULATION ON EUPLOIDY RATES AFTER PGT-A

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Blastocyst, Embryonic development, Preimplantation genetic testing, timing of blastulation, early blastulation

Introduction: Embryo evaluation is one of the most critical processes that affect the clinical outcome in IVF cycles. Conventional morphologic assessment, morphokinetic assessment using time lapse technology or Preimplantation Genetic Testing for Aneuploidies (PGT-A) can be performed in order to choose the most suitable embryo with the higher implantation potential. It is stated that embryos with faster developmental potential, especially early forming blastocysts, show increased euploidy rate and higher implantation potential. Our study investigated the impact of timing of blastocyst formation on euploidy rates after PGTA.

Material and methods: This study was conducted between January 2018 and December 2019 to examine the impact of timing of blastulation on euploidy rates in IVF cycles after PGT-A. PGT-A was performed due to: a) repeated IVF failure, b) advanced maternal age, c) recurrent pregnancy loss. ICSI was implemented in all cases and all blastocysts were cryopreserved with Vitrification procedure awaiting the PGT-A/ NGS result. Single Blastocyst Transfer of euploid blastocyst followed in Thawing cycle and clinical pregnancy rate was monitored. After PGT-A and NGS the embryos were divided in two categories, group A with euploid embryos and group B with aneuploid embryos. The timing of blastulation was investigated and compared between the two groups.

Results: A total of 85 cycles were included in the study and 198 blastocysts were examined after PGT-A and NGS. 69 blastocysts were included in group A and 129 blastocysts in group B. The Mean time of Blastulation in group A was 100,51 hours and in group B was 105,58 hours respectively. 37% of early blastulating (blastocyst formation on day 4) embryos were euploid and 30% of day 5 or 6 blastulating embryos were euploid indicating no statistically significant difference between the two groups ($p>0,05$). The ongoing pregnancy rate of transferring 1 euploid blastocyst is monitored in 79%. Although our primary results show no statistical significant difference between the two groups, this needs further investigation in larger randomized studies as few cases were included.

Conclusion: Our data indicate that embryos which develop earlier in blastocyst stage show similar euploidy rates as later blastulating embryos. Blastocyst morphology is a more critical factor affecting the euploidy rate as studies suggest. The 79% ongoing pregnancy rate of transferring 1 euploid blastocyst indicated the high success rates obtained in IVF after PGT-A and NGS. Additionally, the high success rates show that trophectoderm biopsy no matter how invasive it is as a method is not hazardous for the embryo viability if performed properly. In conclusion, genetic testing combined with time-lapse technology provide further information to improve IVF outcomes.

P-10

IMPACT OF CONCOMITANT PGT-A ON REPRODUCTIVE OUTCOME OF PGT-M AND PGT-SR

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PGT-M/ PGT-SR/Concomitant PGT-A/ pregnancy rate/ spontaneous abortions rate

Introduction: The application of next generation technologies to PGT-M and PGT-SR allows a concomitant PGT-A, expected to improve the reproductive outcome of PGT in patients of advanced reproductive age. The objective of this work was to investigate the reproductive outcome of PGT-M and PGT-SR performed cooncomitantly with PGT-A.

Materials and Methods: A total 762 PGT-M and 331 PGT-SR cycles were performed concomitantly with NGS-based PGT-A. Biopsy material was obtained from the Day-5 blastocysts, using mechanical or laser methods.

Results: Concomitant PGT-A improved the pregnancy rate of PGT-M by 19.6% (from 50% to 69.6%), reducing spontaneous abortions rate by more than two-fold (from 15.6% to 6.2%). Concomitant PGT-A in PGT-SR resulted in an almost two-fold increase of pregnancy rate (from 39.7 % to 66.5%) and two-fold reduction of spontaneous abortion rate (from 16.5% to 8.9%).

Conclusions: Concomitant PGT-A results in improvement of reproductive outcome of PGT-M and PGT-SR, through avoidance of transfer of unaffected embryos with aneuploidies.

P-11

CORRELATION BETWEEN BLASTOCYST MORPHOLOGY AND CLINICAL PREGNANCY OUTCOMES: A RETROSPECTIVE STUDY

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Clinical outcomes in PGT-A, blastocysts morphology, PGT clinical implication

Introduction: Aim of this retrospective study is to investigate the impact of blastocyst morphology towards clinical outcomes.

Materials and Methods: Total of 1803 single frozen embryo transfer cycles were done from January 2018 to March 2021. The number of blastocysts transferred were divided into 2 main groups, pre-implantation genetic tested (PGT) and non-PGT group. From there, it was sub-divided into 4 morphology groups using the Gardner's blastocyst scoring of AA, AB, BA and BB. This study includes day 5 blastocyst and only euploid blastocysts in the PGT group. The clinical pregnancy rates (CPR) and live birth rates (LBR) were analysed using Fisher's exact test to compare the rates between groups and considered statistically significant when *P value* <0.05.

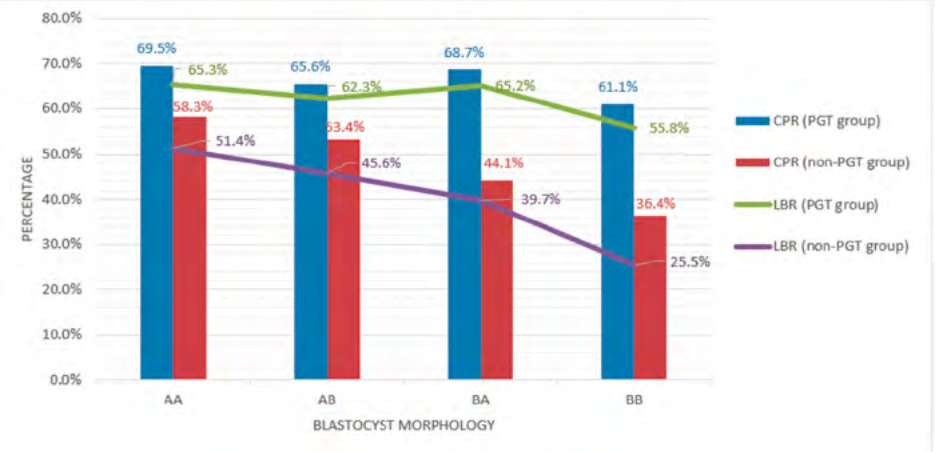
Results: A total of 941 euploid PGT blastocyst and 862 non-PGT blastocyst were transferred. Overall, PGT group shows consistently high CPR and LBR regardless on the blastocyst as shown in *Table 1*. Whereas for non-PGT group, a declining trend was seen for both CPR and LBR, shown in *Figure 1* below.

Table 1: Clinical outcomes of pre-implantation genetic tested (PGT) and non-PGT groups based on blastocyst morphology.

	PGT					Non- PGT					*P value*[a]	*P value*[b]
Blastocyst grade	No. of cases	No. of clinical pregnancy	Clinical pregnancy rates, CPR (%)	No. of live birth	Live birth rates, LBR (%)	No. of cases	No. of clinical pregnancy	Clinical pregnancy rates, CPR (%)	No. of live birth	Live birth rates, LBR (%)	(Comparisons of CPR between PGT and non-PGT group within the same morphology)	(Comparisons of LBR between PGT and non-PGT group within the same morphology)
AA	433	301	69.5	283	65.3	636	371	58.3	327	51.4	0.0002	<0.0001
AB	122	80	65.6	76	62.3	103	55	53.4	47	45.6	0.0760	0.0155
BA	115	79	68.7	75	65.2	68	30	44.1	27	39.7	0.0017	0.0012
BB	113	69	61.1	63	55.8	55	20	36.4	14	25.5	0.0031	0.0003

Note: The morphologic features of blastocysts were assessed on Day 5 before freezing and also on post thawed blastocyst before frozen embryo transfer. Clinical pregnancy rate (%) was calculated as ((No. beta positive patient with ultrasound confirmation of fetal heart)/ (No. of frozen embryo transferred) *100) while live birth rate, LBR (%) was calculated as [(No. live birth)/ (No. of frozen embryo transferred) *100]. The statistical significances between CPR of PGT and non-PGT group within the same morphology group were calculated using Chi-square test (*P value*[a]). While for the statistical significances LBR of PGT and non-PGT group within the same morphology group were calculated using Chi-square test and labelled as *P value*[b]. P<.05 was considered statistically significant.

Figure 1: Comparisons of clinical pregnancy rate (CPR) and live birth rate (LBR) versus blastocyst morphology in both pre-implantation genetic (PGT) and non-PGT group.



The CPR for PGT group is significantly higher compared with non-PGT group for all of blastocyst grade except blastocyst grade AB, which may due to the small case number. The highest CPR in PGT group is morphology AA (69.5%) followed by BA (68.7%), AB (65.6%), lastly BB (61.1%) but these differences in rates are not statistically significant between each other. Contrarily, CPR for non-PGT group is significantly higher when compared between grades AA with BB (58.3% versus 36.4%; p=0.0026), grades AB with BB (53.4% versus 36.4%; p=0.0461) and grades AA with BA (58.3% versus 44.1%; p=0.0283). Moreover, non-PGT CPR is generally lower when comparing with the PGT group even for good graded blastocysts.

The LBR for PGT group is also significantly higher compared to non-PGT group for all blastocysts morphology. The LBR for non-PGT drops from grade AA (51.4%), AB (45.6%), BA (39.7%) and BB (25.5%) while LBR for PGT group is consistently higher throughout all morphology. Within non-PGT group, the LBR for grade AA is significantly higher compared with BB (51.4% versus 25.5%; p=0.0002), as well as between grades AB and BB (45.6% versus 25.5%; p=0.0162). Whereas the differences in LBR for PGT group is not statistically significant between all morphology.

Conclusion: Transferring euploid blastocyst results in higher CPR and LBR for all morphology whereas for non-PGT group, the rates are highly dependent on the blastocyst's morphology. We can conclude that high clinical outcome is achievable by transferring an euploid blastocysts regardless of any morphology.

Additionally, we noticed that blastocysts with grade A trophectoderm in PGT group shows slightly higher clinical outcomes. This could indicate that trophectoderm morphology may give larger impact on clinical outcomes in sustaining pregnancy and reducing miscarriages. However, further study should be done to conclude this hypothesis. Yet, we still suggest to transfer euploid blastocyst with grade A trophectoderm first to achieve the highest possible success rates. Finally, grade AA should be prioritised for transfer in non-PGT group to maximise the success rates.

P-12

PDG AS AN ALTERNATIVE OF PND AND PSYCHOLOGICAL MOTIVATION OF THE PARENTS

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PGD, PND, Depression

Introduction: Couples who choose to undergo artificial reproduction techniques (ART) coupled to preimplantation genetic diagnosis (PGD) are motivated by the desire to prevent the transmission of a genetic disease to their pregnancy. PDG was developed in the mid-1980s as an alternative to prenatal diagnosis (PND) that is carried out on embryos. It has for many years been an established option for chromosomal translocation carriers, but the success rates for having a child varies with the chromosomal rearrangement, the age of the female partner and the ART center. Here, we report the psychological impacts of structural birth defects in offspring and their participation as a motivating factor of the requirements of PGD.

Material and Methods: A Tunisian couple consulted our genetic counselling at the Medical University of Sfax because they conceived during their first pregnancy a malformed offspring. This experience was followed by a parental post-traumatic stress disorder. The couple was seeking a technical solution to avoid such dramatic psychological experience.

Results: The birth defect was identified as a Patau syndrome with an extra copy of chromosome 13 derived from the adjacent-II segregation of a maternal reciprocal translocation t(13;20)(q14;q13). Genetically, this translocation can segregate during meiosis I, by alternate and adjacent-I mode giving normal/balanced gametes as products. In case of other segregation modes, an established chromosomal PND is systematically offered to detect fetal abnormality with a significant rate of accuracy. The depressed female firmly turned down this opportunity as she refused carrying again a malformed fetus (described as a monster) in her womb. During the genetic counselling, the couple was informed about the PGD, which can be offered abroad, as well as the results of this ART technique. They were very satisfied with this solution and they adhered to the ART-PGD protocol.

Conclusion: Our report concluded that there was a paucity of literature about the relation between parental psychological impacts of offspring birth defects as well as the benefits and the risks of PGD. A psychological protocol specific to the announcement of these aspects in genetic counselling should be established.

P-13

THE CORRELATION BETWEEN SPONTANEOUS BLASTOCYST COLLAPSE AND THEIR CHROMOSOMAL STATUS

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collapse, euploid, blastocyst, PGT-A, embryoscope

Introduction: Spontaneous blastocyst collapse is defined when there is a blastocyst volume reduction of ≥50% between the expanded and collapsed blastocyst state. This occurrence can be observed in-vitro and is thought to be a result of the release of blastocoel fluid due to loose cell bindings in the trophectoderm cells (TE). Several studies have reported a reduction in implantation rate when blastocysts with the event of spontaneous blastocyst collapse were transferred (Marcos et al.,2015; Sciorio et al., 2020). However, it is not known how these two events are related. Could aneuploidy be a contributing factor? This is a retrospective study to investigate the correlation between spontaneous blastocyst collapse and their chromosomal status.

Material and Methods: Five hundred and eighty-two (582) patients (age ranged 19 – 47) underwent IVF treatment with Preimplantation Genetic Testing for Aneuploidy (PGT-A) between September 2020 and November 2021 in Alpha International Women's Specialists. Following oocyte retrieval, the oocytes were fertilised and cultured individually until Day 7 using single step medium (Sage 1-Step™,Cooper Surgical,Denmark) in the Embryoscope™ incubator (Vitrolife,Sweden). Blastocyst collapse was evaluated using the EmbryoViewer™ workstation drawing tools. Blastocysts that showed the event of spontaneous collapse were categorised as Group A (Collapsed), and blastocysts without the presence of spontaneous collapse were categorised in Group B (Not-collapsed). The mean age of patients from Group A and B were 34.5 and 33.9 respectively. There was no significant difference in the mean age between these 2 groups ($p>0.05$). Blastocysts which were at least fair graded (Gardner, 1999) were biopsied and vitrified (Cryotec, Japan). The biopsied TE sample had PGT-A and the chromosomal status were determined using Next Generation Sequencing (NGS) (Ion Torrent, USA). In addition, chromosomal mosaicism of the samples was analysed using ReproSeq Mosaic PGS w1.1 workflow. Trophectoderm biopsied samples which were tested to have 20% to 80% aneuploid cells were reported as mosaic.

Results: A total of 1470 blastocysts were included in this study, of which, 159 blastocysts were categorised in Group A and 1311 blastocysts were categorised in Group B. The euploidy rate of blastocysts from Group A and B were 34.0% and 43.3%, whereas the aneuploidy rate of blastocysts from Group A and B were 42.1% and 29.4% respectively. The mosaic rate of blastocysts from Group A and B were 23.9% and 27.3% respectively. Both euploidy and aneuploidy rates between Group A and Group B were statistically significant ($p=0.0269$). However, there was no significant difference observed between the mosaic rate of Group A and B ($p=0.3950$).

Conclusions: This study shows that blastocysts which spontaneously collapsed (≥50% reduction in volume) had a significantly lowered euploidy rate and higher aneuploidy rate compared to blastocysts which did not spontaneously collapse. The increase in aneuploidy rate may have contributed to a lowered implantation rate after the transfer of collapsed blastocysts. We suggest that the presence and absence of spontaneous blastocyst collapse may be used as a selection criterion for embryo transfer. Blastocysts which did not spontaneously collapse should be prioritised for transfer particularly when PGT-A was not done.

P-14

DOES THE TIME INTERVAL BETWEEN BLASTOCYST WARMING AND EMBRYO TRANSFER AFFECT CLINICAL PREGNANCY RATES?

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Euploid FET, blastocyst warming, embryo transfer

Introduction: While the benefits and improved clinical outcome of vitrified-warmed blastocysts transfers are well-documented, there are insufficient studies that examine the influence of the time interval between blastocyst warming and single embryo transfer on clinical pregnancy rates. This retrospective study aims to gain an understanding on this matter.

Material & methods: A total of 497 single Day 5 euploid blastocyst transfers were analyzed between 2020 and 2021. Informed consents were obtained from patients for frozen embryo transfer (FET). The selected blastocysts were warmed using the Cryotec method (Japan) and incubated in Sage 1-Step culture medium (Denmark) before embryo transfer. Embryo transfers were done according to standard protocols. The time interval of blastocyst warming and blastocyst transfer were documented and categorized as follows: ≤60 minutes, 60-120 minutes, 120-180 minutes and >180 minutes. Clinical pregnancy and number of gestational sacs were observed via ultrasound.

Results: All blastocysts survived post-warmed (497/497, 100 %). Clinical pregnancy rates per embryo transfer (CPR/ET) were 71.7 % (109/152) for ≤60 minutes, 72.1 % (194/269) for 60-120 minutes, 73.5 % (50/68) for 120-180 minutes and 87.5 % (7/8) for >180 minutes. The data showed an increasing trend in clinical pregnancy rate with an extended time interval between blastocyst warming and transfer. However, there were no significant differences in clinical pregnancy rates between all groups.

Conclusions: The results of the study demonstrate that the clinical pregnancy rate is not adversely affected by the time interval between blastocyst warming and embryo transfer.

P-15

CLINICAL OUTCOME OF EUPLOID BLASTOCYST TRANSFERS WITH AND WITHOUT DAY-3 LASER ASSISTED HATCHING

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Laser assisted hatching, trophectoderm biopsy, PGT-A

Introduction: Trophectoderm (TE) biopsy for Preimplantation Genetic Testing for Aneuploidy (PGT-A) is a common practice in IVF to select embryos for transfer. There are two strategies to prepare the blastocysts for TE biopsy: 1. Adopting laser assisted hatching (LAH) to create a zona opening on Day 3, thus allowing the TE cells to herniate during blastocyst expansion and subsequently biopsied; 2. Zona opening on Day 5/6, followed by suction of TE cells for biopsy. This is a retrospective study to compare the clinical outcome of euploid blastocyst transfers with and without Day-3 LAH.

Material & methods: A total of 278 patients (age range=19-43, mean age=33.5) underwent elective single euploid frozen blastocyst transfer (SBT) in Alpha IVF & Women's Specialists Centre between January 2021 to October 2021. The transferred blastocysts were divided into two groups: 68 blastocysts had Day-3 LAH (Hamilton Thorne Bioscience, USA) prior to TE biopsy (Group A), while 175 blastocysts were biopsied without prior Day-3 LAH (Group B). All blastocysts were at least fair graded (Gardner's grading) and were biopsied either on Day 5 or 6 of development, followed by Next Generation Sequencing (Ion Torrent, USA) analysis. The blastocysts were vitrified and warmed using the Cryotec method (Cryotec, Japan). Clinical pregnancy and gestational sacs were observed via ultrasound.

Results: The clinical pregnancy and implantation rates of Group A were 60.6% (43/71) while Group B were 74.9% (155/207). The miscarriage rates of Group A and Group B were 9.3% (4/43) and 12.9% (20/155) respectively. Group B showed a significantly higher clinical pregnancy and implantation rates compared to Group A ($p<0.050$). There was no significant difference in miscarriage rate between the two groups ($p>0.050$).

Conclusions: Based on our findings, the transfer of euploid blastocysts without Day-3 LAH showed a higher clinical pregnancy and implantation rates when compared to those with Day-3 LAH. Hence, TE biopsy without prior Day-3 LAH is recommended to achieve better clinical outcomes. Nevertheless, further studies with a larger sample size should be carried out to validate these results.

P-16

A STUDY OF ANEUPLOIDY RATES DURING IN VITRO FERTILIZATION WITH PREIMPLANTATION GENETIC TESTING

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Preimplantation genetic testing, morphological grade, cryopreservation time, clinical outcomes

Introduction: The aneuploidy greatly decreases the efficiency of in vitro fertilization (IVF), which results in arrested developments, embryo fragmentation, pregnancy loss and abnormal birth defects. Maternal age and meiotic abnormalities are main contributing pathophysiological factors of chromosomal abnormality. However, even good quality embryo transfer in IVF using best morphological grades doesn't always show successful pregnancy. So confirmation of euploid embryos using preimplantation genetic testing-Aneuploidy (PGT-A) can prevent recurrent implantation failure and recurrent pregnancy loss. We aimed to study the aneuploidy of embryos with blastocyst development grade, cryopreservation time and clinical outcomes.

Material and methods: This is a retrospective study using the medical records of 138 patients who underwent IVF procedures with preimplantation genetic testing between October 2018 and November 2021 in a single IVF center. Successful ICSI cycles were examined under PGS with 24 chromosomes screening and divided into 3 groups: euploid, aneuploid and mozaic. Single embryo transfer (SET) used euploid blastocyst. All PGT-A groups were compared with the blastocyst development data and clinical outcomes.

Results: The comparison of the PGS result and blastocyst morphology grade showed an increased tendency of aneuploidy ($p<0.01$, $R= -0.82$) and mosaicism ($p<0.004$, $R= -083$) with a poor grade of blastocysts.

We have divided into 3 groups depending on time to cryopreservation as follows: upto 120 hours, from 121 to 130 hours and above 131 hours. The result showed the rate of normal chromosomal embryos decreased with the increase of time such as aneuploidy ($p<0.03$, $R= -0.9$) and mosaic ($p<0.02$, $R= -0.75$).

There is no significant difference for euploid blast transfer, between whether good or fair or poor morphology grades and implantation rates. But embryos with poor morphology grade showed decreased rate of ongoing pregnancy (72% of good grades and 60% of poor grade embryos) compared with other groups.

Conclusions: Our study result showed that the decrease of morphology grade directly correlated with decrease of euploidy, whereas decreased rate of aneuploidy and mosaic had indirect correlation. The rate of euploid embryos decreased with the increase of cryopreservation time, whereas the rate of aneuploidy and mosaicism had increased, which showed strong indirect proportionality. The comparison of normal blastocyst transfer, morphology grade and implantation rates showed non-significant results. However, there is a significant tendency for poor morphology grade and ongoing pregnancy.

P-17

FIRST PREGNANCY IN GEORGIA USING PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY AND ENDOMETRIAL RECEPTIVITY ANALYSIS

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Embryo transfer, preimplantation genetic testing, endometrial receptivity analysis

Introduction: Molecular biological technology has revolutionized assisted reproduction. Many infertile couples have conceived and delivered healthy babies through the use of two molecular biology techniques: Preimplantation Genetic Testing for Aneuploidy (PGT-A) and Endometrial Receptivity Analysis (ERA). Progress of these molecular techniques outside of Western Europe, Eastern Asia, Australia and the United States has been slow. Our case report demonstrates success using these molecular techniques for the first time in Georgia.

Material & methods: A 42 year-old patient presented for treatment of secondary infertility. Her first pregnancy (at 21 years) was complicated with preeclampsia. She delivered a healthy baby via cesarean section (CS). Upon presenting at our clinic (21 years later), she reported 2 years of secondary infertility, including one unsuccessful autologous IVF cycle with a single embryo transferred..

With us, she underwent a donor oocyte cycle (due to age and poor ovarian reserve) using trophectoderm biopsy and PGT-A with NGS. From 8 blastocysts, 4 euploid embryos were identified. Despite apparent adenomyosis, 2 euploid embryos were transferred in an frozen embryo transfer (FET) cycle. After this step, a timed endometrial biopsy was performed and sent for ERA (Igenomix). Results of the ERA reported that personalized embryo transfer (ET) should be performed 145±3 hours, after progesterone initiation.

Results: The next ET was planned according ERA result. Two euploid embryos were transferred 24 hours later than our norm. A positive β-hCG was seen 12 days after ET. One amniotic sac and one embryo was seen with ultrasound at six weeks. Pregnanc24 hours later than we typically perform FETs y progressed with no complication. At 39 weeks, a healthy female newborn was delivered by CS.

Conclusions: This first report of a live birth following the use of both PGT-A and ERA in Georgia confirms the utility for molecular biological techniques in achieving live birth, even with patients who have complicated medical history. This demonstrates that modern technology can be applied successfully in a less-affluent country where IVF is affordable.

P-18

THE LEVEL OF MOSAICISM AND CHROMOSOMAL ABNORMALITIES IN DIFFERENT AREAS OF MOSAIC HUMAN BLASTOCYSTS

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Preimplantation genetic testing, aneuploidy, mosaicism, next generation sequencing, in vitro fertilization

Introduction: Mitotic errors might lead to the formation of the cell clones with unequal chromosomal sets within the same embryo. This phenomenon has been described as embryonic mosaicism. Since the very first publications on preimplantation genetic testing for aneuploidy (PGT-A), researchers have faced a high mosaicism level in the human embryos obtained by in vitro fertilization cycles. Meanwhile, mosaicism of trophectoderm (TE) cells does not correspond to that one within the inner cell mass (ICM). The nature of this mosaicism and its high impact on embryo development draws attention to this issue. In this work, we studied the chromosome number in three different areas of the human blastocyst with mosaicism in the trophectoderm cells detected using next generation sequencing (NGS).

Material & methods: All procedures were fulfilled according to the World Medical Association's Declaration of Helsinki and approved for the research (Project №AAAA-A19-119021290033-1). Oocytes of 16 clinically healthy fertile voluntary participants with a normal karyotype at the age of 20-32 years were fertilized in vitro by sperm of 11 clinically healthy donors with a normal karyotype from the International Centre for Reproductive Medicine sperm bank. Early human embryos were cultured in COOK sequential culture media solutions for 5-6 days up to blastocyst stage and graded in accordance with Gardner classification. After morphological evaluation blastocysts of high quality were subjected to TE biopsy for PGT-A by NGS and cryopreserved by vitrification in Kitazato medium according to recommended protocol. Embryos with detected single or multiple chromosomal mosaicism in TE cells were thawed and incubated until completed expansion. Such blastocysts were sectioned in three parts with two containing TE cells and one with ICM and adjacent TE cells. Each sample was analyzed separately by NGS.

Results: Six human blastocysts with detected by PGT-A mosaicism in their TE cells were sectioned in three parts and analyzed. Our data indicate that the proportion of aneuploid cells in biopsy taken for PGT-A analysis does not necessarily reflect true chromosomal status of the whole embryo and cannot be extrapolated to that one in ICM cells. The karyotype of cells form different parts of mosaic embryos shows low concordance. Abnormalities in mosaic embryos are unpredictably diverse. Our preliminary results indicate that if mosaic chromosomal imbalance was detected in blastocyst, the probability of an euploid chromosome set in other parts is low. Notably, qualitative and quantitative characteristics of the mosaic state can be discordant between different parts of the same embryos, and in the samples containing TE adjacent to ICM the mosaicism tends to increase, which may have physiological significance for the implantation. The results of our study evidently support the conclusion that mosaicism revealed in blastocyst reduces the likelihood of finding the euploid chromosome set in the other parts of the embryo.

Conclusions: There is no absolute concordance between the level of mosaicism and the type of chromosomal abnormalities, detected in different parts of mosaic preimplantation human embryos, whereas in the samples containing TE cells adjacent to the ICM the mosaic rate tends to increase.

P-19

IS PATERNAL AGE ASSOCIATED WITH PLOIDY STATUS OF PREIMPLANTATION EMBRYO?

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Preimplantation genetic testing, aneuploidy, mosaicism, paternal age

Introduction: The impact of advanced maternal age on embryo ploidy status has been well studied but the association of advanced paternal age is confounding. This becomes an emerging issue as couples tend to delay child bearing age nowadays. Our study aimed to investigate the effect of paternal age on embryo ploidy status in young and old maternal age populations using Preimplantation Genetic Testing-Aneuploidy (PGT-A).

Material and methods: This is a retrospective review of 190 PGT-A cycles with a total of 1074 conclusive blastocyst biopsies done in our centre from July 2018 to December 2019. Only day 5/6 blastocysts which scored A and B for both trophectoderm (TE) and inner cell mass (ICM) using Gardner and Schoolcraft system were selected for biopsy. DNA libraries were constructed from biopsied TE using Ion Reproseq PGS Kit and sequenced on next generation sequencing Ion Torrent PGM™ platform according to manufacturer's instruction. Embryos with <20% abnormal cells were reported as euploid, 20-80% as mosaic, and >80% as aneuploid. The study was divided into 2 main groups according to maternal age, Group A (< 35 years) and Group B (≥ 35 years). Each group was stratified into 2 subgroups based on paternal age, Group 1 (< 40 years) and Group 2 (≥ 40 years). The mean ages are as follow: 28.6±4.3 (young maternal), 38.9±3.1 (old maternal), 34.2±3.9 (young paternal) and 45.6±5.4 (old paternal). The number of conclusive biopsies were 362, 279, 211 and 222 for Group A1, A2, B1 and B2 respectively. Fisher's exact test was used for statistical analysis, with significant value at $p<0.05$.

Results: Comparison between Group A1 and A2 demonstrated no significance difference between euploidy rates (45.3% vs 44.4%), aneuploidy rates (21.8% vs 26.5%) and mosaicism rates (32.9% vs 29.0%). However, different findings were observed for older maternal age group. Significant difference in euploidy rates (35.5% vs 24.3%, p=0.0117) and aneuploidy rates (44.5% vs 60.8%, p=0.0008) was observed in group B1 and B2 respectively. There was no significant difference in mosaicism rates (19.9% vs 14.9%) between two paternal ages in older maternal age group (p=0.2038).

Conclusions: Our results indicated that paternal age appeared to have no major impact on ploidy status in younger maternal age group. However, there is a significant decrease of euploidy rate in older maternal age group when the paternal age is ≥ 40 years. Age-related fertility decline for men may be more subtle than women but is present. The negative influence of man's aging may be compensated by maternal repair mechanisms in viable, healthy oocyte when they partnered with young women. Nevertheless, more studies should be carried out to ascertain the correlation between paternal age, aneuploidy and mosaicism.

P-20

DOES APPLYING ADDITIONAL LASER ASSISTED DRILLING DURING TROPHECTODERM BIOPSY CAUSE HIGHER MOSAIC RATE IN BLASTOCYSTS?

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Trophectoderm biopsy, mosaic rate, laser assisted drilling

Introduction: In Alpha IVF, laser assisted hatching were done on day 3 after ICSI for all pre-implantation genetic testing for aneuploidy (PGT-A) cycles. Trophectoderm (TE) biopsy techniques used were laser+pulling (L+P), laser+flicking (L+F) and flicking only(F). At the time of biopsy, an extra step of creating additional artificial opening by using laser assisted drilling may be required when (a) blastocyst has very little herniated cells; (b) inner cell mass is at the hatching point or biopsy site. Our internal study showed that biopsy using different techniques (L+P, L+F and F) does not affect mosaic rate. This prospective study was designed to evaluate the effect of additional laser assisted drilling on the mosaic rate.

Material and Methods: This study was conducted between 11th March – 19th August 2019, 443 patients had undergone oocyte retrieval and blastocyst culture up to 6 days after intracytoplasmic injection (ICSI) was done. Laser assisted hatching were done on day 3 post-ICSI. Biopsy were done on day 5 and/or day 6. Laser pulse length used during LAH and biopsy was fixed at 400ms. A total of 824 hatching blastocyst (BG5) and fully hatched blastocyst (BG6) with at least Grade A and Grade B TE quality (Gardner's grading) were included in this study. Of which were separated into 3 groups: (A)BG6 (n=79), (B) BG5 without additional additional laser assisted drilling during biopsy (n=713) and (C) BG5 with additional laser assisted drilling (n=32). The number of biopsied cells were ranged from 5-10 cells. Biopsied cells were tested using Next Generation Sequencing (Ion Torrent, USA) for PGT-A.

Results: The mosaic rates for Group A, B and C were 19.0% (15/79), 23.4% (167/713) and 39.5% (15/38) respectively. Mosaic rates of Group A and B were comparable (p=0.4807, whilst Group C had significant higher mosaic rate than Group A and B (p=0.0238 and p=0.0319 respectively). The mean age of Group A, B and C were 31.1, 31.4 and 27.1 respectively. The mean age between these 3 groups were not statistically significant (A vs B, p=0.0713; A vs C, p=0.06727; and B vs C, p=0.4408).

Conclusions: Applying laser assisted drilling during TE biopsy to create additional zona opening will produce more mosaic blastocysts.

P-21

LESSONS FROM THE 2019 GENQA PREIMPLANTATION GENETIC TESTING EQA FOR ANEUPLOIDIES AND STRUCTURAL CHROMOSOMAL ABNORMALITIES

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Preimplantation, Genomic, Testing, PGT, EQA

Introduction: Genomics Quality Assessment (GenQA) has provided external quality assessments (EQAs) for preimplantation genetic testing (PGT) EQAs for over 10 years. EQAs cover a wide range of PGT approaches; testing monogenic disorders, polar body testing, structural rearrangements and aneuploidy detection by variable methods.

The outcomes from the EQAs help identify sub-optimal testing and issues with reporting results and allows an improvement in the standard of service for couples undergoing PGT. The 2019 EQAs for PGT aneuploidy (PGT-A) and structural chromosomal rearrangement (PGT-SR) detection in blastomere and trophectoderm samples by Next Generation Sequencing (NGS) and array testing identified the over-interpretation of results with regards to mosaicism and the reporting of abnormalities below the resolution of the platforms used.

Material & methods: Laboratories were provided with three clinical case scenarios and corresponding DNA samples for each EQA and were required to perform routine analysis and submit a clinical report. The genotyping results, clinical interpretation in the context of the cases provided and clerical accuracy of the reports was assessed by a panel of assessors against peer-reviewed marking criteria and current best practice guidelines.

Results: There were 75 participants in the PGT-A and 65 participants in the PGT-SR EQA. In each EQA, 4 participants (PGT-SR 6.2% (4/65) and PGT-A 5.3% (4/75)) incorrectly reported mosaicism for both whole chromosomal and structural non-mosaic abnormalities. Furthermore, the PGT-SR EQA identified 4.6% (3/65) of laboratories inappropriately reporting abnormalities below the limit of detection of the platform used.

Conclusions: GenQA provides a service that measures the clinical accuracy of PGT results reported by the participating laboratories and helps to ensure a high standard of care. A panel of expert advisors examined the issues arising from the 2019 EQAs and provided feedback to individual participants regarding why they had arisen and how they could be addressed. This educational aspect of EQA promotes improvements to the services provided by the PGT laboratories.

P-22

CLINICAL OUTCOMES AFTER TRANSFER OF MOSAIC BLASTOCYSTS IN THE ABSENCE OF EUPLOID EMBRYOS

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mosaicism, preimplantation genetic testing for aneuploidy (PGT-a), next-generation sequencing

Introduction: The evolution of assisted reproductive technologies has been joined by the development of advanced molecular techniques for preimplantation genetic diagnosis of aneuploidies (PGT-a). This has allowed the classification of the preimplantation embryos as euploid, aneuploid and, more recently, as mosaic. Chromosome mosaicism is typically defined as the presence, in a biopsied cells sample, of two or more cell lines with different chromosome sets. Until recently, any embryo with chromosomal abnormalities was not considered for transfer. However, recent research has shown some level of success in the transfer of mosaic embryos and some pregnancies resulting in healthy babies have been reported. In the absence of chromosomally normal embryos there is a trend now to consider the transfer of mosaic embryos, however, more evidence is needed to conclude whether this is a safe and efficient approach.

Material & methods: This retrospective study aims to analyse the results obtained after transferring blastocysts diagnosed by next-generation sequencing (NGS) as mosaic. A total of 40 mosaic blastocysts were transferred into 38 patients who underwent a PGT-A cycle between October 2018 and October 2021. None of these patients had a chromosomally normal blastocyst available for transfer and thus they were counselled for mosaic blastocyst transfer. For the analysis of the results, the type, grade (>50% high, <50% low) of mosaicism and the aneuploidy involved were considered to evaluate the implantation, clinical pregnancy, and miscarriage rates resultant from the transfer of mosaic blastocysts.

Results: The clinical pregnancy rates resultant from the transfer of mosaic blastocysts (n= 38 patients, 55.26%) were statistically equivalent (p value = 0.469) to those obtained when euploid embryos were transferred (n=164 patients, 61.6%) during the same period of the study. Similarly, no differences (p value = 0.728) were found in terms of implantation rates between the group of mosaic (n=22/40, 55%) and euploid (n=120/205, 58.5%) blastocysts. Regarding miscarriage rates, no differences were found (p value = 0.707) either between groups (n=3/21, 14.28% and n=11/101, 10.9%, respectively). The implantation rate between low-grade (n=19/29, 65.52%) and high-grade (n=3/11, 27.7%) mosaic embryos showed significant differences (p value = 0.040). None of the embryos with a complex mosaicism resulted in a clinical pregnancy. Better implantation and abortion rates were observed for mosaic embryos with monosomy compared to those with trisomy. Of the 21 pregnancies established from blastocysts diagnosed as mosaics, 15 have resulted in 16 healthy babies, while 3 are still on going.

Conclusions: The results of this study confirm the reproductive potential of blastocysts diagnosed as mosaic. While research remains inconclusive about the long-term impacts of babies born from mosaic blastocyst, accumulated scientific evidence suggest that mosaic blastocysts could be considered for transfer in the absence of chromosomally normal embryos, especially those with low grade, monosomy and simple aneuploidy. However, this decision should always be very carefully counselled with the patients and with high attention given to each potential outcome.

P-23

MOSAICISM: WHAT IS HIDDEN FROM THE NGS?

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next generation sequencing, mosaicism, preimplantation genetic test for aneuploidy, FISH

Introduction: Nowadays mosaicism is one of the main problem in preimplantation genetic test for aneuploidy (PGT-A) and for interpretation in programs of assisted reproductive technologies. Next generation sequencing (NGS) enables high-accuracy identification of a wide range of chromosomal abnormalities in early embryos including mosaicism. But a certain part of chromosomal abnormalities still remains inaccessible for detection even using such novel diagnostic tools as NGS.

Materials and Methods: The study was performed in the Medical Centre IGR from 2016 to 2019 and involved 3382 embryos samples obtained from 668 women (mean age=33.7±5.4 years) who were treated with IVF/ICSI cycles. For PGT-A a trophectoderm (TE) biopsy was performed on the post- fertilization hours 120 or 144. 1134 samples were diagnosed using Ion S5 (Thermo Fisher Scientific, USA) and 2248 samples - using fluorescent *in situ* hybridization (FISH) in chromosomes 9, 13, 15, 16, 17, 18, 21, 22, X, Y.Statistical analysis was carried out using Shapiro-Wilk test for normality, T-test for arithmetical mean, Chi-square test.

Results: Among analyzed samples the following variants were identified: normal – 43.5%(n=978) and 43.7%(n=495), aneuploidy – 26.4%(n=593) and 53.7%(n=609) samples including 4.8%(n=55) of segmental aneuploidy, mosaicism – 25.8(n=580) and 1.7%(n=19) samples, polyploidy – 4.3%(n=97) and 0 samples by FISH and NGS, respectively. Results were not obtained from 0.9%(n=11) samples by NGS because of DNA degradation.

In group of embryos with mosaicism detected by FISH different combinations of nondisjunction were found. The largest number was a combination of diploid/polyploid cells(n=300) and among them, for example, diploid/tetraploid (n=178) variant may be explained by the fact that part of cells is preparing for division. This combination can also be true mosaicism and cause at least the violation in the chorionic villi formation. Variants such as disomy/monosomy/trisomy (n=35) and trisomy/monosomy (n=26) by one certain chromosome were also identified by FISH. With the use of NGS it is not possible to identify the above mentioned mosaicism variants, although it may have clinical consequences. But other combinations such as disomy/monosomy (n=128) and dysomy/trisomy (n=90) may be revealed by both FISH and NGS methods (n=12 and n=7, respectively). Data analysis showed that proportion of normal embryos is the same in studies using different methods of diagnostics, but the proportion of aneuploid and mosaic embryos demonstrated statistically significant difference (SSD)($p<0,001$).

Interesting that the average number of nuclei from one TE sample analyzed by FISH was different in embryos with different types of chromosomal status that may depend on embryo morphological quality. There was no SSD found between the cell number of normal and mosaic samples (7.5±2.9vs.7.4±3.2,p=0.489), but aneuploid (7.5±2.9vs.5,6±2.9,p=0.0001) and polyploid (7.5±2.9vs.4,7±2.5,p=0.0114) had SSD compared to normal samples.

Conclusions: Based on these results we can summarize that among embryos defined as euploid by NGS there are some that carry mosaicism as well as among the embryos designated as normal by FISH there are aneuploid embryos because of limited chromosome number of the FISH panel. So, the most expedient approach for the embryos selection is to combine several methods for the comprehensive study of embryo's genetic parameters.

P-24

RELATIONSHIP BETWEEN THE QUALITY OF TROPHECTODERM CELLS AND THE RATE OF MOSAICISM

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Mosaicism, TE quality, TE biopsy, PGT-A

Introduction: The incidence of embryo mosaicism in IVF has been a widely discussed topic in recent years. Previously, we reported that the use of different biopsy techniques during trophectoderm (TE) biopsy did not affect the mosaicism rate (Chan et al. 2020). Moreover, the euploidy and aneuploidy rates among different blastocysts quality were studied (Wang et al. 2018). However, the correlation between mosaicism and TE quality was not widely studied. Hence, we sought to determine if the quality of TE cells affect the rate of mosaicism in this study.

Materials and methods: This retrospective study was conducted from January 2017 to November 2021 in Alpha IVF & Women's Specialists Centre, assessing the relationship between mosaic rate and TE quality. A total of 16,985 blastocysts with a mean age of 32.4 (age range: 17.0 - 47.0) were divided into 3 groups: 3,549 blastocysts were categorized in Group I (TE grading A); 12,211 blastocysts in Group II (TE grading B) and 1,225 blastocysts in Group III (TE grading C). The TE cells were graded according to the Gardner's grading system as: Grade A - many cells forming a cohesive layer; B - few cells forming a loose layer and C - few large cells. Biopsy techniques such as lasering, pulling and/or flicking were used to perform TE biopsy on day-5 and/or day-6 of development for Preimplantation Genetic Testing for Aneuploidies (PGT-A) with Next Generation Sequencing (NGS) (Ion Torrent, USA). Biopsied samples were amplified and DNA libraries were constructed according to manufacturer's specifications. Chromosomal mosaicism was analyzed using ReproSeq Mosaic PGS w1.1 workflow. Biopsied samples with 20-80% of aneuploid cells were considered mosaic according to PGDIS guideline (PGDIS, 2016).

Results: From the pool of blastocysts that were biopsied, the mosaic rate for Group I, II and III were 16.4% (581/3549), 19.6% (2393/12211) and 20.5% (251/1225) respectively. This data shows a significantly lower mosaic rate in Group I when compared to Group II (p= 0.0001) and Group III (p= 0.0012). However, no significant difference was seen when comparing between Group II and Group III (p= 0.4768).

Conclusion: In this study, it was shown that blastocysts with grade C trophectoderm cells show a higher mosaicism rate in comparison to TE grade A and B.

P-25

TESTING MULTIPLE EMBRYO SAMPLES IN THE PGT-A FOLLOW-UP STUDY PROVES MOSAIC NATURE OF SEGMENTAL ANEUPLOIDIES

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PGT-A, mosaicism, segmental aneuploidy, NGS

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P-26

EMBRYO QUALITY IS A DETERMINANT CRITERION FOR SELECTING THE MOST ELIGIBLE MOSAIC FOR TRANSFER

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mosaicism, preimplantation genetic testing for aneuploidy (PGT-a), next-generation sequencing, embryo quality, implantation

Introduction: Several studies show the benefit of transferring mosaic embryos when there are no euploid embryos to transfer, and they still result in ongoing pregnancies and what is more important is that they result in healthy babies.

Studies and guidelines suggest prioritizing mosaic embryos based on maternal age, chromosomes impacted, grade of mosaicism, number of chromosomes involved, type of mosaic (segmental vs complete, monosomy vs trisomy) but embryo quality is never part of these criteria.

Studies claim that mosaic implantation rate is lower than euploid embryos, but they never show if both populations are comparable in terms of quality.

Material & methods: This is a retrospective observational study performed in a private centre between February 2018 and February 2021. The study includes the data analysis of 264 euploid blastocysts and 41 low risk mosaic blastocysts (defining low risk regarding chromosome syndromes and under 50% grade of mosaicism). All transferred in single embryo transfer (SET) to 299 patients after PGT-A (mean maternal age 38,9 years).

PGT-A with NGS technology was offered to patients of advanced maternal age and/or with repeated IVF failures. Trophectoderm biopsies were performed on day 5 and/or day 6 embryos, with laser assistance.

Blastocyst morphology was scored in 3 groups: A: excellent (AA, AB, BA), B: good (BB), C: average and poor-quality embryos (BC, CB, CC). (Gardner-Schoolcraft classification)

Low risk mosaic embryo transfer was offered to patients with no euploid embryos to transfer.

Results: We found no significant differences between both populations (euploid and mosaic embryos) in terms of embryo quality (Chi^2 p-value =0,681) so we were able to compare the overall implantation of similar quality populations.

Despite euploid implantation being higher as described in most studies, no statistical differences (Chi^2 p-value = 0,308) were found in terms of implantation rates between mosaic (51,2%) and euploid (60,2%) blastocysts during the same period. There are no differences between the mean age of both groups (39,7 vs 38,8 years, respectively).

The implantation rates for euploid blastocysts were 78,7% (n=47), 63,4% (n=164) and 33,9% (n=53) in the A, B and C blastocyst quality groups, respectively, showing high significative differences among the three groups.

The implantation rates of low-risk mosaic blastocysts were 85,7% (n=7), 50,0% (n=28) and 16,7% (n=6) in the A, B and C blastocyst morphology groups, respectively, showing also still significant differences despite the small population. (Chi^2 p-values according to implantation: Euploid =0,000; Mosaic=0,045)

We have also compared the three quality categories between both populations showing no significative differences (Chi^2 p-values according to quality: A=1,000; B=0,209; C=0,653), concluding that same quality embryos behave the same way despite being euploid or mosaic.

Conclusions: Embryo quality has always been a strong biomarker to predict implantation, and this is also true for mosaic embryos as well. We aim to demonstrate that embryo quality is determinant for selecting the most eligible mosaic for transfer.

We cannot compare implantation potential of euploid embryos with mosaic embryos without describing both populations in terms of quality. This simple concept should be considered in future mosaic research publications.

P-27

NON TRANSLOCATED CHROMOSOMAL ANEUPLOIDY IN PREIMPLANTATION GENETIC TESTING FOR STRUCTURAL REARRANGEMENTS (PGT-SR) CARRIERS

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PGT, Translocation, Aneuploidy, Robertsonian, Reciprocal

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P-28

COMPLEX CHROMOSOMAL REARRANGEMENT AND PGT-SR

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Complex Chromosomal Rearrangement (CCR), PGT-SR, fertility, sperm-FISH

Introduction: Complex Chromosomal Rearrangement (CCR) carriers are at high risk for chromosomally unbalanced pregnancies, which may lead to recurrent miscarriages or affected offspring. The nature of each rearrangement, including the number of chromosomes and breakpoints involved, have significant implications on the percentage of unbalanced gametes following meiotic segregation of CCRs. Thus, prior knowledge and detailed characterization of such rearrangements in CCR carriers can lead to the utilization of assisted reproduction technologies, such as In Vitro Fertilization (IVF) and Preimplantation Genetic Testing for structural rearrangements (PGT-SR), in order to prevent the transmission of unbalanced chromosomal rearrangements to the offspring.

Materials and methods: A number of cytogenetic and molecular methods, including chromosomal G-banding analysis, Fluorescence In Situ Hybridization (FISH), sperm-FISH, and whole genome sequencing (WGS), were previously employed to investigate a CCR family with fertility problems, an affected male and non-affected female offspring, and phenotypically normal parents. The non-affected daughter of age 35, at the time of investigation, underwent controlled ovarian stimulation and IVF for three independent cycles yielding only two 5-day trophectoderm biopsies in each cycle. PGT-SR was carried out using the 24Sure+ array platform.

Results: Initial chromosomal G-banding analysis and FISH identified a rare paternally-transmitted three-way CCR [t(6;7;10)(q16;q34;q26)] in a phenotypically normal father and daughter. Further sperm-FISH investigations in the father, analyzing the possible segregation patterns of the derivative chromosomes, revealed that only two combinations out of sixty-four (3.1%) result in balanced gametes. Subsequently, a cryptic chr6 breakpoint was identified by WGS, thus creating an interstitial chr6 segment which was translocated on der(10). This additional complexity suggested that the number of possible segregation patterns is even higher, and consequently, the percentage of resulting balanced gametes becomes even lower. Because of the complex nature of the paternally-inherited CCR, and after genetic counselling, the daughter opted for IVF and PGT. PGT-SR was carried out and revealed abnormal CCR derivative profiles in all six biopsies examined. In the case of a normal/balanced result in PGT, prenatal diagnosis should have been performed to rule out unbalanced rearrangements as a result of the additional breakpoint and possible recombination at the resulting interstitial segment, which are beyond the resolution of array or NGS.

Conclusion: The current study presents a rare case where a phenotypically inconsequential CCR is stably transmitted from a fertile male carrier to his daughter. Collectively, results highlight the importance of investigating CCRs using a combination of conventional and molecular based techniques in order to delineate their full complexity. Combining diagnostic testing in CCR carriers with the use of PGT-SR along with appropriate reproductive genetic counseling can be a valuable tool for couples who are affected and undergo IVF procedures. In cases with very complex rearrangements and unfavorable PGT-SR results alternative fertility options, could be recommended to CCR carriers to achieve a normal pregnancy/child.

P-29

PGT-M BY EXCLUSION ON A FAMILY WITH HIGH RISK FOR PRNP PRION DISEASE

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Familial prion disease, Gerstmann-Sträussler-Scheinker syndrome, PRNP gene, PGT-M by exclusion

Introduction: A couple with a history of familial prion disease, Gerstmann-Sträussler-Scheinker syndrome (GSS) which is autosomal dominant, was referred for PGT-M. The father of the perspective mother had died of the disease. Molecular analysis revealed the presence of the five octapeptide repeat insertion mutation in the PRNP gene.

After counseling, the couple requested PGT-M by exclusion since the prospective mother did not wish to know her carrier status.

Material and Methods: A six-point linkage-based assay was originally designed in silico and tested on the family. Four informative STR sites were identified (2 proximal and 2 distal to the gene) and the assay was validated using single cells (lymphocytes) before implementation.

Two IVF cycles were performed within a period of two years and, in both cycles, day-5 trophectoderm biopsies were taken and analyzed. Biopsied embryos were cryopreserved until use.

Results: The first cycle identified two out of seven embryos at low risk for prion disease, since they had not inherited a grand-paternal (high-risk) allele. Both were transferred resulting in biochemical pregnancy which was lost.

In the second cycle eleven embryos were tested and six were identified as low-risk. Embryo transfer resulted in a twin pregnancy with healthy delivery. Supernumerary low risk embryos are kept in cryopreservation.

Conclusion: We have successfully designed, set up and applied a novel bespoke PGT-M assay for familial prion disease - PRNP associated Gerstmann-Sträussler-Scheinker syndrome (GSS). The success of the assay prompted other members of the family to express interest in having PGT-M.

P-30

HOW EFFECTIVE IS DAY 3 CLEAVAGE-STAGE BIOPSY FOR PRE-IMPLANTATION GENETIC TESTING FOR MONOGENIC DISEASE?

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cleavage, blastocyst, biopsy

Introduction: To carry out preimplantation genetic testing for monogenic disease (PGT-M), trophectoderm (TE) biopsy has become increasingly common and has replaced cleavage-stage biopsy in many centres. Trophectoderm biopsy allows more cells to be safely removed from the embryo making genetic testing more robust. However, fresh embryo transfer may not be possible due to time required for diagnosis, and freezing/thawing could potentially reduce the efficacy of this technique. The aim of this retrospective analysis was to evaluate the effectiveness of day 3 cleavage-stage biopsy for PGT-M compared to current results for TE biopsy in our centre.

Material and Methods: Retrospective analysis of data was carried out for 110 cycles of PGT-M between 2013 and 2018 using day 3 cleavage-stage biopsy. A comparison was made with results achieved from 32 cycles of PGT-M using TE biopsy (Day 5/6) on fresh embryos carried out between 2019-2021. Insemination was by ICSI for all cycles. Transfer of an unaffected blastocyst was performed fresh on day 5 for cleavage-stage biopsy and after freezing/thawing for all TE biopsy cases and all subsequent frozen embryo transfer (FET) cycles. Genetic analysis was performed using whole genome amplification (Repli-G mini kit by Qiagen) and polymerase chain reaction for subsequent haplotyping and genome analysis.

Results: For cleavage-stage biopsy, a median (range) of 12 (2-25) oocytes were collected; 10 (2-21) oocytes injected and 6 (0-16) embryos biopsied (84%) on day 3. Of the 110 cycles, there were 46 clinical pregnancies giving a rate of 42% per oocyte recovery or 49% (46/94) per embryo transfer. In 16 cycles (14.5%), there was no embryo transfer: 2 due to failed fertilisation; 5 due to poor embryo development; and 9 due to no genetically suitable embryos for transfer. In 45% (49/110) of cycles, there was vitrification of surplus embryos on day 5/6 and following FET's the cumulative clinical pregnancy rate (per cycle with at least one transfer) was 73% (69/94) (up until December 2021).

For TE biopsy, a median (range) of 9.5 (1-23) oocytes were collected, 9 (0-18) oocytes injected and 4 (0-9) blastocysts biopsied (54%). Of the 32 cycles, 9 had no embryos to transfer: 3 due to failed fertilisation/no oocytes to inject; 1 due to no embryos suitable to biopsy; and 2 due to no embryos genetically suitable for transfer. Three cycles had embryos suitable for transfer but have not yet had an FET. The remaining 23 cycles have had at least one FET and the cumulative clinical pregnancy rate was 70% (16/23) (up to December 2021).

Conclusion: This observational study demonstrated excellent clinical and cumulative pregnancy rates using cleavage-stage biopsy for PGT-M, with cumulative pregnancy rates as good as TE biopsy. This supports the use of cleavage-stage biopsy as an effective and alternative strategy for performing PGT-M, especially in centres where there may be issues performing TE biopsy. This study includes a low number of fresh TE biopsy and FET cycles, and further data would be needed to assess which biopsy protocol is more effective.

P-31

ANALYSIS OF THE TANDEM STATUS OF MICRODUPLICATION COPY NUMBER VARIANTS FOR PREIMPLANTATION GENETIC TESTING

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CNVs, PGT, tandem

Introduction: Although the mutational spectrum of microduplications has been studied, the organization of copy number variants (CNVs) is still not clear. In a paucity of studies microduplications were generally reported as tandem in direct orientation while the rest were adjacent and insertional duplications. Since analysis of CNVs for preimplantation genetic testing (PGT) require linkage analysis, location of the microduplication is essential in order to prevent misdiagnosis.

Aims: To analyze the “tandem status” of patients that required PGT for microduplication CNVs in our unit.

Materials and Methods: For all patients requiring PGT for microduplications since the year 2017, analysis of the tandem vs non tandem orientation was evaluated by FISH analysis of metaphase cells from the CNV carrier.

Results: 29 CNV carrier patients (18 females and 11 males) were evaluated for tandem status. 23(79%) CNVs were recurrent microduplications (RCM), and 6(21%) were non-recurrent microduplications (NRCM). Among the RCM: 22q11.2 (30%); 16p11.2 (17%); 1q21.1; 7q11.23; 15q11.2; 16p13.11; 17q12; Xq28. The NRCM were: 6p21.1, 7q11.22, 7q33, 21q22.3, Xq13.1, Xq22.2.

All the tested microduplications were found in tandem, a CNV state which is compatible with linkage analysis for PGT.

Conclusions: While in the literature there is debate regarding the tandem status of microduplications, we found that all of our cases were tandem. Testing of the tandem status is important mainly for rare CNVs in order to prevent misdiagnosis by misapplication of linkage analysis for PGT. FISH testing of a larger cohort of microduplication-bearing samples is needed in order to confirm our results.

P-32

IDENTIFICATION OF SMALL SEGMENTAL ANEUPLOIDIES IN HUMAN PREIMPLANTATION EMBRYOS

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segmental aneuploidy, microdeletion, below detection limit; microarray

Introduction: Preimplantation genetic testing for aneuploidy (PGT-A) of all 24 chromosomes has become a common practice to improve implantation and clinical pregnancy rates in patients undergoing *in vitro* fertilization. Array comparative genomic hybridization (aCGH) and next generation sequencing (NGS) enable identification of whole chromosome aneuploidy and large chromosomal abnormalities. For prenatal and postnatal diagnosis, both techniques are also utilized for the detection of submicroscopic chromosomal imbalances – microdeletions and microduplications. In contrast to the 30-80% risk of sporadic whole chromosome aneuploidy that depends on maternal age, large segmental aneuploidies are independent of maternal age but observed in ~10-15% of embryos subjected to PGT-A. These segmental aneuploidies include *de novo* segmental gains and losses seen in ~10% of blastocysts, and genomic imbalances resulted from a known or yet undiagnosed chromosome abnormality in the parents. Preimplantation genetic testing for structural rearrangement (PGT-SR) is recommended to such couples, however, ~40% of coupes with recurrent miscarriages and infertility have normal karyotype analysis despite the presence of a structural rearrangement. Moreover, *de novo* disease-causing submicroscopic chromosome imbalances affect ~0.3% of newborns and are likely more prevalent among preimplantation embryos. Current technologies are limited to identification of chromosomal imbalances larger than 5-10 Mb in size. Embryos affected by cryptic pathogenic aberration that are below current limits of detection are routinely implanted.

Materials and methods: The 60K CGH GenetiSure microarray platform (Agilent Inc., Santa Clara, CA) has been utilized for PGT-A using the Cytogenomics "Single Cell Aberration Method" for detection of large aberrations (>10 Mb in size). Screening for segmental copy number variations was performed on 200 embryos from 59 couples negative for a whole chromosome and mosaic aneuploidies. In addition, 18 embryos from 4 couples referred for PGT-SR were evaluated for segmental aneuploidies resulted from the parental structural rearrangements as well as *de novo* deletions/duplications. Seven microdeletion-positive DNA samples were amplified from suspensions containing 5-10 cells derived from lymphocyte of individuals carrying microdeletions ranging from 0.6 to 5 Mb in size. To evaluate the sensitivity in detection of microdeletion conditions, the microdeletion-positive samples were tested by aCGH utilizing the "Single Cell Small Aberration Method" designed for CNV evaluation that are less than <10 Mb in size.

Results: Large *de novo* segmental aneuploidies were observed in 20/200 (10%) embryos from PGT-A group and 2/18 (11%) embryos from PGT-SR group ranging from 5.5 Mb to 160 Mb in size. Using the "Single Cell Small Aberration Method" analysis, all aberrations were detected among microdeletion-positive samples. Screening of embryonic samples for the known disease-causing CNV revealed a *de novo* pathogenic 1.4 Mb deletion 17p12 in a single embryo.

Conclusions: Segmental imbalances in the embryos and fetuses have a significantly lower potential for implantation leading to a diagnosis of idiopathic infertility or discovered by karyotype or microarray analysis after a miscarriage. Detection of microdeletions and duplications associated with human pathologies may improve the odds of *in vitro* fertilization procedure and reduce incidence of microdeletion syndromes.

P-33

NEXT-GENERATION SEQUENCING ENABLED CHROMOSOMAL ANALYSIS FOR 2MB OF CHROMOSOMAL SEGMENT IN PREIMPLANTATION EMBRYOS

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NGS, PGT-SR, FISH

Introduction: The chromosome analysis for preimplantation genetic testing for structural rearrangement (PGT-SR) has changed from fluorescence in situ hybridization (FISH) to next-generation sequencing (NGS). It has been reported that accurate chromosome analysis cannot be performed for small chromosomal segments (10 Mb or less) using NGS, compared to FISH, which is easy to diagnose using telomere probes.

Here, we report a reciprocal translocation couple who has 120 kb and 2.57 Mb of chromosomal segments could obtain a baby by PGT-SR using the modified NGS method.

Case: A 37-year-old woman, gravida 5 para 1. In her daughter with developmental delay, the chromosome was diagnosed as normal by G-banding, but duplication of the long arm of chromosome 17 (17q25.3) was detected using FISH. Reciprocal translocation of chromosomes 2 and 17 in one of the couple was revealed by FISH, suggesting that the microduplications in their offspring were derived from parental translocation. Preliminary chromosome analysis using NGS was carried out towards PGT-SR for the couple after four miscarriages. Accurate chromosome analysis of the offspring using microarray method revealed a copy number loss of 120 kb in the short arm of chromosome 2 and a copy number gain of 2.57 Mb in the long arm of chromosome 17. It was difficult to detect unbalanced translocations by conventional NGS analysis with 1 million leads and 1 Mb reading window. Modified NGS, which increased the number of reads to 4 million and reduced the reading window to 100-500 kb, allowed unambiguous detection of the 2.57 Mb duplication of chromosome 17, but failed to detect a small deletion in chromosome 2. The preliminary results showed that NGS could be used for PGT-SR in this case. PGT-SR was conducted after approval from the Japan Society of Obstetrics and Gynecology. Six blastocysts were first analyzed using conventional NGS. Two of the three blastocysts with balanced chromosomes had aneuploidy, and the remaining three were determined to have unbalanced chromosomes due to adjacent Ⅰ. Additionally, modified NGS was performed on 4 blastocysts, excluding 2 aneuploid blastocysts, to verify the results obtained using conventional NGS. The modified NGS showed that microduplication of chromosome 17 was clearly detected in all unbalanced blastocysts, and no deletion or duplication was detected in one blastocyst to be diagnosed as balanced. The balanced and euploid blastocyst was transferred, leading to pregnancy.

Conclusion: Adjustment of the analysis conditions in NGS allowed the diagnosis of a small chromosomal segment of about 2 Mb in PGT-SR. Before conducting PGT-SR for small chromosomal segments, it is necessary to conduct preliminary inspection to confirm that accurate analysis using NGS is possible.

P-34

SUCCESSFUL PREGNANCY OF A RARE NON-CLASSIC CONGENITAL ADRENAL HYPERPLASIA PATIENT AFTER PREIMPLANTATION GENETIC TEST

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PGT-M, NCAH, CYP21A2, IVF, pregnancy

Introduction: Non-classic congenital adrenal hyperplasia (NCAH) due to 21-hydroxilase deficiency is autosomal recessive disorder characterized by androgen excess. The vast majority of NCAH patients seeking medical attention have 21-hidroxylase (21-OH) deficient type associated with CYP21A2 mutations. Over 200 CYP21A2 mutations have been reported and approximately 10 of them account for the majority of affected alleles. Here, we describe a successful pregnancy, after IVF cycle and preimplantation genetic test, of a NCAH patient presenting with one heterozygote deletion of the gene CYP21A2 plus a rare substitution in the promoter region of the gene (-102 G>A), and a partner who also has one heterozygote mutation in the same gene (Q318X).

Material and Methods: The couple presented with one-year of infertility and seeking IVF treatment and PGT for monogenic disease (PGT-M) for CAH or NCAH. She (31 yo) was in use of corticoid, presented regular menstrual cycles and had NCAH diagnosis confirmed by genetic test, with a heterozygote deletion in CYP21A2 gene plus a rare substitution in the promoter region of the gene, -102 G>A. He (32 yo) was also tested and a heterozygote mutation (Q318X) was detected. A direct diagnosis of Q318X mutation and an indirect diagnosis using informative Small Tandem Repeats (STRs) were optimized for the couple. Two independent diagnoses were applied to each embryo during PTG-M. The causative mutation detection was performed by Primer Chain Reaction (PCR), and the mutant site was interrogated using the MiniSequencing Reaction. A total of six STRs (RING3, TAP1, D6S1666, TNFa, D6S265 and D6S1683) were found surrounding the gene CYP21A2 and used as indirect diagnosis. The STRs were chosen if contained a tetranucletide repetition core, linked to the gene (upstream or downstream) and having the highest heterozygosity value. Genomic DNA were extracted from peripheral blood from the couple and both sets of parents and STRs were classified as being informative (both couple heterozygotes), partially informative (one of the couple homozygote and the other heterozygote) or not informative (both homozygote).

Results: Two ovarian stimulation cycles were performed to optimize the number of embryos for PGT-M. It was obtained a total of 6 blastocysts, that were biopsied and frozen. Four embryos were identified as carrier of two mutations (maternal and paternal), one embryo was carrier of only one maternal mutation, and one was carrier of both maternal mutations and the wild paternal allele. The combined PGT-A study revealed four euploid embryos, one mosaic aneuploid embryo and one triploid embryo. Artificial endometrial preparation was performed for the transfer of two euploid male carrier embryos. Pregnancy was achieved and confirmed around 6 weeks of pregnancy with transvaginal ultrasound, showing implantation of both gestational sacks but only one of them with heart bit. The baby was born elective C-section at 39 weeks of gestation.

Conclusion: Genetic testing and counseling are important for women with NCAH. The association of assisted reproductive technologies with the use of PGT can help this couples to have healthy babies. To identify severe mutations and investigate the partner is an essential part of the treatment.

P-35

A COMPREHENSIVE PRE-IMPLANTATION GENETIC TEST FOR ALPHA AND BETA THALASSEMIA DISEASE

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PGT-A, PGT-M, thalassemia

Introduction: In Vietnam, frequency of alpha-thalassemia carriers, beta-thalassemia carriers and both alpha-thalassemia and beta-thalassemia carriers are 2.1%, 1.4% and 0.03%, respectively. For couples who come to PGT-M as a treatment for healthy offspring, carriers with both alpha and beta thalassemia mutation may want to screen for all detectable mutations for the best embryo selection. We have developed a test which is able to detect alpha-thalassemia and beta-thalassemia mutations in one single biopsy. Besides, the PGT-A test could also be performed on the same biopsied sample.

Material and methods: Biopsied samples from Day-5 embryos underwent Whole genome amplification reaction (WGA) using PicoPLEX WGA Kit (Takara Bio USA, Inc.).

For beta-thalassemia mutations detection, specific primers were designed for amplification of coding region of HBB gene and six interested STRs which meet the requirement of heterozygosity > 0.3 and located within 1Mb flanking the HBB gene.

For alpha-thalassemia mutations detection, two chosen STRs were located in deletion regions of α^{SEA} mutation. In addition, specific primers for GAP-PCR were also designed as the second strategy for α^{SEA} mutation detection, which help to give the final call in some complicated cases.

gDNA of reference samples (including parents and siblings/relatives) and WGA product were used as template for targeted PCR. Interested STRs were run on Capillary Electrophoresis system while other PCR products and WGA product were undergone library preparation step (Nextera XT DNA Library Prep Kit, Illumina, Inc.) followed by sequencing on Miseq system. Bluefuse Multi software was used for CNV calling (for PGT-A test), Miseq Reporter software was used for SNPs genotyping and STRs were analyzed by GeneMapper ID-X software.

Results: We reported here one case of three embryos in which the father carries both α^{SEA} thalassemia mutation and CD26 beta-thalassemia mutation, the mother carries α^{SEA} thalassemia mutation. PGT-M test resulted in one embryo with heterozygous of α^{SEA} mutation and CD26 mutation, two embryos with heterozygous of α^{SEA} mutation and no CD26 mutation detection. In this case, no proband child's DNA was provided but linkage analysis may be performed between three embryos for allele-dropout detection. This result was combined with PGT-A result for the selection of embryo transfer.

Conclusions: We have successfully designed a PGT-M test which is able to combine with PGT-A test and simultaneously detect alpha-thalassemia and beta-thalassemia mutation, allows the best decision for embryo transfer.

P-36

ONE CASE OF PREIMPLANTATION GENETIC TESTING FOR MARFAN'S SYNDROME IN VIETNAM

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Marfan's syndrome, FBN1, PGT-A, PGT-M, single nucleotide polymorphism

Introduction: Marfan's syndrome (MFS) is a systemic disorder of connective tissue caused by mutations in the fibrillin 1 gene (FBN1) which is located on the chromosome 15q21 and encodes the extracellular matrix protein fibrillin-1. The disorder is inherited in an autosomal dominant pattern, which means one copy of the affected gene is sufficient to cause the disease. We reported here a case of a couple in which the husband is a carrier of nonsense FBN1 mutation c.3585T>A (Cys1195Ter) and was diagnosed with Marfan's syndrome, underwent an IVF cycle combined with preimplantation genetic testing (PGT).

Material and methods: Ten embryos was first screened for aneuploidy with PGT-A test. Euploid embryos were then performed the PGT-M test that allowed us to select unaffected embryos for transfer. For this PGT-M test, specific primers were designed to amplify the FBN1 mutation and interested single nucleotide polymorphism (SNP) which is located as far as 200kb upstream and 200kb downstream of FBN1 gene. Only heterozygous SNPs of the husband were selected for linkage analysis.

Embryo biopsy was performed on day 5 followed by whole genome amplification (WGA) reaction using PicoPLEX WGA Kit (Takara Bio USA, Inc.). WGA product was used as starting material for PGT-A test and also for amplification of the mutation and appropriate SNPs. Genomic DNA samples of the parent were extracted from peripheral blood and were used for amplification of the mutation and the SNPs as well. WGA and PCR product then were used for library preparation using Nextera XT DNA Library Prep Kit. Sequencing was performed on Miseq Sequencer (Illumina, Inc.). Raw data was analyzed by Bluefuse Multi software for CNV calling and Miseq Reporter software was used for mutation detection and SNPs genotyping.

Results: The PGT-A results showed that six out of ten embryos was aneuploidy. For that reason, 4 euploid embryos were tested for c.3585T>A mutation. By the use of SNPs near the FBN1 gene for linkage analysis, PGT-M test showed one unaffected embryo and three affected embryos. The unaffected euploid embryo was selected for transfer that resulted in ongoing pregnancy.

Conclusions: We have succesfully performed a case of PGT-M test for Marfan's syndrome that was able to combined with PGT-A test. This combination helped to reduce the cost and the turnaround time of the PGT test for Vietnamese patient.

P-37

NGS FOR ALL TRANSLOCATIONS? CUSTOMIZED ANALYSIS ALGORITHMS ENABLE DETECTION OF SEGMENTAL CHROMOSOMAL IMBALANCES

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PGT-SR, Aneuploidy, Translocation, NGS, Microarray

Introduction: PGT for the detection of chromosomal rearrangements using Next Generation Sequencing (NGS) has been applied with a resolution of ≥ 5-20 Mb, which is the declared detection limit of commercially available kits. However, still some patients carry chromosomal rearrangements below the detection limit. Therefore, utilization of a customized analysis approach is required for the effective use of this technology for the detection of a broad range of chromosomal imbalances. The aim of this study is the detection of relatively smaller unbalanced chromosomal segments (≥1 Mb) that are below the manufacturer's recommended resolution limits.

Material & methods: PG-Seq Kit 2.0 (PerkinElmer) was used for the NGS library generation using genomic DNA samples of 4 patients with unbalanced chromosomal rearrangements. Sequencing was performed on MiSeq (Illumina) platform and the data were analysed using PG-Find Analysis Software. Six small unbalanced chromosome segments ranging from 0.5 Mb to 4.5 Mb previously detected with microarray analysis were included in this study. Three of the patients carrying these chromosomal rearrangements generated products of familial subtelomeric translocations and one patient had a product of familial paracentric inversion. Reanalysis of some samples, for which the standard analysis fails to detect target rearrangements, was performed by altering the filtering parameters in order to focus on target regions in depth. Each samples was evaluated in the framework of the size of the segmental gain/loss (Mb), copy number of each segment, concordance with the microarray results and different analysis algorithms.

Results & conclusions: NGS detection capacity of unbalanced segments that are previously identified with microarray analysis was 7 out of 8 (87.5%). 6 out of 8 (75%) variants were ≤4.5 Mb and the rest were 8.6 Mb and 17.9 Mb (Table 1). One of the variants was very small (0.5Mb) and could not be detected with NGS. Evaluation of the individual data quality demonstrated that the optimum number of reads for the detection of segmental gains/losses ≥1 MB was 500000 per sample. This study shows that altering analysis algorithms makes NGS a qualified tool for the detection of unbalanced chromosome rearrangements ≥1 Mb. Further studies can be designed by lowering the sample size to 24 in order to increase the number of reads per sample, which may enable the detection of chromosomal imbalances bellow 1 Mb. Moreover, further studies are also required using PGT samples to detect the detection limit of NGS in this context.

Microarray Result	CNV Size	Standard Algorithm	Customized Algorithm
arr[GRCh37] 18q23(76577645-78012829)x1 arr[GRCh37]3q26.33q29(179847885-197837049)x3	1,4 Mb 17,9 Mb	No Yes	Yes Yes
arr[GRCh37] 22q11.21(18894835_21505417)x1 arr[GRCh37] 3q11.1q11.2(93575285_94728404)x4	2,6 Mb 1,15 Mb	No No	Yes Yes
arr[GRCh37] 22q11.1q11.21(17397498_18628078)x1 arr[GRCh37] 22q11.21q11.22(18661724_23208022)x4	1,2 Mb 4,5 Mb	No No	Yes Yes
arr[GRCh37]4p16.3p16.1(72447_8732731)x1 arr[GRCh37] 19p13.3(259395_770382)x3	8,6 Mb 0,5 Mb	Yes No	Yes No

Table 1: Detection of CNVs using customized analysis algorithms is presented.

P-38

SUCCESSFUL PRE-IMPLANTATION GENETIC TESTING USING KARYOMAPPING FOR INFANTILE NEUROAXONAL DYSTROPHY TYPE 1 (PLA2G6 C.2098C>T)

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infantile neuroaxonal dystrophy type 1, embryo selection, haplotyping, karyomapping, pre-implantaton genetic testing for monogenic disease (PGT-M)

Introduction: Infantile neuroaxonal dystrophy (INAD) type 1 or neurodegeneration with brain iron accumulation type 2B is caused by mutation in PLA2G6 gene on chromosome 22q13.1. It is inherited in an autosomal recessive fashion. The patients with homozygous genotypes have spasticity, cerebral and cerebellar atrophy, progressive psychomotor regression, seizures, nystagmus and optic atrophy with the onset in their first 2 years of life (OMIM #256600, #610217). Pre-implantation genetic testing (PGT) is an alternative to traditional prenatal diagnosis (PND) giving couples at risk of having affected offspring a chance to start with a pregnancy with disease-free baby. Recent karyomapping assay is useful in providing information of monogenic genotyping and chromosome analysis at the same time. This study aimed to perform and compare karyomapping and DNA analysis for PGT-M of PLA2G6 in clinical PGT.

Material and methods: A family experienced two pregnancies affected with INAD came through for genetic counselling and PGT treatment. They decided to join the project following thoroughly counselling and informed consent was obtained. Their first child died at the age of 7 and their second child started having symptoms at the age of 3. Whole exome sequencing (WES) revealed PLA2G6 (NM_003560) c.2098C>T mutation. DNA of their second child was used as reference in karyomapping assay. The patient underwent routine IVF procedures. Embryo biopsy was performed on Day-5 post-fertilization and the biopsied trophectoderm underwent whole genome amplification. SNP array with karyomapping analysis was carried out for haplotyping and copy number variation (CNV). Novel multiplex fluorescent PCR with mini-sequencing protocol was developed and tested for mutation analysis to compare with karyomapping results. Polymorphic markers linking to PLA2G6 gene i.e. D22S272, D22S276, D22S279 and D22S282 were also analyzed for back up linkage analysis results and contamination identification.

Results: Nine healthy embryos were subject to PGT. Karyomapping results showed 2 normal, 6 heterozygous and 1 affected embryos. Fortunately that all embryos were chromosomally balanced (4 male and 5 female). Mini-sequencing and four linked polymorphic markers analyses were concordant to karyomapping results. No foreign DNA contamination were detected. One normal male embryo with good morphology was transferred and ongoing singleton pregnancy was resulted.

Conclusions: In this study, we present the success of clinical PGT-M cycle of new mutation, PLA2G6 c.2098C>T, using karyomapping in a family suffering with INAD in their two children. Novel multiplex fluorescent PCR using mini-sequencing and four linked polymorphic markers were developed to validate karyomapping assay. Karyomapping was proved to be quick, convenient, accurate and useful for monogenic haplotyping and chromosome analysis in the embryos. There is no need to develop new protocol for every new disease or new mutation. Time and expenses can be saved from new PGT protocol development. The family can be assured that their offspring will be spared from not only monogenic disease but also chromosome abnormality.

P-39

PRE-IMPLANTATION GENETIC TESTING BY KARYOMAPPING FOR POLYCYSTIC KIDNEY DISEASE (ADPKD) USING EMBRYOS AS REFERENCES

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haplotyping, karyomapping, pre-implantaton genetic testing for monogenic disease (PGT-M), autosomal dominant polycystic kidney disease (ADPKD), mini-sequencing

Introduction: Autosomal dominant polycystic kidney disease (ADPKD, OMIM 173900) is caused from mutation within PKD1 gene on chromosome 16p13.3. Subjects with heterozygous mutation have renal cysts, liver cysts and intracranial aneurysm. Most dangerous complication is end-stage kidney failure. Pre-implantation genetic testing (PGT) is an alternative to traditional prenatal diagnosis (PND) for couples to start a pregnancy with a disease-free baby. In this study, haplotyping using aSNP and karyomapping analysis was performed alongside with novel PCR protocol using mini-sequencing and microsatellite analyses for clinical PGT-M of PKD1.

Material and Methods: A family suffering and at risk of having offspring affected with ADPKD came through for genetic counselling and PGT treatment. They decided to join the project following thoroughly counselling and informed consent was obtained. The husband and his sister were both affected with ADPKD and carried novel deletion in exon 15 of PKD1 gene, NM_001009944 (c.4902delG), therefore, she was planned to be reference in haplotyping analysis. The patient underwent IVF procedures. Embryo biopsy was performed on Day-5 post-fertilization and biopsied trophectoderm underwent whole genome amplification. SNP array with karyomapping analysis was carried out for haplotyping and copy number variation (CNV). Novel multiplex PCR with mini-sequencing protocol was developed and tested for mutation analysis to compare with karyomapping results. Informative polymorphic marker, D16S475, linking to PKD1 gene was also analyzed for back up linkage analysis results and contamination identification.

Results: Twenty two embryos with good quality were biopsied on Day 5 and subjected to PGT. During karyomapping analysis, haplotypes of reference DNA, husband's sister, turned out to be total identical to him, consequently her DNA was not informative for using as reference in karyomapping assay. In this case, mini-sequencing analysis results has become useful, one of the normal and one of the affected embryos were used as references for karyomapping assay. Karyomapping results revealed 7 normal, 11 affected and 4 with intragenic recombination embryos. Mini-sequencing and microsatellites analyses for linkage analysis gave concordant results in all embryos. In addition, karyomapping demonstrated two embryos with abnormal chromosomes, i.e. 46,XX/45,XX -22 and 45,XX -19. Therefore, five normal (two male and three female) embryos with balanced chromosomes were fulfilled for transfer. Polymorphic marker analysis showed no extraneous DNA contamination.

Conclusions: Clinical PGT-M cycle using karyomapping incorporating with mini-sequencing was performed for a family suffering from ADPKD and at risk of having ADPKD offspring. Karyomapping provides a universal haplotyping analysis for monogenic disorders and chromosome balance information at the same time. DNA of a member of the family is needed as informative reference for haplotyping analysis. However, in this case the only reference was not informative because the reference and her brother shared identical alleles from their parents. In case of absence of informative reference, one of the embryos with known genotypes from DNA analysis can be used as reference. Both karyomapping and DNA analysis help in confirming each other's results and omit the risk of misdiagnosis from allele drop out (ADO) of the traditional DNA analysis.

P-40

PREIMPLANTATION GENETIC TESTING IN SLOVENIA: 15 YEARS' EXPERIENCE AT THE UMC LJUBLJANA

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PGT, chromosome structural rearrangement, monogenic disease, clinical outcome

Introduction: Preimplantation genetic testing (PGT) has been available in Slovenia since 2004. We conducted a retrospective study to present clinical outcomes and the development of techniques and strategies in the PGT program in couples with chromosome and monogenic diseases in Slovenia from 2004 to 2018.

Materials and methods: Couples carriers of chromosome structural rearrangement or monogenic disease were eligible for PGT. After the stimulation, the retrieved oocytes were fertilized by IVF or ICSI, the latter being always used in cycles for monogenic diseases and NGS-based 24-chromosome screening. First, two blastomeres were biopsied from a cleavage-stage embryo on day three, and one or two unaffected embryos were transferred on day five. In these cycles, FISH analysis for chromosome structural rearrangements or sex selection in X-linked disorders were performed, while multiplex PCR with linkage analysis and mutation detection assay was used in monogenic disease testing. In 2017, we implemented a blastocyst biopsy and NGS based 24-chromosome screening. All biopsied embryos were cryopreserved until the results of PGT were available, and a single unaffected embryo was transferred, as appropriate.

Results: A total of 178 couples were treated at our institution from January 2004 to December 2018. The most frequent indications for PGT were single gene disorder and chromosome rearrangement (82 and 85 couples), followed by aneuploidy screening and sex selection in X-linked diseases (11 and 10 couples). There were 332 PGT cycles with oocyte retrieval. An embryo transfer was possible in 216 cycles and resulted in 74 clinical pregnancies. Altogether, the clinical pregnancy rate per cycle and embryo transfer was 21% and 31% in years 2004-2016 (blastomere biopsy cycles), and 25% and 43% in years 2017-18 (blastocyst biopsy cycles), respectively. Sixty-five unaffected children were born, while 15 pregnancies ended in spontaneous miscarriage (13%). No cases of misdiagnosis or adverse neonatal outcomes were reported.

Conclusions: Our results are comparable to the most recent ESHRE PGD Consortium data published and demonstrate that PGT is an efficient and reliable tool. The PGT has improved reproductive care in couples with chromosome rearrangements and monogenic diseases in Slovenia.

P-41

NOVEL ISSUES IDENTIFIED THROUGH TWELVE YEARS OF EXTERNAL QUALITY ASSESSMENTS FOR PGT FOR MONOGENIC DISORDERS

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Preimplantation, Testing, PGT, EQA, Monogenic

Introduction: Genomics Quality Assessment (GenQA, previously UK NEQAS for Molecular Genetics), has provided external quality assessments (EQAs) for preimplantation genetic testing for monogenic disorders (PGT-M) for over 12 years. Eight disorders including recessive, dominant and X-linked diseases, incorporating triplet repeat disorders have been provided and has identified common and novel issues which will be described to promote accuracy PGT.

Material & methods: The EQA format follows a PGT case and is provided as two stages. In stage one, DNA samples for a “mock” couple wishing to undergo PGT are distributed along with an “affected” family member. Laboratories are required to perform a feasibility study to determine if they can offer PGT, detailing the testing strategy to be employed. Stage 2 requires the testing of cells which mimic embryos. Laboratories can test either single cells (blastomere) or a set of six cells (trophectoderm). Fully interpretative reports and results for markers used are required. The genotyping result, clinical interpretation and reporting are assessed for each stage, using peer-reviewed marking criteria.

Results: Pilots were delivered in 2008 and 2009 for cystic fibrosis. In 2011, performance criteria were applied and the first accredited EQA delivered to 15 laboratories participated. Participation has significantly increased over the years, with 68 laboratories participating in the 2021 EQA. A common concern has been the understanding of informativity of markers and the reporting of misdiagnosis rates. The delivery of triplet repeat EQAs identified differing approaches to analysis, either including direct repeat testing or only indirect marker testing, both of which raised difficulties. The move from hg18 to hg19 highlighted the issues associated with positioning of markers, in particular for Huntington disease. The 2014 EQA witnessed the implementation of SNP genotyping as a testing strategy and the subsequent change from testing blastomere cells to trophectoderm cells which has continued through the years bringing new challenges to the laboratories.

Performance issues remain as evidenced in the 2021 EQA with two laboratories (3%) reporting incorrect results for embryo testing and four laboratories (6%) reporting interpretation errors.

Conclusions: GenQA has been providing PGT-M EQAs for over 12 years. By alternating the disorders and different modes of inheritance various aspects of testing has been addressed and learning outcomes highlighted to ensure the correct embryo results are reported. Whilst the incidence of genotyping/interpretation errors remains low, they are reported annually, demonstrating the need for continual EQA participation.

P-42

EXPANDING APPLICATION OF NON-DISCLOSURE PREIMPLANTATION GENETIC TESTING (PGT) FOR A LARGER SPECTRUM OF MONOGENIC DISORDERS

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Non-disclosure PGT-M, Expanding applications, Huntington's disease, Early-onset Alzheimer's disease, Breast Cancer

Introduction: Non-disclosure Preimplantation Genetic Testing (PGT) is an option for those at-risk for an autosomal dominant disorder who do not wish to know their own status but would like to reduce the risk of their embryos inheriting a given disease. Non-disclosure presents the opportunity for those at-risk to reduce it without determining their disease status. It was first introduced in for Huntington's disease (HD). However, there has been recent interest from patients and providers for additional indications that utilize non-disclosure PGT. The purpose of this study was to review the use and outcome of patients who underwent non-disclosure PGT.

Methods & Materials: Here we present a retrospective analysis of identified a total of 13 conditions in which non-disclosure PGT had been pursued.

Results: We performed 210 PGT cycles for HD, with the transfer of 263 genetic predisposition-free embryos, yielding 104 clinical pregnancies and birth of 101 children. In 107 of these cycles patients preferred not knowing the actual diagnosis of their own genotypes, thus choosing non-disclosure PGT-M. However, non-disclosure PGT-M is no longer restricted to HD, and has now been applied to 12 other conditions: Amyotrophic Lateral Sclerosis (2 cycles), Spinocerebellar Ataxia Type 3 (3 cycles), Spinocerebellar Ataxia Type 6 (2 cycles), Hereditary Breast and Ovarian Cancer syndrome (6 cycles), Cerebral Autosomal Dominant Arteriopathy and Subcortical Infarcts with Leukoencephalopathy (CADASIL; 1 cycle), Charcot-Marie-Tooth disease 1A (1 cycle), Early-onset Alzheimer's disease (2 cycles), Gerstmann–Sträussler–Scheinker syndrome (2 cycles), Myotonic Dystrophy (1 cycles), Multiple Endocrine Neoplasia Type 4 (3 cycles), Myofibrillar Myopathy (2 cycles), and GRN-related frontotemporal dementia (1 cycles).

Overall, 133 non-disclosure PGT-M cycles were performed for 95 patients, and resulted in the transfer of 146 unaffected or genetic predisposition free embryos in 123 cycles (1.19 embryos per transfer on the average), resulting in 79 clinical pregnancies (64.2% pregnancy rate) and birth of 73 unaffected or genetic predisposition free children.

Conclusions: The data demonstrate the expanding application of non-disclosure PGT-M, including late-onset disorders, hereditary cancer predispositions and conditions with variable expressivity.

P-43

PREIMPLANTATION GENETIC TESTING FOR MENDELIAN CONDITIONS (PGT-M) STRATEGIES FOR VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS)

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PGT-M, Variants of Uncertain Significance (VUS), Genetic Counseling

Introduction: Advances in genomic testing strategies including Next Generation Sequencing (NGS) and Whole Exome Sequencing (WES) have increased the number of Variants of Uncertain Significance (VUS) that are identified by molecular testing and presented for PGT-M. VUS poses clinical challenges in interpretation of clinical significance and subsequently reproductive options including PGT-M. We present our strategies for undertaking PGT-M for VUSs.

Methods& Materials: Retrospective review of PGT-M cases and development of processes for undertaking PGT-M where a VUS was identified.

Results: Of a total of 44 VUS cases presented, 36 were accepted for PGT-M and 8 declined, due to inadequate genotype/phenotype correlation in the context of family history. For cases that were accepted for PGT-M, a letter of support and recommendation were required from the referring provider as well as documentation of prior genetic counseling regarding the molecular finding. The data show the importance of clinical history and additional family member testing in assessing feasibility of PGT-M. In cases of dominant inheritance, VUS may segregate in affected and unaffected family members, supporting the decision for PGT-M, while in the others, when VUS is identified in children and one of their parents, PGT-M is not required, because the patient and parents' phenotype did not correlate adequately with the expected phenotype of the gene identified. However, in the majority of cases VUSs require additional interpretation and correlation with family and clinical history to determine if PGT-M is an appropriate option.

Conclusions: The above framework for considering PGT is utilized for VUS patients in our experience, which is based on a system of clinical and technical review to determine feasibility of PGT-M on a case by case basis depending on molecular findings.

P-44

DETECTION OF HETEROZYGOUS WHOLE EXON DELETIONS IN EMBRYOS USING QF-PCR

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QF-PCR, STR haplotyping, CNV, de novo, PGT-M

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P-45

COMBINED PGT-A AND PGT-M FOR ABCA3 GENE MUTATION IN A SINGLE BIOPSY

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preimplantation genetic testing, PGT-A, PGT-M, single gene disorder, monogenic disease

Introduction: ATP binding cassette subfamily A member 3(ABCA3) is critical for synthesis of pulmonary surfactant. This surfactant spreads across the lung tissue and facilitates breathing. Mutation in ABCA3 gene leads to pulmonary surfactant metabolism dysfunction (OMIM #610921). This autosomal recessive disease causes severe, often fatal respiratory problems in infants. A healthy male offspring was reported after transferred an unaffected embryo by using combined preimplantation genetic testing for aneuploidy (PGT-A) and monogenic disease (PGT-M) for ABCA3 gene in a single biopsy.

Material and methods: A couple with ABCA3 gene mutation underwent infertility treatment in TMC Fertility at Thomson Hospital Malaysia in July 2019. Female patient is heterozygous c.737C>T (p.Pro246Leu), while her partner is heterozygous c.3364G>A(p. Glu1122Lys). They previously had an affected child who inherited both mutations. Informed consents were obtained from the couple after explaining to them about the flow of procedures and its' limitations. Controlled ovarian stimulation with antagonist protocol was performed. Intra-cytoplasmic sperm injection was performed on 20 matured oocytes. Eleven blastocysts were biopsied and vitrified. Whole genome amplification (WGA) of the embryo biopsied cell was performed with Ion SingleSeq™ kit (Thermo Fisher Scientific). For PGT-A analysis, the WGA libraries were processed and sequenced on next generation sequencing Ion Torrent PGM™ platform according to manufacturer's instruction. Embryos reported as euploid and transferable mosaic were further tested with PGT-M. For PGT-M analysis, genomic DNA was extracted from peripheral blood of the couple and affected child with QIAamp DNA Mini Kit (QIAGEN). The purified WGA libraries of embryos, and genomic DNA from the parent-child trio were further processed by PGDSeq kit (Bioarray SL). Flanking sequence of the ABCA3 mutation region was target amplified, purified and quantified individually to construct PGT-M libraries. The PGT-M libraries were then pooled in equimolar amount, isothermal amplified and enriched before sequenced on same platform. Sequencing raw data was processed by Ion Torrent Suite™ Server, with Homo sapiens hg19 as reference genome for alignment, a target BED file to define regions of interest and a hotspot BED file to define regions of known mutations. Plugin "coverageAnalysis" and "variantCaller" were launched to generate coverage data, and to detect the single nucleotide polymorphism (SNP), respectively.

Results: Out of 11 PGT-A tested embryos, 6 were reported as euploid or transferable mosaic. Among the 6 embryos, 1 was reported as unaffected, 4 as heterozygous, and 1 as affected after PGT-M. The unaffected embryo was transferred. Single intrauterine gestational sac was detected by ultrasound scanning during 6 weeks of pregnancy. According to the information given by the patient, prenatal diagnosis indicated that the fetus had an unaffected genotype. A healthy baby boy was delivered by cesarean section at 39 weeks of gestation without any complication.

Conclusions: The combined of PGT-A and PGT-M technique has minimized the risk of embryo damage by performing single biopsy on the same embryo. Likewise, this combined technique has resulted a healthy male offspring which free of hereditary disease.

P-46

SOLVING A DE-NOVO MICRODELETION PGT-M CASE BY COMBINING LONG AND SHORT RANGE PCR WITH NGS

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PGT, de novo variant, microdeletion, NGS, SNPs

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P-47

INTRODUCING CELLS FROM URINE AS RELIABLE ALTERNATIVE TO BUCCAL CELLS IN PRECLINICAL EVALUATION OF PGT-M

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PGT-M, single cell evaluation, monogenic disease

Introduction: The targeted amplification for Preimplantation Genetic Testing for monogenic diseases (PGT-M) established in our laboratory relies on multiplex PCR for the co-amplification of the pathogenic variant and suitable genetic markers (here short tandem repeats; STRs). Fragment length analysis and mini-sequencing allow for the determination of low- and high-risk haplotypes in the preclinical work-up. Each individually developed assay is validated using 50 single- or few-cell samples. Buccal cells have previously been employed for single cell evaluation in PGT-M. Yet, due to the Covid-19 pandemic isolating those cells poses an additional risk factor of potential infection for medical staff. In search of a reliable and lower-risk alternative, we assessed cells isolated from urine.

Material & methods: Single or few cells were isolated from 10-20 ml fresh urine samples by centrifugation (200×g, 5 min) followed by 3 washing steps of the cell pellet in DPBS. The cells were resuspended in 1-2 ml DPBS and 20 µl of the solution were transferred under mineral oil-covered BSA droplets (10 mg/ml) in a culture dish. Individual cells were picked using a PGD stripper under an inverted light microscope, lysed and amplified using the established multiplex PCR assays in our laboratory.

Results: Ample quantities of cells with normal epithelial-like morphology were obtained from urine samples. As proof of principle-study, 36 urine (u) cells from 3 individuals were analyzed using a STR-based testing protocol targeting 10 STR loci in the vicinity of the *CFTR* gene. Compared to a previous experiment using 54 buccal (b) cells accuracy (u: 99.7%; b: 100%) and amplification efficiency across loci (u: >97.2%; b: >96.3%) were highly concordant. The allele dropout (ADO) rates varied between experiments (u: 0-8.3%; b: 0-2.8%), but were still in compliance with the ESHRE good practice recommendations for PGT-M. Having passed the initial quality criteria, we proceeded with a preclinical work-up for the *PKHD1* locus comprising 50 urine cell samples from 2 individuals. Analysis revealed an accuracy of 100%, amplification efficiencies >98% and ADO rates <4% when evaluating 11 STR markers and 2 pathogenic variants.

Conclusions: Compared to buccal cells, isolation of cells from urine is less time-consuming, follows a simpler protocol and provides a lower risk of potential infection with Covid-19 for the medical staff. Taken together with the convincing results of our initial studies, single or few cells isolated from urine represent a robust alternative source to buccal cells for assay validation in the preclinical work-up of PGT-M.

K.O-M and L. W. contributed equally.

P-48

PILOT STUDY OF MITOCHONDRIAL DNA QUANTIFICATION IN ANEUPLOID AND EUPLOID EMBRYOS AFTER PGT-M

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PGT-M, WGA, mtDNA,

Objective: Many studies have performed that standard morphological evaluations of embryos cannot provide a reliable prediction of chromosome status. Therefore, this study is focused on relationship between mitonchodrial DNA (mtDNA) quantification to maternal age, sex of embryo, chromosomal aneuploidy and implantation rate.

Material and Methods: Trophectoderm (TE) was biopsies from embryo blastocysts and performed by whole genome amplification (WGA) using multiple displacement amplification (MDA). The DNA of TE samples were subjected to PGT-M analysis to exclusion of monogenetic diseases. Embryos, classified without monogenetic disorders, were subsequently analysed by next generation sequencing (NGS) to aneuploidy analysis and mtDNA quantification.

Results: The patients undergoing IVF cycle were divided into five age groups (22-26, 27-30, 31-34, 35-39 and 40-48) and in which total 769 blastocysts were generated. Influence of maternal age on embryonic development via mtDNA level was not significantly proven. Embryos were evaluated as 58 % euploidy (448) and 42 % aneuploidy (321). It was establish that chromosomally abnormal blastocysts tended to contain significant larges amount of mtDNA (approximately double) compared to euploid blastocyst. The relationship between sex of the embryo and mtDNA quantity was also examined and no significant difference in mtDNA levels and between male (390) and female (379) embryos did not observed. Furthermore, 140 embryos were selected and observed for dependence of mtDNA level on implantation rate. In 33 case were confirmed pregnancy 21 gravidities based on UZ (mtDNA *P=0,0009)* and 12 biochemical gravidities (mtDNA *P=0,0093)*; implantation rate was not known in 107 embryos. Every sixth embryo underwent successful implantation.

Conclusion: In this study, several factors of embryonic development associated with change of mtDNA levels were demonstrated. MtDNA quantification in embryos have provided conclusive data for great potential for in vitro fertilization (IVF).

P-49

PREIMPLANTATION GENETIC TESTING (PGT) FOR MONOGENIC DISEASES

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PGT-M, In vitro fertilization (IVF), Monogenic disorders, Short tandem repeat (STR), Minisequencing

Introduction: Preimplantation Genetic Testing is an assisted reproductive technique that enables us to perform genetic analyses at the embryo stage. Several embryos are generated through the IVF procedure and those that are free from targeted genetic disorders (i.e. unaffected embryos) are selected for transfer into the uterine cavity. Preimplantation genetic testing serves as an important alternative to prenatal diagnosis in the sense that, it prevents the transmission of single gene disorders to the newborn and enables the couples to give birth to a healthy child. Additionally, the technique also allows the birth of a potential donor for stem cell transplantation for the affected sibling, when coupled with HLA matching.

Material and Methods: In our center, we have performed preimplantation genetic testing for 781 couples within a time period of 5 years (between Jan 2017-Dec 2021). In all cases, the parents are either affected or carriers for a specific disease caused by an alteration in a single gene. Before the PGT procedure, a preclinical set-up study was first conducted. Conventional PGT-M utilizes short tandem repeat (STR) sequences which require knowledge of the targeted genetic locus and custom design of primers for polymerase chain reaction (PCR) amplification. The amplification of STR marker regions was performed in a fluorescent multiplex PCR, which is followed by a minisequencing reaction for the detection of mutation specific to the disease.

Results: Within a 5-year period, 781 couples underwent PGT procedure and a total of 5909 embryos were screened in order to identify the healthy and unaffected ones suitable for transfer. In 106 out of 781 couples, HLA matching was also performed in combination with the mutation analysis. Overall, we were able to diagnose 192 different monogenic diseases. The most commonly studied diseases among these were hemoglobinopathies (beta-thalassemia, sickle cell anemia), SMA (spinal muscular atrophy), cystic fibrosis, Down syndrome and DMD (Duchenne muscular dystrophy). Preimplantation genetic testing of monogenic diseases (PGT-M) is technically challenging, since extremely small amount of biomaterial is used (i.e. only 1 cell for blastomere biopsy), which in turn, increases allele dropout (ADO) rates, contamination and failed amplification (FA) events. For this purpose, a two-round nested PCR technique was established to increase the efficiency and specificity from a very low template DNA material. Minisequencing technique was applied successfully in all cases for the detection of mutation, by designing primers specific to the mutation in concern. Furthermore, in addition to performing linkage analysis by using the STR marker regions in proximity to the mutation site, maternal contamination markers were also used in all cases in order to rule out possible contamination events during embryo biopsy.

Conclusions: Preimplantation genetic testing provides an efficient and accurate means of diagnosing any monogenic disease in the embryo stage and ensures the birth of healthy offspring by determining the unaffected embryos, as long as the genetic basis of the disease is identified in the couples. Use of the minisequencing is proved to be a relatively simple, cost-effective and more specific technique for mutation analysis compared to other methods being used.

P-50

PROVIDING PREIMPLANTATION GENETIC TESTING FOR MONOGENIC DISORDERS (PGT-M) TO PATIENTS USING DONOR OR POSTHUMOUS SPERM

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PGT-M, Sperm, Donor, Karyomapping, Posthumous

Introduction: PGT-M performed by linkage analysis is based on the identification of informative markers spanning the "defective" gene (upstream: 5', intragenic, downstream: 3') causing a monogenic disorder. This method requires parental DNA to be analysed alongside embryo biopsies in order to provide a diagnosis. Parental DNA is usually derived from a population of diploid cells such as lymphocytes present in peripheral blood or saliva. Accessibility to paternal peripheral blood or saliva might not be possible in the case of a patient pursuing PGT-M using donor sperm, or sperm from a deceased partner. Unlike diploid cells that contain the same recombination events throughout the whole population, each sperm cell has a different recombination event, meaning that the DNA profile in the male partner's sperm sample may be different to that of his somatic cells. The feasibility of performing PGT-M by linkage analysis using DNA from a population of haploid sperm cells as a source of paternal DNA was investigated.

Materials and methods: The use of sperm DNA was validated retrospectively on 10 PGT-M cases (33 embryos) that were originally analysed using paternal DNA derived from blood. Each case was re-analysed using DNA derived from sperm from the corresponding man.

DNA from both sources was extracted (QIAamp mini kit, Qiagen) and PGT-M conducted using the validated Infinium Illumina SNP arrays (Karyomapping). Interpretation of results was performed with BlueFuse Multi software where the Single Nucleotide Polymorphism (SNP) genotyping call rate was used to perform quality control (QC) and informative SNP markers were used to diagnose the embryo for the monogenic disorder and to detect chromosomal abnormalities.

Results: The average number of relevant informative SNP markers was comparable between the blood and sperm DNAs (5': 34 vs 33, intragenic: 3 vs 3, 3': 40 vs 40, respectively). The average call rate was also similar for DNAs extracted from either source (98% blood DNA vs 97% sperm DNA). Based on these results, monogenic disease diagnosis was correctly determined in all cases using sperm DNA (33/33 embryos, 100% concordance), when compared to blood DNA. In total, 10 different genomic regions were analysed (MLH1, CFTR, OCRL, FBN1, HBB, DMPK, C1CAM, BRCA1, TBX5 and PKD1), one region per case, with the exception of HBB which appeared in two cases. Additionally, all chromosomal abnormalities (16/16 types, 100% concordance) detected using blood DNA were also correctly identified when performing analysis using sperm DNA.

Conclusions: We demonstrate the first use of haploid sperm as an accurate alternative to diploid lymphocytes as a source of paternal DNA needed to conduct PGT-M by Karyomapping. This is a crucial step towards providing better access to vital PGT-M for patients undergoing IVF treatment with a sperm donor or for posthumous use, something that may otherwise prove impossible. There is no reason to suspect regions other than those tested would have a differing result, but caution should be observed.

P-51

MSOME OF TERATOZOOSPERMIA COUPLED TO INTRACYTOPLASMIC MORPHOLOGICALLY-SELECTED SPERM INJECTION AND PREIMPLANTATION GENETIC DIAGNOSIS

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IMSI, PGT

Introduction: Macrocephalic spermatozoa syndrome with multiple tails (MSS) was first described in 1977 in its classical form (type I) which comprises a combination of oligoasthenoteratozoospermia as well as sperm heads of increased size with irregular shapes and multiple flagella. Molecular cytogenetics reveals chromosomes disorders in spermatozoa with tetraploidy. Molecular genetics reveals the presence of a microdeletion at the exon 3 of AURKC gene in the majority of type I forms. Three recurrent other mutations in MSS were also described in AURKC gene. Here, we report the case of an infertile couple who consulted our genetic counselling for MSS type I and its management by specific artificial reproduction technique coupling motile sperm organelle morphology examination (MSOME) and intracytoplasmic morphologically selected sperm injection (IMSI).

Material & methods: The couple was referred to us for genetic exploration after twelve years of male infertility and the failure of two attempts of intracytoplasmic sperm injection (ICSI). This couple was then addressed abroad for a management by MSOME-IMSI.

Results: The 43-year-old male had an extreme oligospermia (less than one million spermatozoa / ml) with a monomorphic teratospermia. In fact, 90% of spermatozoa were macrocephalic with multiple tails. The sperm migration test allowed us to select 10% of normal forms. The two precedent ICSI attempts failed because of a default of fertilization in the first attempt and a failure of implantation in the second (transfer of an only obtained embryo). Several semen analyses done abroad confirmed the partial form of the MSS of our patient. During this recent attempt, subnormal spermatozoa were selected by MSOME and used for IMSI. This resulted in two embryos of poor quality. Only one of the two embryos could continue its development until the moment of the transfer. A tentative of preimplantation genetic diagnosis (PGD) took place but the DNA of the biopsied embryo showed obvious fragmentation stopping any exploration. Unfortunately, after transfer, no pregnancy could be obtained. Molecular study of the AURKC gene by PCR simplex done in our laboratory, confirmed the c.144delC microdeletion in the homozygous state in our patient. According to the literature, correlation between SSM type1, c.144delC, tetraploidy and contraindication of reproductive technologies (ART) are already documented but after several sessions of genetic counseling, the couple still refuses to abandon the project of conceiving their own progeniture and continues to seek ART. In this context, we suggested sperm exploration using flow cytometry and fluorescence in-situ hybridization (FISH) to estimate the percentage of tetraploid spermatozoa. These investigations may contribute to delay the significant psychologic impact of this infertility and its harmful prognosis.

Conclusions: Careful selection of normal-looking spermatozoa by MSOME has previously been evaluated in several AURKC c.144delC deleted patients. FISH analyses performed on selected spermatozoa of these patients showed that aneuploidy was constant. In contrast, in MSS patients type II, the prognosis is better. In fact, the rate of euploid spermatozoa must be assessed to estimate the rate of MSOME-IIMSI-PGD success.

P-53

NON-INVASIVE PGT-A (NIPGTA) RELIABILITY USING DIFFERENT TECHNIQUES FOR CHROMOSOMAL ANALYSIS

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niPGT-A, cell free DNA,spent medium

Introduction: An ideal progress for PGT-A would be to bypass the embryo biopsy step and predict euploidy through noninvasive methods. Different studies reported variable success and concordance rates. Discordances may be due to embryonic mosaicism, preferential elimination of aneuploidy cells, DNA contamination and the technique used for DNA analysis. The niPGTA efficacy has been restricted by technical complications associated with the low DNA quantity and quality, presenting technical challenges for genetic analysis. Low abundance and poor integrity DNA seem to be more successfully overcome by some strategies of amplification and genetic analysis than others. Therefore, amplification technology adopted, as well, the chromosomally genetic test used could be key for the reliability of niPGTA. The aim of this work was to evaluate whether the accuracy of niPGT-A could be improved by different chromosomal analysis techniques.

Material & methods: A prospective blinded study was performed (September 2018-December 2019). We included 92 TE PGT-A biopsies. Their correspondent spent embryo culture medium (SCM) were divided in two aliquots and evaluated by two different chromosomal genetic analyses(n=184). Negative controls were included (n=8). Finally, to investigate the diagnostic discrepancies, TE-aneuploid embryos were re-analyzed using TE and ICM biopsies (n=18). In total 302 chromosomal analyses were performed. TE biopsies results were compared blindly with the SCM results from the same embryo. Embryos were cultured in continuous media(Global Total LP) until D3, washed three times and cultured again until embryo biopsy. Embryos were biopsied on D5 or D6. TE analysis were carried out using Veriseq (Illumina®). SCM collection was done following TE biopsy and samples were stored at -80ºC. SCM chromosomal analysis were performed using Veriseq (Illumina®) and NICS (Yikon®).

Results: We obtained genetic results in 96.8% of TE-samples vs 90.4% in both SCM-techniques($p>0.05$). The mosaicism rate was higher in SCM regardless the technique used: 30.4% SCM-NICS, 28.3% SCM-Veriseq vs 14.1% TE-biopsies ($p<0.05$). Consistency in the diagnosis between both SCM-techniques were 95.2%. Regarding diagnostic concordance, no significant differences were obtained between both SCM-technique (74.6% SCM-NICS vs 72.3% SCM-Veriseq; $p>0.05$). Considering embryos biopsied on D6 these rates reached up the 92.0% and 86.5%, respectively; being the sensitivity for both SCM-techniques for D6 biopsed embryos was 100% and the specificity 77% and 61.5% respectively. Analysing the full chromosome concordance, the cytogenetic results were exactly the same as the TE-biopsy in 45.2% SCM-NICS and 41.7% SCM-Veriseq. Moreover, in 20.1% SCM-NICS and 23.3% SCM-Veriseq the results were discordant only in mosaicism diagnosis. The rest were partial and complementary discordances. No significant differences were reported for these parameters between embryos biopsied on D5 or D6. Moreover, we reanalysed TE-aneuploid embryos, 55.6% of the discrepancies were due to maternal DNA contamination, 22.2% to embryo mosaicism, 11.1% to low resolution in SCM-NICS and 11.1% to low resolution in both techniques.

Conclusions: Diagnostic concordance between PGT-A and niPGTA is independent of the genetic analysis technique. Interestingly, it was higher for blastocyst that had biopsied on D6. Therefore, niPGTA may be influenced by factors such as DNA-contamination and embryo mosaicism. Optimization of culture conditions and medium retrieval could improve the realibility of niPGTA.

P-54

ADDRESSING MATERNAL CONTAMINATION PROBLEM ON NIPGT-A, IS IT POSSIBLE TO DETECT IT BY GENOTYPING?

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Maternal contamination, noninvasive PGT-A, SNP, embryo cell-free DNA

Introduction: PGT-A studies (Preimplantation Genetic Testing for Aneuploidies) are a widespread tool for in vitro fertilization (IVF) treatments, which can be very beneficial for couples with a specific indication like advanced maternal age, recurrent miscarriage, and or recurrent implantation failure. Despite the benefits of the PGT-A being clear, the discussion continues as this technique is appropriate for every couple that undergoes IVF treatment. One of the reasons to consider carefully the conventional use of PGT-A approach relies on the fact that requires a trophectoderm (TE) biopsy of the embryo (day 5 or 6 after fertilization) to collect 5-8 cells for subsequent WGA amplification (Whole Genome Amplification). Nowadays, there is still controversy around the possible damage of this technique on the developing embryo and the consequent effects on implantation[1].

Following the trends in medicine, PGT-A approach is changing towards non-invasive techniques. Several groups have published their results on concordance rates between the TE biopsy and the spent culture medium (SCM), ranging from 65 to 85%[2–4]. In those studies, before SCM collection, assisted hatching or vitrification was performed, which makes the technique, in fact, slightly invasive. Additionally, they address the low concordance rates as a limitation of this technique, concluding that maternal contamination may be the underlying cause for the low concordance. A recent multicenter study reports a concordance rate of 78% with the non-invasive material collection, but they show significant intercenter variability, ranging from 72.5 to 86.3% concordance and increasing uncertainty with niPGT-A results of 46, XX[5]. Therefore, it is clear that every clinic desiring to implement the niPGT-A as a service needs to validate its concordance ratio and address the possible maternal contamination problem. In short, maternal contamination is a source of variability that must be limited as much as possible to increase the concordance index between the biopsy and the medium and standardize the technique. For this reason, it is necessary to develop a tool that allows the detection of samples with a high probability of being affected by maternal contamination making the niPGT-A a highly standardized and reproducible methodology.

Material & methods: Here we present a strategy based on SNP (Single Nucleotide Polymorphism) to evaluate maternal DNA contamination in the SCM. More than 30 SCM were processed and analyzed to address maternal contamination and to study their ploidy concordance rate, comparing with corresponding TE biopsy. The protocol followed for SCM collection implies refreshing the medium on day 4 and collecting the specimen on day 6. After that, SCM and TE biopsy are amplified by WGA protocols followed by a modified protocol for SCM samples. Finally, SCM were also processed for target amplification and sequenced with the Ion Torrent platform.

Results: In this work, we develop a maternal contamination score based on allele frequency in the medium, which can help to evaluate the risk of maternal DNA contamination in the samples.

Conclusions: This may be a tool to detect maternal contamination on niPGT-A studies.

P-55

VERIFICATION OF THE CONTENT OF EMBRYONIC CULTURE MEDIUM BY GENOTYPING USING STR MARKER

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Ni-PGT-A, parental DNA, STR

Introduction: The success of assisted reproduction treatments is based on the selection for transfer of the best embryos. The most reliable method to assess the chromosomal status of preimplantation embryos is preimplantation genetic testing for aneuploidies PGT-A.

However performing an invasive procedure like embryo biopsy (EB) could affect viability. To avoid this limitation, non-invasive methods based on the analysis of the cell-free DNA released by the embryo during the latest stages of preimplantation development has been proposed. The objective of our study is to asses if non-invasive PGT-A may be possible by assessing the origin of DNA present in spent blastocyst media before undergoing EB for PGT-A.

Material and methods: Our study was done on 12 blastocyst media sample from 3 differents couples. We analysed all samples using AmpFLSTR Identifiler Kit. We used also parental DNA to define the origin of detected DNA from blastocyst media sample.

Embryo culture media was collected from single embryo culture droplets, then stored at -20°C. Spent embryo culture media samples from 10ul-30ul culture droplets were whole genome amplified using SurePlex. The quantification and the quality of DNA yield was assessed by gel electrophoresis and high sensitivity Qubit instrument (ThermoFisher).

Results: Data analysis and allele calling was performed with GeneMapper and the correspondant analysis software IDv3.2.1.

Our study done on 12 blastocyst media sample for PGT-A application, using Fifteen STR (short tandem repeat) loci, failure and diagnostic non concordance rates.

This result is due to significantly different allele drop-out rates between maternal and paternal loci, we showed that maternal DNA exceeded the paternal one, suggesting DNA contamination from cumulus cells.

Conclusion: Current study suggest that spent culture media is unreliable for embryo's genetic assessment, and the non-invasive methods need rigorous validation prior to clinical applications.

P-56

CELL-FREE EMBRYONIC DNA TESTING IN COMBINED SPENT EMBRYO CULTURE MEDIUM AND BLASTOCOEL FLUID FOR ANEUPLOIDIES

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Spent embryo culture medium, blastocoel fluid, cell-free embryonic DNA, non-invasive PGT-A, laser assisted hatching

Introduction: There is growing interest in the use of minimally invasive and non-invasive preimplantation genetic testing (mi/NIPGT) based on cell-free embryonic DNA (cfeDNA) from blastocoel fluid (BF) or/and spent embryo culture medium (SEM). However, despite the promising results, concordance rate for ploidy between cfeDNA and trophectoderm (TE) biopsy, inner cell mass (ICM) and/or whole blastocysts is still controversial issue. Moreover, it is still debated origin of cell-free embryonic DNA in spent medium from human embryo (TE DNA or both TE DNA and ICM DNA). The objective of this study was to evaluate the efficacy and accuracy of NIPGT-A using cfeDNA in combined SEM+BF samples in comparison to corresponding TE biopsy PGT-A.

Material & methods: Laser assisted hatching (LAH) of fresh embryos was performed on Day 4. Day 4 embryos from LAH group and from control group (NO AH) were put in an individual fresh 15µL droplets of Sage 1-StepTM medium. On Day 5/6/7 fully expanded blastocysts were collapsed by a single laser pulse at the junctions between TE cells to release BF. The blastocysts were moved to a biopsy dish and combined SEM+BF medium samples (10µL) were collected. SurePlex kit (BlueGnome) was used for whole genome amplification (WGA) of both SEM+BF and TE biopsy samples. All WGA products were conducted PGT-A using NGS by Illumina NextSeq® 550 platform. CNV analysis is performing with new NxClinical software (BioDiscovery). Results were statistically evaluated using the Chi Square/Fisher exact test. The embryos were classified as putative mosaic blastocysts if the level of mosaicism ranged from 30% to 70% aneuploid DNA in the TE biopsy and SEM+BF sample. For this study, the mosaicism threshold was set at 50% (i.e., level of <50% aneuploid DNA was considered as euploid blastocyst and ≥50% as aneuploid).

Results:

1. All SEM+BF samples were amplified.
2. LAH was not associated with the average WGA-DNA concentration in SEM+BF medium (36.8 ± 20.1 ng/µL vs. 39.8 ± 17.0 ng/µL for LAH vs. no AH, respectively; P=0.3).
3. Informative NGS results in SEM+BF LAH group were 86.2% (56/65) and 93.7% (163/174) in SEM+BF NO AH group (P=0.07).
4. Informative NGS results were obtained from 99.2% (244/246) of TE biopsies. and from 91.6% (219/239) of SEM+BF samples (7 samples with maternal DNA contamination for NIPGT).
5. Average cfeDNA quantity in SEM+BF is similar in euploid and aneuploid embryos (40.1 ± 19.2 vs. 41.6 ± 17.1, respectively; P = 0.6).
6. Concordance rates for euploidy (118/134) and aneuploidy (77/84) were not statistical significantly different between SEM+BF and TE biopsy groups (88.1 vs. 91.7%, respectively).
7. Sensitivity and specificity for NIPGT-A were 91.7% and 88.1%, respectively.

Conclusions: LAH did not influence cfeDNA concentration and efficiency of informative NGS results in combined SEM+BF. CfeDNA in combined SEM and BF effectively reflects the chromosomal status of euploid and aneuploid TE biopsy samples. In addition, the ICM is in direct contact with BF, so cfeDNA in the combined SEM+BF media o*riginates from both TE and ICM*, and we assume that this approach may better represent ICM chromosomal status.

P-57

POTENTIAL GENE MUTATIONS WITH RECURRENT OOCYTE MATURATION DISORDERS AND FERTILIZATION FAILURES

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Oocyte, fertilization, wee2, ooep, infertility

Introduction: Aiming at recurrent oocyte maturation disorders, fertilization failures and early embryonic development abnormalities during the IVF treatment, we speculate that these patients are more likely to have genetic mutations. For these patients, we can find mutant genes through whole exon sequencing technique, and provide candidate genes of fertility dysfunction for future PGT.

Material and methods: We collected peripheral blood from patients with problems of oocytes maturation and recurrent fertilization failure at IVF centre of Run Run Shaw Hospital and Zhejiang Provincial Key Laboratory of Reproductive Disorders. Whole exon sequencing was performed. Besides, based on the results of Sanger sequencing of the patients' whole family, the inheritance patterns of mutations were determined. At the same time, *in vitro* cell experiments were used to verify gene function and analyze the harmfulness of specific gene mutations.

Results: The mutated genes that cause oocyte maturation disorders were found: *Ooep* (c.G110C (p.R37P) and c.C109G (p.R37G)); cause fertilization failures: *Wee2* (c.115_116insT (p.Q39Lfs *) 5), c.C1459T (p.R487W), c.T1576G (p.Y526D) and c.G585C (p.K195N). These mutations cause abnormal mRNA transcription and are related with abnormal protein structure.

Conclusions: The above gene mutations can be used for future PGT and help couples to have fertility offspring.

P-58

PGT-M AFTER CARRIER SCREENING WITH FAMILY HISTORY IN CONSANGUINITY COUPLES

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Carrier screening, consanguinity couples, monogenic disorders, autosomal-recessive, PGT-M

Introduction: Target carrier screening is currently used for detection of pathogenic variants, requiring preimplantation genetic testing for monogenic disorders. Introduction of this approach allowed offering genetic counseling and preimplantation genetic testing to a number of at risk couples described below.

Materials and Methods: 38 couples were included, through familiar history - 11, consanguineous marriages -2, miscarriages - 6, ethnicity - 4, and prospective counseling-14.

To identify heterozygous carrier, the samples were sequenced using the Illumina HiSeq2500 system with 100 bp paired-end reads of the expanded panethnic panel Horizon274 (Natera, USA), including 254 autosomal recessive and 20 X-linked hereditary diseases.

For preliminary stage of PGT-M, individual test systems using the Sanger sequencing with region-specific single-nucleotide primers and collateral molecular genetic diagnostics with closely spaced STRs have been developed. Trophectoderm biopsy was performed on the 5th day of embryo development. Whole genome amplification (WGA) was performed using a Qiagen kit (REPLI-g SC WGA) with quality control of WGA products using a Qubit fluorimeter and agarose gel electrophoresis. Trophectoderm samples were examined using prepared individual panels for family pathogenic variants and aneuploidy using GenetiSure microarrays to the Agilent protocol.

Results: Carrier of pathogenic variants in one or several genes of hereditary diseases were detected in 27 patients (35.5% of the examined). 44,4% (12 people) of these couples were carriers of two or more diseases. Heterozygous carriers of pathogenic variants in one gene were found in two consanguineous couples. One cousins couple with miscarriages were carriers three hereditary metabolic diseases (phenylketonuria, Pompe disease and Zellweger Spectrum Disorders, PEX-10 related). Polycystic Kidney Disease was detected in other second degree cousin couple, with a history of pregnancy termination. In addition to bilateral polycystic kidney disease, there was an indication of ventriculomegaly in the fetal autopsy examination report. Genetic Counseling showed that the parents belong to a Dagestanian ethnic group. Pan-ethnic panel analysis was recommended. No pathogenic variants were found in the *PKHD1* gene responsible for the development of autosomal recessive polycystic kidney disease. Both partners were heterozygous carriers of pathogenic variant in a completely different gene - the centrosomal protein *CEP290* (OMIM 610142). Homozygous state of this gene lead to development of severe forms of primary ciliopathies - autosomal recessive diseases manifesting by a combination of brain abnormalities, kidneys (often polycystic) and eyesight problems (such as Meckel, Joubert, Senor-Loken, Barde-Bidel syndromes).

PGT-M was conducted for both families in combination with aneuploidy testing (PGT-A).

Conclusions: Genetic counseling and a target carrier screening provides information on the pathogenic variants, with possibility of application of PGT-M. Genetic counseling to optimises the examination of these couples allowing preventing hereditary disorders, especially in consanguineous couples.

P-59

COMPREHENSIVE CARRIER SCREENING STRATEGY FOR CHALLENGING GENOMIC CONDITIONS

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Carrier Screening, NGS, Pseudogenes

Introduction: Universal carrier screening test has become a widespread approach for detecting couples at increased risk of having an affected child due to ethnicity or consanguineous marriage. Initial approach of hot spot screening for common mutations has been replaced by NGS-based extended screening tests covering 100-400 diseases. With the recent developments in genomic technologies, condition specific carrier screening has been replaced by expanded carrier screening (ECS) approach, which enables screening of a large number of genetic diseases independent of ethnic background. Recent idea is that specificity/sensitivity and uniform coverage are more important than the number of genes involved in the panel. In this study, the validation data of 64 patients with carrier screening panel targeting coding regions of 420 genes are presented.

Material & methods: A multiplex sequencing panel targeting 100% of coding bases plus flanking regions for 420 genes associated with Mendelian disorders was created through the Ion AmpliSeq™ Designer. The panel assesses SNVs, indels, and CNVs addressing >28,000 ClinVar non-benign variant loci as well as novel variants across the entire targeted coding region of each gene. 33 high value genes have specially optimized designs and algorithms to enable single-exon CNV and enhanced variant calling. As a part of multicenter validation study for Ion Torrent CarrierSeq ECS Kit (Thermo Fisher Scientific) DNA samples from 32 patients with previously reported variant results in different genes were processed and the data were analysed using Carrier Reporter Software. Quality performance was tested by variety of parameters, including percentage of bases covered within the target loci, uniformity of target coverage, ability to call SNV, indel, and CNV variants. In addition, 32 clinical samples with known variants including pseudogene mutations as well as CNV variants were tested. 18 genes were selected for the study including GBA, HBA1/HBA2, CYP21A2, CYP11B1, SMN1/SMN2, VWF, which are difficult to analyze due to presence of pseudogenes, gene conversions and paralogues. CFTR and DMD genes are added for their valuable CNV information.

Results & conclusions: 31 out of 32 clinical samples passed the acceptance criteria (>200X read depth and >93% uniformity). Results revealed that 27 of 35 previously detected variants are called by the analysis algorithm. 8 false negatives were detected in 6 genes (CYP21A2, GLA, GBA, CYP11B1, HBA1/HBA2, ITGB3, VWF). Additionally, potential false positive calls were detected majority of which lies in gene coversion regions (ABCC6, CBS, CYP21A2). Detection of all CNV's is encouraging and potential single exon–level resolution in genes such as CFTR and DMD will be crucial for clinical samples. 8 false negatives mainly lie in gene conversion regions and majority of overcalls are due to gene conversion algorithm sensitivity (e.g. target genes CBS, ABCC6, CYP21A2, CYP1B1). These results suggest that a correction algorithm for gene conversion is required. After the initial analysis special algorithms were adopted, which enabled detection of all variants in 8 false negative samples making the test 100% sensitive for challenging variants. These special analysis algorithms also aid to overcome false positives by adjusting gene conversion sensitivity.

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ESTIMATION OF LATENT GENETIC LOAD OF SINGLE-GENE DISORDERS AMONG THE POPULATION OF UKRAINE: PILOT STUDY

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preconception screening, carrier screening, genetic risk, genetic load, PGT-M

Introduction: Single-gene disorders with recessive and X-linked inheritance mechanism are an extremely important medical problem and a burden on the public health system. It is known that most cases of congenital anomalies in newborns are associated with recessive inheritance (up to 5 cases per 1,000 newborns) [1]. Mutations can be latently inherited for generations asymptomatically, causing risk only in the case of corresponding partner combinations. It is believed that each person is a latent carrier of 7-14 recessive single-gene disorders, and the a priori risk constitutes 1/44 of couples or 1/175 of pregnancies [2].

Preconception genetic screening is an effective mechanism for detecting latent mutation carrier that underlies further reproductive family planning (conscious risk-taking, prenatal or preimplantation genetic testing of embryos). However, the spectrum of the mutation carrier may vary depending on the ethnicity of the patients. The spectrum of latent carriers of mutations among the population of Ukraine remains unexplored.

Material & methods: Prospective testing of patients who applied (06.2021-12.2021) at the stage of planning a natural or ART pregnancy and did not have a positive family history of hereditary pathology, neurodevelopmental disorders, congenital anomalies, infertility, recurrent pregnancy losses. There were 116 tests, 47 closed diagnostic programs carried out. The closed program represented cases when both partners were examined or when the examination of the first partner of a couple did not reveal mutation carriage, so the diagnosis of the second partner was not performed.

Genomic DNA obtained from the sample is enriched for targeted regions using a hybridization-based protocol, and sequenced using Illumina NGS-technology. Unless otherwise indicated, all targeted regions are sequenced with ≥50x depth or are supplemented with additional analysis. The study included 302 genes, the full list of which is available at: www.ivf.com.ua/carrier-test.pdf.

Results: No mutations were detected in 22 cases (18.97%). Mutation carriers were detected in 94 cases (81.0%). Of these, only cases of ≥ 1 pathological mutation – 81 (69.8%), only cases of mutation with low penetrance – 13 (11.2%). By number of pathological mutations: 1 mutation – 36 cases (31.0%), 2 – 27 (23.3%), 3 – 16 (13.8%), 4 and 5 – 1 (0.9%) each. The most common genes in which mutation carriage was identified were HFE (31), GALT (15), CFTR (11), SMN1 (9), GJB2 (8), PAH (7), BTD (6), WNT10A (6), CYP21A2 (5), DHCR7 (4), FMR1 (4), F5 (4), PMM2 (3), SERPINA1 (3).

Out of 47 closed diagnostic programs, 3 cases of pathogenic mutation carriage coincidence in partners within one gene were identified (6.4%).

Conclusions

1. The conducted testing allowed for the prevention of 3 high-risk pregnancies.
2. The frequency of recessive single-gene pathology is underestimated.
3. The ongoing testing (in collaboration with other laboratories) will allow to characterize epidemiological features and calculate the genetic risk, taking into account the ethnicity of patients.

Reference list:

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