18TH INTERNATIONAL CONFERENCE ON PREIMPLANTATION GENETICS

PGDIS

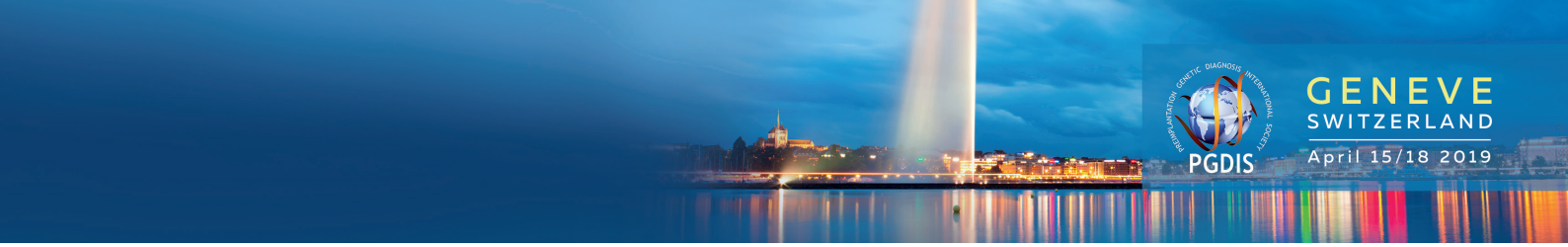
PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

GENEVE
SWITZERLAND

April 15/18 2019
www.pgdis2019.org

FINAL PROGRAM



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Welcome message

Dear Colleagues,

It is our great pleasure to welcome you to the 18th International Conference on Preimplantation Genetics, organized by Preimplantation Genetic Diagnosis International Society in Geneva Switzerland.

We prepared an exciting scientific program, based on the most recent developments in Preimpantation Genetics, and, as usual, put together a multidisciplinary group of leading experts in reproductive biology, genetics and embryology, involved in the development of Preimplantation Genetic Testing (PGT) technology. In addition to reviewing the current progress and limitations of PGT technology, new areas of its application and future prospects will be addressed, such as the application of whole genome sequencing, emerging technologies towards embryos therapy, feasibility of PGT for epigenetic disorders, reliability of alternative approaches to PGT.

We do hope that you will enjoy the scientific discussions and the social program in the city of Geneva, located in the heart of Europe at the shores of the biggest lake in Western Europe, near the foot of Mont-Blanc, the continent's highest peak, which will provide you with an unforgettable experience.

Prof. Stylianos Antonarakis, MD, PhD
Chairman of Organizing Committee

Prof. Anver Kuliev, MD, PhD
Executive Director of PGDIS

General Information

Exhibition Office & Secretariat

VIAJES PACÍFICO S.A.

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CICG Centre International de Conférences Genève

> Rue de Varembé 17, 1211 Genève, Suiza

> Tel: +41 22 791 91 11

Language

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

EACCME Accreditation

The European Accreditation Council for Continuing Medical Education (EACCME).
Granted 18 European CME credits (ECMEC)

EACCME credits

Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity. The EACCME credit system is based on 1 ECMEC per hour with a maximum of 3 ECMECs for half a day and 6 ECMECs for a full-day event.

Poster Session

> All posters will be presented in paper format

> Posters should be 90 cm in length and 120 cm in height, and they should be displayed by the authors in the poster area in the slot corresponding to the code number provided in the confirmation letter

> Posters must be put in display on Tuesday April 16th 2019 between 9.00 to 10.30 (fixing support will be supplied by the Techinical Secretariat) and they will remain in the Posters' area until Thursday, April 18th , when they will have to be removed by authors no later than 12.00.

> The Technical Secretariat is not responsible for those posters that will not be removed at the end of the Congress

> Poster discussion will take place during the Lunch and Posters Sessions indicated in the Program. During these sessions posters' authors are requested to stand next to their poster to answer potential questions from participants.

General Information

Congress badges

All participants, accompanying persons and exhibitors must wear the Congress identification badges. Entrance to meeting rooms, posters 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 ask to 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.

MONDAY 15th APRIL

09.30-13.30
SALLE 3

PerkinElmer
For the Better

PERKINELMER PRECONFERENCE COURSE

PerkinElmer is delighted to announce our partnership with PGDIS as a Major Sponsor of the upcoming PGDIS Conference in Geneva, Switzerland, April 15-18, 2019. As a major sponsor of this Conference, PerkinElmer will be hosting a pre-congress course as detailed below. Our hope with this unique pre-congress course is to update the field on the most recent advances in Preimplantation Genetic Testing (PGT) and to broaden your horizons with selected talks from other fields that also deal with single to small numbers of cells including prenatal diagnostics, cancer and forensics to name a few.

Provisional Program & Call for abstracts*

1. Introduction and Welcome
2. Experience with automation of PG-Seq for PGT-A with Robotic Liquid Handling
3. Experience using PG-Seq for combined PGT-A and PGT-M with Target Sequence Enrichment
4. Non-invasive PGT-A (niPGT-A) Initial findings from a Global Research Study
5. Selected Abstracts from Participants * (topics to include)
   - > Use of novel methods for expanded PGT
   - > Use of novel single cell tools for comprehensive testing of circulating tumor and fetal cells
   - > Use of novel cell free DNA analysis tools
6. Future Advancements in the use of genetics in IVF
7. Closing Remarks

*PerkinElmer is calling for abstracts from complementary research and applied fields that analyze single cells and cfDNA including non-invasive prenatal diagnosis, cancer diagnosis and forensics. This unique format will provide course participants with an opportunity to hear about advances in related methods that could be applied to biopsy-based or non-invasive PGT. Expect to be inspired! Abstracts selected for presentation in the PCC will be 15 minutes in length. The deadline for abstract submission is February 1, 2019 and notification of abstracts selected for participation will be made no later than March 1, 2019 (submit according to format described on the Conference website).

We are truly excited and honored to be such a large part of PGDIS 2019 and look forward to having you join us in Geneva next April!

09.30-13.30
SALLE 4

ThermoFisher SCIENTIFIC

THERMO FISHER SCIENTIFIC PRECONFERENCE COURSE

TIME	TOPIC AND SPEAKER
09.30-09.40	Introduction and welcome
09.40-10.10	Building brighter futures together: An overview of the Thermo Fisher Scientific Reproductive Health portfolio George Hii, Director Global Market Development, Reproductive Health, Thermo Fisher Scientific
10.10-10.40	Validation of the Applied Biosystems™ Carrie Scan™ preconception expanded carrier-screening assay: the most comprehensive CNV carrier screening solution for challenging diseases such as SMA, DMD, and thalassemia Doron Behar PhD, CEO Igentify, USA
10.40–11.10	Ion™ ReproSeq™ PGT-A simple and scalable next-generation sequencing workflow for aneuploidy analysis Colin Davidson PhD, Product Manager, Reproductive Health, Thermo Fisher Scientific
11.10-11.30	Coffee break
11.30-11.55	Ion ReproSeq in PGT-A applications Dr Fabien Murisier, Directeur, Fertas SA, Lausanne, Switzerland Embryologist at Fertas, CPMA and Fasteris, Switzerland
11.55-12.20	PGT-A experience: managing the increasing workload and implementing PGT-M with Ion AmpliSeq™ technology Murat Cetinkaya,MD, PhD, ART and Reproductive Genetics Center Memorial Hospital, Istanbul, Turkey
12.20-12.45	PGT for all CFTR Sicilian mutations Sandrine Chamayou PhD, Director, Laboratories of IVF and Preimplantation Genetic Diagnosis, Infertility clinic HERA, Catania, Italy
12.45-13.10	Late breaking news Title and presenter to be confirmed soon
13.10- 13.30	Closing remarks and summary
13.30	Close of Meeting

Thermo Fisher Scientific is proud to be part of PGDIS 2019 and looks forward to welcoming you to the preconference workshop in Geneva
https://www.thermofisher.com/rh

09.30-13.30
SALLE 3

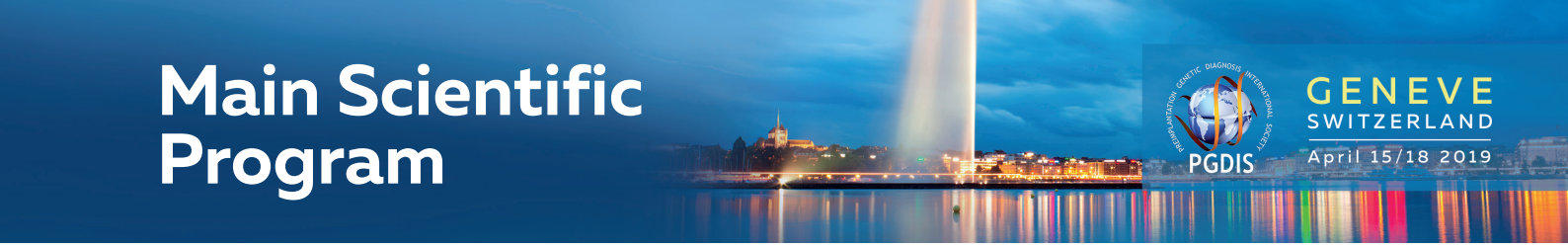
Agilent

AGILENT PRECONFERENCE COURSE

14:30-18:30	PROGRAM	PRESENTER
	> Agilent's human reproductive genetics portfolio	Heidi Kijenski Global Marketing Director Human and Reproductive Genetics Agilent Technologies
	> Comprehensive pre-implantation genetic testing using a genome-wide NGS approach Introduction: Implementation of OnePGT in clinical labs around the world	Jessie Theuns, PhD Product Manager Reproductive Genetics Agilent Technologies
	> Multi-centre evaluation of OnePGT, a generic, NGS-based approach for automated haplotyping and copy-number assessment, both combined or independently, in human single blastomere and trophectoderm samples	Joris Vermeesch, PhD Professor Chair, Department of Human Genetics Universitaire Ziekenhuizen Leuven
	> Evaluation and implementation of OnePGT for monogenic conditions in the UK	Pamela Renwick, PhD Center for Preimplantation Genetic Diagnosis Guy's and St Thomas' NHS Foundation Trust Great Maze Pond, London, UK
	> Implementation of Agilent OnePGT solution in a routine clinical setting in the Netherlands	
	Coffee Break	
	> The power and advantages of microarrays for PGT-SR/A: ease, speed, and resolution	Valentina Maran, PhD Product Manager, CGH Portfolio Agilent Technologies
	Customer presentation	
	> Clarigo: Enabling NIPT for all	José Tijsen, PhD Global Marketing Director, Prenatal Agilent Technologies
	Customer presentation	
	Panel discussion	All
	Tapas and drinks in main area	

OnePGT is For Research Use Only. Not for use in diagnostic procedures.
Clarigo is for In Vitro Diagnostic Use.

Main Scientific Program



DAY 1 – TUESDAY 16th APRIL — SALLE 2

08:15-08:30	**OPENING AND WELCOME** S Antonarakis, A Kuliev
08:30-09:00	**PLENARY LECTURE** Whole Genome Sequencing: Application to PGT S. Antonarakis
09:00-10:30	**SESSION 1 - PROGRESS TOWARDS UNIVERSAL PGT** Chairs: D Wells & S Rechitsky 09:00-09:30 How close we are to Comprehensive PGT D Wells 09:30-10:00 D Leigh 10:00-10:30 PGT for De-Novo Mutations S Rechitsky
10:30-11:00	**COFFEE BREAK**
11:00-11:30	**PLENARY LECTURE** Real-time imaging of preimplantation embryo development N Plachta
11:30-13:00	**SESSION 2 - ORIGIN AND MECHANISMS OF ANEUPLOIDY** Chairs: A Handyside & J Vermeesch 11:30-12:00 Mechanism of maternal age dependence M Herbert 12:00-12:30 Origin of mosaicism and segmental variations Eva Hoffmann 12:30-13:00 Meiomapping A Handyside
13:00-14:00	**LUNCH BREAK**

14:00-15:30 **SESSION 3 - FREE COMMUNICATIONS OC-01 to OC-06**
Chairs: K Xu & A Thornhill

OC-01 SUCCESSFUL HEMATOPOIETIC STEM CELL TRANSPLANTATION IN 62 CHILDREN FROM HEALTHY SIBLINGS CONCEIVED AFTER PREIMPLANTATION HLA MATCHING
Umay Kara, B.; Gavaz, M.; Kumtepe Çolakoğlu, Y.; Yelke, H.; Pirkevi Çetinkaya, C.; Çetinkaya, M.; Kahraman, S..

OC-02 PATHOGENIC DNA VARIATION WITHIN ACMG SECONDARY FINDINGS GENES IN 18,000 HEALTHY INDIVIDUALS USING CLINICAL EXOME SEQUENCING FOR CARRIER SCREENING
Mir Pardo, P.; Jiménez-Almazán, J.; Alonso, R.; Panadero, J.; Hernández-De-Diego, R.; Abellán, C.; Simón, C.; Martín, J..

OC-03 SELECTIVE TRANSFER OF EUPLOID NONCARRIER EMBRYOS WITH THE USE OF LONG-READ SEQUENCING IN PREIMPLANTATION GENETIC TESTING FOR RECIPROCAL TRANSLOCATION
Chow, J.F.C.; Cheng, H.H.Y.; Lau, E.Y.L. Yeung, W.S.B.; Ng, E.H.Y.

OC-04 PGT-M FOR DE NOVO MUTATIONS – HAPLOTYPE DETERMINATION USING MORPHOLOGICALLY POOR EMBRYOS
Stock-Myer, S.; Tang, P.; Twomey, A.; Kohfahl, A.; Shi, E..

OC-05 NOVEL APPROACH ENABLING THE SIMULTANEOUS DETECTION OF SNV AND CNV FOR PGT-M AND PGT-A USING A SINGLE-TUBE ASSAY.
Kimura, Y.; Laliberte, J. Kamberov, E.; Viotti, M.; Victor, A[3]; Brake, A.; Zouves, C.; Barnes, F.; Farmer, A.

OC-06 PGT-A METHOD ALLOWING COMBINED PGT-M FOR DETECTION OF HETEROPLASMY AND ESTIMATION OF MTDNA MUTATION LOAD IN EMBRYO BIOPSIES.
Myers, S.; Jasper, M.

COFFEE BREAK

16:00-16:30 **PLENARY LECTURE**
Status and Impact of Prospective Carrier screening on PGT application
J.L. Simpson

16:30-17:30 **SESSION 4 - PGT IN DIFFERENT SOCIAL SETTINGS**
Chairs: S Kahraman & S Hamamah

16:30-17:00
Experience of Nationally-based PGT–
S Hamamah

17:00-17:30
PGT in Switzerland after Popular vote and Change of Legislation
F Murisier

DAY 2 – WEDNESDAY 17th APRIL — SALLE 2

09:00-09:30	**SUMMARY OF HIGHLIGHTS OF PREVIOUS DAY AND INTRODUCTION TO SECOND DAY** A Handyside
09:30-10:00	**PLENARY LECTURE** Gene regulatory network in the context of genome instability in human embryo development T Voet
10:00-10:30	**SESSION 5 - DEVELOPMENTS IN PGD-A (PART I)** Chairs: R Scott & D Cram Update of Randomized Controlled Studies on PGT-A Impact on Reproductive Outcome R Scott
10:30-11:00	**COFFEE BREAK**
11:00-13:00	**SESSION 5 - DEVELOPMENTS IN PGD-A (PART II)** 11:00-11:30 Pregnancy outcome of transferred embryos with mosaicism and segmental variations F Fiorentino 11:30-12:00 Impact of severe male infertility on chtomosome status of embryos and pregnancy outcome in younger women S Kahraman 12:00-12:30 NIPT for PGT follow-up J Vermeesch 12:30-13:00 Updated PGDIS recommendations for replacement of mosaic embryos D Cram
13:00-14:00	**LUNCH BREAK**

14:00-15:30

SESSION 6- FREE COMMUNICATIONS OC07 to OC 12
Chairs: G Harton & C Strom

OC-07 IMPACT OF CHROMOSOMAL MOSAICISM IN IVF OUTCOMES: EXPERIENCE FROM TWO HUNDRED MOSAIC EMBRYOS TRANSFERRED PROSPECTIVELY
Spinella, F.; Biricik, A.; Minasi, M.G.; Varrichhio, M.T.; Corti, L.; Viganò, P.; Baldi, M.; Surdo, M.; Cotroneo, E.; Fiorentino, F.; Greco, E.

OC-08 SEGMENTAL ANEUPLOIDIES SHOW MOSAIC PATTERN REDUCING PREDICTIVE VALUE COMPARED TO HIGH WHOLE CHROMOSOME ANEUPLOIDIES REPRESENTATIVENESS
Girardi, L.; Romanelli, V.; Fabiani, M.; Cimadomo, D.; Rienzi, L.; Ubaldi, F.M.; Serdarogulları, M.; Coban, O.; Findikli, N.; Boynukalin, K.; Bahceci, M.; Patassini, C.; Poli, M.; Rubio Lluesa, C.; Simón, C.; Capalbo, A..

OC-09 ASSESSMENT OF ANEUPLOIDY AND MOSAICISM CONCORDANCE BETWEEN DIFFERENT TROPHECTODERM BIOPSY SITES AND THE INNER CELL MASS EVALUATED WITH NEXT-GENERATION SEQUENCING
Tufekci, M.A.; Cetinkaya, M.; Cinar Yapan, C.; Kumtepe Colakoglu, Y.; Yelke, H.; Pirkevi Cetinkaya, C.; Kahraman, S..

OC-10 COMPARISON OF VARIOUS ANEUPLOIDY TYPES AMONG TWO TROPHECTODERM BIOPSIES AND A REST OF THE EMBRYO
Navrátil, R.; Horák, J.; Horňák, M.; Kubíček, D.; Oráčová, E.; Janíčková, N.; Veselý, J.; Veselá, K..

OC-11 HIGH CONSISTENCY IN EMBRYO ANEUPLOIDY TESTING OF UNIFORM, MOSAIC AND SEGMENTAL ANEUPLOIDIES WITH THE APPLICATION OF A VALIDATED ALGORITHM
Garcia Pascual, C.; Navarro Sánchez, L.; Navarro Gayá, R.; Rodrigo Vivó, L.; Garcia Herrero, S.; Campos Galindo, I.; Peinado Cervera, V.; Jiménez Almazán, J.; Simón Vallés, C.; Rubio Lluesa, C..

OC-12 WHAT TO DO WHEN THERE IS NO EUPLOID EMBRYOS? TRANSFER MOSAIC EMBRYOS OR PROPOSE ANOTHER PGT-A CYCLE?
Cetinkaya, M.; Pirkevi Cetinkaya, C.; Tufekci, M.A.; Cinar Yapan, C.; Kumtepe Colakoglu, Y.; Yelke, H.; Kahraman, S..

15:30-16:00 **COFFEE BREAK**

16:00-17:30

SESSION 7: EMERGING TECHNOLOGIES
Towards Embryo Therapy
Chairs: S Antonarakis & C Simon
CRISPR-Based Editing– Any prospect for PGT?
H O'Neill
Panel Discussion

DAY 3– THURSDAY 18th APRIL — SALLE 2

08:30-09:00	**SUMMARY OF HIGHLIGHTS OF PREVIOUS DAY AND INTRODUCTION TO THIRD DAY** JL Simpson
09:00-09:30	**PLENARY SESSION** Clinical Impact of Maternal-Embryonic Communication at Implantation C Simon
09:30-11:00	**SESSION 8 - FREE COMMUNICATIONS OC13 to OC 18** Chairs: C. Rubio & T Gordon OC-13 CORRELATION BETWEEN ANEUPLOIDY, MOSAICISM AND MORPHOKINETIC DEVELOPMENT IN 1550 BIOPSIED BLASTOCYSTS Kumtepe Çolakoğlu, Y.; Tüfekçi, M.; Çınar Yapan, Ç.; Umay Kara, B.; Gavaz, M.; Yelke, H.; Pirkevi Cetinkaya, C.; Cetinkaya, M.; Kahraman, S.. OC-14 MOSAIC EMBRYO TRANSFERS – A COMPILED ANALYSIS OF PUBLISHED DATA Viotti, M. OC-15 THE INCIDENCE AND ORIGIN OF CHROMOSOME ANEUPLOIDIES IN HIGH QUALITY KARYOMAPPING SNP PROFILES Hornak, M.; Horak, J.; Kubicek, D.; Navratil, R.; Zwinsova, B.; Tauwinklova, G.; Oracova, E.; Vesela, K.. OC-16 INTRACYTOPLASMIC SPERM INJECTION IS NOT NECESSARY AS A PREVENTIVE MEASURE AGAINST PATERNAL CELL CONTAMINATION IN PREIMPLANTATION GENETIC TESTING Lynch, C.; Cater, E.; Charitou, M.; Forbes, H.; Griffin, D.; Gordon, T. OC-17 RECOMBINANT VERSUS URINARY GONADOTROPHINS: A PILOT STUDY TO EVALUATE PLOIDY STATUS OF EMBRYOS DERIVED FROM IVF. Leong, D.L.; Chen, J.J.; Wong, P.S.. OC-18 THE INSIGHTS OF EMBRYO MOSAICISM IN IVF CYCLES Coll, L.; Parriego, M.; Garcia-Monclús, S.; Rodríguez, I.; Boada, M.; Coroleu, B.; Vidal, F.[2]; Veiga, A.
11:00-11:30	**COFFEE BREAK**
11:30-12:00	**PLENARY LECTURE: PROSPECT OF PGT FOR EPIGENETIC DISORDERS-SINGLE CELL METHYLOME AND EPIGENETIC ANALYSIS** G Kelsey

12:00-13:30	**SESSION 9 - ALTERNATIVE APPROACHES TO PGT** Chairs: S Munne & L Gianaroli 12:00-12:30 NIPGT multicenter concordance study C Rubio 12:30-13:00 Blastocellic Fluid-based NIPGT L Gianaroli 13:00-13:30 Further Data on Feasibility of PGT without IVF S Munne
13:30-13:45	**CLOSING REMARKS AND PRESENTATION OF NEXT PGDIS CONFERENCE** A Handyside, A Kuliev

Book of Abstracts

PROGRESS TOWARDS UNIVERSAL PGT- HOW CLOSE ARE WE TO COMPREHENSIVE PGT?

Don Leigh
NGS Pty Ltd, Australia
Calmette Hospital & 1st People's Hospital, Kunming, Yunnan, China

"Are we there yet?"- these are the words that many parents would have heard when taking children on some sort of trip. The journey began over 30 years ago when the first PGTs were done.

It was simple back then- the answers required were generally simple (or at least straightforward) and you only went looking for directed answers. PGS was for chromosomes and PGD was for a gene(s)- and generally, never the two would meet. Early chromosome testing was based on FISH and the targets were polar bodies or blastomeres from cleavage embryos- apart from some technical expertise in getting the sample, analysis was essentially down to the operator's ability to count to 1 (2nd PB) or 2 (1st PB and blastomeres). Life was good- but unfortunately the results were often not so productive with implantation rates resisting change and only some groups reporting decreased miscarriage rates for some patient cohorts. The naysayers reached a crescendo around 2005 when it was proclaimed that not only was PGS not helping but it was hurting patient's chances of a pregnancy. Three years of gloom followed with claim and counter claim on the appropriateness of PGS. PGD fared a bit better with the simple logic of Mendelian genetics and obvious success in outcomes- although some mishaps in the early days led some groups to possibly over emphasize and focus on DNA contamination, ADO and recombination events- life for PGD though was generally good. The darkness of those 3 years of doubt and dispute were substantially dispelled when a British Knight led the field into the new era of comprehensive chromosome screening where every chromosome could now be seen to be both problematic and mischievous.

Finally, the full impact of identifying any and all chromosome changes was seen- a dramatic increase in implantation rate and decreased miscarriage rates for most groups tested. There were still some shadowy areas of understanding but the light was again shining and things were, maybe, good again. Was it just a breakthrough in analysis that flicked the switch? No, there were some underlying bits to the puzzle that were not given the amount of the credit for the improvements that they possibly deserved. Two other steps in addition to CCS were essential for the realization of these new outcomes- vitrification with concomitant cryo-transfer and blastocyst stage biopsy.

The naysayers retreated, not totally but sufficiently far to encourage the bulk of the world to adopt the new processes. "Are we there yet?". No, but getting closer. In the meantime, PGD continued on its singular path but now it had to also face the multitude of positive findings associated with the benefits of CCS- find not only the correct genetics for an embryo but also the correct chromosome compliment.

How to combine the two for the best of both world's outcomes? Approaches to improved PGS (now referred to as PGT-A, PGT-SR) and combining PGD (now PGT-M) into a single will be discussed. "Are we there yet?"

PREIMPLANTATION GENETIC TESTING (PGT) FOR DE NOVO MUTATIONS (DNM)

S Rechitsky, A Kuliev
Reproductive Genetic Innovations, 2910 MacArthur Boulevard, Northbrook, IL 60062, USA

Key words:
PGT-M, De Novo Mutations, Single Sperm Typing, Polar Body Analysis

Introduction:
PGT-M may presently be applicable for any inherited disorder for which sequence information or relevant haplotypes are available for the detection by direct mutation analysis or haplotyping in oocytes or embryos. However, these approaches cannot be applied in cases of de novo mutations (DNM) in parent(s) or affected children, as neither origin nor relevant haplotypes are available for tracing the inheritance of this DNM in single cells biopsied from embryos or in oocytes. On the other hand, with the improved awareness of PGT, an increasing number of couples request PGT, without any family history of the genetic disease that has been first diagnosed in one of the parents or in their affected children. So special PGT strategies are required for the genetic conditions determined by DNM.

Materials and Methods:
We developed PGT strategies for DNM which were applied for 277 families with 83 different genetic conditions. The majority, 256 of them, were determined by dominant mutations, with only 4 by autosomal recessive and 21 - by X-linked DNM. It is of interest, that despite the expected predominance of dominant DNM of a paternal origin, with the increasing proportion of older paternal partners in the modern society, there was a comparable proportion of DNM of the paternal (138 couples) and maternal origin (110 couples). The latter presents a particular challenge in developing a PGT strategy, frequently requiring polar body testing to determine maternal haplotypes. The other challenge is presented by gonadal mosaicism detected in increasing number of PGT parents. In addition, up to 10% of the tested DNM (29 patients) were first detected in the affected children, with no evidence of detectable mutation in parents, despite the finding the corresponding mutant haplotype associated with normal allele.

Results:
PGT strategies for these families were different depending on the origin of DNM, and included an extensive DNA analysis of the parents and affected children prior to PGT, with the mutation verification, polymorphic marker evaluation, whole- and single-sperm testing, and PB analysis in order to establish the normal and mutant haplotypes, without which PGT cannot be performed. In cases of DNM of paternal origin, the DNM was first confirmed on the paternal DNA from blood and total sperm, followed by single-sperm typing to determine the proportion of sperm with DNM and relevant normal and mutant haplotypes. For a higher reliability of testing, the relevant maternal linked markers were also detected, to be able to trace for possible shared maternal and paternal markers.

In cases of DNM of maternal origin, DNM was first confirmed in maternal blood, and PGT was performed, when possible, by PB analysis, to identify the normal and mutant maternal haplotypes. Also, in order to trace the relevant paternal haplotypes, single-sperm typing was performed, whenever possible, for avoiding misdiagnosis caused by possible shared paternal and maternal markers. In cases of DNM-detected first in children, the mutation was verified in their whole blood DNA, followed by testing for the mutation in paternal DNA from blood, total and single sperm. So, in contrast to previous PGT practice, performing PGT for DNM required extensive preparatory DNA work before performing the actual PGT, with the additional tests including single-sperm analysis and the requirement of performing sequential PB1 and PB2, followed by blastocyst analysis.

Overall, we performed 516 PGT cycles for DNM for 277 couples, which resulted in pre-selection and transfer of 678 DNM-free embryos in 464 cycles (average of 1.4 embryos per transfer) yielding 262 (56%) unaffected pregnancies, with only 25 (9.5%) spontaneous abortions, and birth of 265 healthy children, confirmed to be free of DNM tested

Conclusions:
This is the world's largest series of PGT for DNM, which could not be performed by traditional approaches, due to unavailability of family history and lack of any affected family member to identify the origin of mutation and trace the inheritance of the mutant and normal alleles in oocytes and embryos. However, as demonstrated the specific strategies may be developed in search for the possible origin of DNM and relevant haplotypes as the basis for developing a PGT design for each particular couple with DNM, allowing a highly accurate pre-selection of oocytes and embryos free from DNM.

IMAGING HOW THE PREIMPLANTATION EMBRYO FORMS IN REAL TIME

Nicolas Plachta
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plachtan@imcb.a-star.edu.sg

Key Words:
Preimplantation embryo; single cell dynamics; molecular events in real time.

Preimplantation development has typically been studied using fixed specimens. To reveal the dynamics that form the embryo, we established advanced imaging technologies to visualize single-cell dynamics and molecular events in real time within the mouse embryo. With this approach, we discovered how transcription factors bind to DNA in single cells of the embryo, to regulate the first cell fate decisions during embryonic development. We also unveiled new forms of actin and microtubule cytoskeletal organization, which control how cells become polarized during compaction and how they interact with each other to establish the first forms of tissue architecture, the pluripotent and trophectoderm lineages, and the blastocyst cavity. Together, our findings uncover dynamic mechanisms controlling how the mammalian embryo forms.

HOW AND WHEN DO OOCYTE CHROMOSOMES FALL APART DURING FEMALE AGEING?

Mary Herbert
Newcastle Fertility Centre, International Centre for Life, Newcastle upon Tyne, UK

Mammalian female meiosis commences in utero and is marked by reciprocal exchange of DNA between parental homologues to form bivalent chromosomes.

Thereafter, oocytes remain arrested in prophase of meiosis I and are recruited for growth and ovulation throughout reproductive life. Oocytes ovulated later in life are derived from a depleted stock and are characterised by an extended period of arrest in prophase of meiosis I.

Extending this beyond ~35 years in humans is associated with an increased incidence of meiotic segregation errors giving rise to aneuploid oocytes, most of which are not compatible with life. Notable exceptions are trisomies of chromosome 21 and the sex chromosomes.

Bivalent chromosomes are stabilised by cohesin complexes containing the meiosis-specific alpha-kleisin subunit, Rec8. Cleavage of Rec8 by separase results in resolution of bivalents to their four constituent chromatids. This occurs in two steps: Rec8 is first cleaved on arms during anaphase I, giving rise to dyads chromosomes, and then on centromeres during anaphase II, giving rise to single chromatids. In female mammals, anaphase I occurs shortly before ovulation while/whereas anaphase II occurs after the fertilising sperm enters the egg.

Studies on human oocytes indicate a strong correlation between female ageing and premature resolution of centromeric cohesin (). Consistent with this, we and others have previously reported that female ageing in mice is characterised by depletion of Rec8 from oocyte chromosomes ().

This is accompanied by reduced recruitment of SgoL2, which protects centromeric Rec8 from cleavage by separase until anaphase II (). Together, these events are sufficient to explain the prevalence of premature loss of centromeric cohesin in oocytes from older females. We are therefore interested in understanding the timing and mechanisms of cohesin depletion during female ageing. Our recent work indicates that cohesin is gradually depleted from oocyte chromosomes during the prolonged arrest at prophase of meiosis I, before oocytes are recruited for growth. Interestingly, the age-related decline in chromosome-associated Rec8 exceeds that of Smc3, which is a subunit of mitotic as well as meiotic cohesin complexes. This suggests that cohesin complexes containing alpha-kleisin subunits other than Rec8 are present on oocyte chromosomes and might be less vulnerable to depletion during female ageing. In addition, we have tested the idea that leaky inhibition of separase during the prolonged arrest at prophase of meiosis I contributes to gradual loss of Rec8. In support of this possibility, we can detect separase in oocytes before they are recruited for growth.

We find, however, that deletion of separase specifically in oocytes does not prevent the decline in Rec8 levels observed during female ageing. Together, these findings indicate that cohesin loss occurs gradually during the prolonged prophase arrest and that this occurs by a separase-independent mechanism.

POLAR BODY ANALYSIS FOR PREIMPLANTATION GENETIC TESTING

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The use of polar bodies for preimplantation genetic testing for both single gene defects (PGT-M) and aneuploidy (PGT-A) was pioneered by Verlinsky and colleagues beginning in the late 1980s. For many years, it was applied clinically to diagnose the inheritance of maternal mutations and, using fluorescence in situ hybridisation (FISH) with chromosome specific probes, to detect maternal meiotic segregation errors resulting in aneuploidy. Most clinics now culture to the blastocyst stage and with the advent of vitrification, biopsied blastocysts can be cryopreserved efficiently while the samples are sent to a genetics lab for testing. However, polar body biopsy continues to be used in several European countries with legal or ethical restrictions on the testing of embryos.

Although the second polar body can persist to the blastocyst stage, both polar bodies are eventually lost and do not form part of the embryo. Removing both of the polar bodies is therefore minimally invasive. Polar body analysis by NGS-based copy number analysis or SNP genotyping and either karyomapping or meiomapping is highly effective for both aneuploidy and linkage-based testing for maternal mutations. The advantage for PGT-A, in particular, is that only maternal meiotic aneuploidies are identified avoiding the problem of interpreting intermediate copy number changes associated with chromosome mosaicism in multiple cell biopsies. Polar body biopsy therefore remains a valuable alternative strategy for PGT.

IMPACT OF EXPANDED CARRIER SCREENING (ECS) ON UPTAKE OF PREIMPLANTATION GENETIC TESTING FOR MONOGENIC DISORDERS (PGT-M)

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Key Words:
Expanded Carrier Screening (ECS); Preimplantation Genetic Testing for Monogenic Disorders (PGT-M)

One of important problems in the health care application of technological developments in genomics is the availability of prospective identification of those at need for these technologies. The development and implementation of prospective carrier screening programs in the last few years is expected to increase the uptake of PGT-M. But most of couples at genetic risk are unaware of their genetic profiles to request PGT, which has been improved with the upgrade of traditional ethnicity-based screening to the expanded carrier screening (ECS). Recent advances in genomic technologies allow for multiplexed platforms with ability to screen for thousands of mutations and variants at a reasonable cost. The present ECS panels differ greatly in scope and include over thousand conditions and variants of variable penetrance, which are commercially available, offering the possibility of a more comprehensive and efficient risk evaluation. However, although the potential ECS to increase the number of couples presenting for PGT-M is obvious, no data are available to evaluate their actual impact. Thus this report evaluates the impact of ECS on the uptake of PGT-M, based on our ongoing PGT-M experience, which represents the world's largest series in one center.

A total of 643 (54.6%) PGT-M cases were performed through ECS referral during the years 2016-2018, as part of our overall PGT-M practice of 1188 cases, including referrals through traditional approach during the same period of time. To evaluate the impact of ECS on the PGT-M uptake, we analyzed the dynamics of increase of at-risk couples presenting for PGT-M through ECS overall, as well as separately for each genetic condition tested, compared to the baseline referrals through the traditional approach.

The analysis showed an increase of ECS based referrals to PGT-M from 35.1% (133 of 322 cases) in 2016 to 53.8% (210 of 392 total) in 2017, and 63.3% (300 of 474) in 2018. Among the most frequently referred conditions were cystic fibrosis (CFTR) (131 of 182, 72%), the uptake of which increased from 50% in 2016 to 82.7% in 2018; Deafness (GLB2) (65 of 81, 80.2%) with the uptake growing from 31.2% in 2015 to 86.4% in 2018; Fragile X (FMR1) (102 of 128, 80%), the uptake growing from 73% in 2016 to 84% in 2018; Thalassemia and sickle cell disease (HBB) (58 of 99, 58.6%), increasing from 45.8% in 2016 to 60.9% in 2018. So, the overall number of prospective PGT-M cases for the last three years more than doubled after ECS-based referral, with a similar dynamics for each condition tested (up to 80% increase of referrals through ECS). This may become the major source for performing PGT-M in the near future, allowing to offer PGT-M prospectively before the birth of an affected child.

The data show significant increase of the PGT-M uptake following ECS, demonstrating the utility for offering PGT-M prospectively to the couples at risk.

GENE REGULATORY NETWORK IN THE CONTEXT OF GENOME INSTABILITY IN HUMAN EMBRYO DEVELOPMENT

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Key Words:
Genetic instability; Single-cell genome-plus-transcriptome sequencing; Mosaicism

Genomic instability is common in preimplantation embryos of a variety of species. Up to 80% of human cleavage stage embryos following in vitro fertilization acquire genetic mosaicism, but some may develop to normal individuals after uterine transfer. The causative mechanisms of this chromosome instability as well as the impact of acquired genetic anomalies on embryo development are not understood and remain speculative.

Using novel methodologies for single-cell genome-plus-transcriptome sequencing (G&T-seq) of all cells of human preimplantation embryos from the fertilized egg to the blastocyst stage, we disclose insight in the gene regulatory network of preimplantation embryo development and how this is impacted by aneuploidy.

Single-cell DNA CNV analysis revealed frequent missegregations of whole chromosomes as well as segmental rearrangements in all embryonic cell stages. Using these single-cell genomic profiles in combination with cell cleavage imaging of the developing embryos, we could deduce the origin of the abnormalities and construct cell lineages. Single-cell gene expression analysis of the same cells, classified the cells according to the embryonic developmental stage, the expression activation of the embryonic genome and disclosed the functional impact of acquired numerical and structural chromosome aberrations on development of the human embryo. Furthermore, the data reveals which genetic anomalies contribute to the epiblast cells in the blastocyst that provide the ectodermal, mesodermal and endodermal cell lineages, the building blocks of our organs.

PREGNANCY OUTCOME OF TRANSFERRED EMBRYOS WITH MOSAICISM AND SEGMENTAL VARIATIONS

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Mosaic embryos are characterized by the presence of chromosomally different cell lines within the same embryo. Typically, mosaic embryos were not transferred in IVF treatments because, similar to aneuploidy embryos, they were considered abnormal. However, in a recent study we have demonstrated that euploid/diploid mosaic embryos hold the potential to implant and result in the birth of healthy babies. As a consequence, the transfer of these embryos is now offered as an option for women who undergo IVF resulting in mosaic embryos but no euploid embryos.

Although the impact of mosaicism on the implantation and developmental potential of embryos is not fully known, it is reasonable to assume that it is likely to influence the clinical outcome of IVF treatments. We also hypothesized that the extent of mosaicism and the type of aneuploidy involved may affect the IVF success rate. However, no definitive conclusion could be drawn because our study was small and the data available were insufficient to test this hypothesis.

The aim of this study was to assess whether the extent of mosaicism and the type of the type of aneuploidy may influence the development potential of mosaic embryos. To test this hypothesis we enlarged our previous study, offering the transfer of mosaic embryos, at different aneuploidy percentage and aneuploidy content, to 200 women for which the IVF/PGT cycle resulted in no euploid embryos available for transfer.

Comprehensive chromosome screening was performed using high resolution next generation sequencing (NGS) methodology. The clinical outcome obtained after transfer of mosaic embryos with low (<50%) and high (≥50%) aneuploidy percentage or with different types of aneuploidy involved in mosaicism, was compared with that resulting from a control group of 500 euploid blastocysts.

We found that the reproductive potential of a mosaic euploid/aneuploid blastocyst is inversely correlated with the abnormal-to-normal cells ratio. Mosaic embryos with a high percentage of chromosomally abnormal cells (≥50%) resulted in a statistically significant reduction in clinical pregnancy rate/ET, implantation rate, and live-birth rate compared with mosaic embryos with a lower aneuploidy percentage (<50%). In contrast, embryos with lower aneuploidy percentage (<50%) have a clinical outcome similar to euploid embryos. In addition, we found that the type of aneuploidy involved in mosaicism correlates with the clinical outcome, as embryos with single or double monosomy have significant higher chances in developing into healthy euploid newborns as compared to other types of aneuploidy. The transfer of mosaic blastocysts with complex aneuploidies or segmental rearrangement showed the worst clinical outcome. No statistically significant differences in clinical results were found between mosaic embryos with monosomy and the euploid control group.

In conclusion, the results of our study demonstrated that mosaic embryos have poorer clinical outcomes compared with euploid embryos and that their implantation and developmental potential is influenced by the extent of mosaicism and the aneuploidy content.

IMPACT OF SEVERE MALE INFERTILITY ON CHROMOSOMAL STATUS OF EMBRYOS AND PREGNANCY OUTCOME IN YOUNGER WOMEN.

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The relationship between severe male infertility and embryo aneuploidy has long been a subject of interest and there have been a limited number of studies. Our study used next generation sequencing (NGS), to evaluate the chromosomal status of blastocyst stage embryos and evaluated embryo morphokinetics and pregnancy outcomes in young women (<35) according to severe male infertility subgroups.

Severe male factor (SMF) is defined as sperm concentration below 5 million per ml. This covers a very wide range from 5 million down to non-obstructive azoospermia (NOA), in which sperm production is severely impaired. In our study, SMF cases were divided into the following 5 subgroups according to sperm concentration; 1) between five million and one million, 2) between one million and one hundred thousand, 3) less than one hundred thousand (cryptozoospermia), 4) Obstructive Azoospermia (OA) and 5) Non-obstructive Azoospermia (NOA). The control group was composed of males with normal sperm parameters (>39 million and >40% motile sperm in the ejaculate). 228 severe male infertility cases and 67 control cases with normal sperm parameters. In all groups female age was <35 and PGT-A and morphokinetic evaluation were performed.

The PGT-A results showed that the overall aneuploidy rate of patients with SMF was 55.3% and the mosaicism rate was 13.2%. In the control group these figures were 55.8% and 6.7% respectively, a significantly higher mosaicism rate (p=0.001) was observed in the SMF group. Significantly higher chromosomal aneuploidy rates were found in couples with cryptozoospermia, in patients with OA and in patients with NOA compared to other male subgroups .

Furthermore, embryo morphokinetic evaluation was carried out using time lapse imaging to investigate embryo development. NOA patients reached the first cleavage significantly later than the control group (27.78h vs. 26.55h, respectively; p=0.0347). There was also a difference between obstructive and non-obstructive azoospermia patients in the time to reach blastocyst, OA patients achieving a blastocyst significantly earlier than NOA (tB= 102.20h vs. tB=104.91h, respectively; p=0.0297).

The cumulative live birth rates of SMF groups were compared. Significantly lower cumulative live birth rates were found in couples with cryptozoospermia (43.7%), in patients with OA (46%) and in patients with NOA (46.1%) compared to other SMF subgroups with five million and one million (58.1%) and between one million and one hundred thousand (71.4%) subgroups

In conclusion, the chromosomal status of embryos in severe male infertility cases with young female partners (<35) was not significantly different from that of the control groups. However, the mosaicism rate was significantly higher. Higher chromosomal aneuploidy rates were found in the most severe form of male infertility subgroups with cryptozoospermia, OA and NOA. Morphokinetic evaluation showed that NOA patients reached the first cleavage significantly later than the control group indicating that SMF impairs early embryonic competence. Finally, a significantly negative effect on clinical outcomes was observed in the three most severe male infertility (cryptozoospermia, OA and NOA) subgroups.

GENOME-WIDE PROFILING AND HAPLOTYPING OF CELL-FREE DNA ENABLING COMBINED NON-INVASIVE PRENATAL DIAGNOSIS OF INHERITED MONOGENIC DISEASES AND ANEUPLOIDY.

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Keywords:
NIPT, Haplotyping, Haplarithmisis, PGD, OnePGT

Non-invasive prenatal diagnosis for the common trisomies 21, 18 and 13 has become standard of care in many countries world-wide. We developed methods that enable not only the detection of those common trisomies but leverage the identification of all fetal and maternal genomic and chromosomal disorders. As of July 2017 NIPT is reimbursed for all pregnant women in Belgium, so the outcome of this screening program will be presented, as well as our innovative NIPT pipeline.

Non-invasive prenatal diagnosis (NIPD) for monogenic diseases has only been tested on a small number of genes using a locus-specific approach and has not become widely implemented. Here, we present a new method that enables the deduction of fetal haplotype to detect inherited monogenic diseases and aneuploidy. Using targeted sequencing in a genome-wide level of resolution, parental haplotype inheritance in the fetus was determined non-invasively with high accuracy based on cffDNA haplotyping analysis.

In all clinical cases, the haplotypes blocks linked with the wild-type allele were correctly identified, and a maternally inherited trisomy case was properly deduced. The method is generic and can deduce the presence or absence of any inherited disease allele. Moreover, it leverage the genome wide fetal haplotype at relatively low cost.

Since genome wide haplotyping of embryos (karyomapping or OnePGT) enables the reconstruction of genome-wide haplotypes, copy-number profiles and are able to determine the segregational origin of haplotypes, those methodologies are being implemented as a generic method for preimplantation genetic testing (PGT) in many IVF laboratories. This non-invasive prenatal haplotyping approach can also confirm that the fetus is derived from the embryo transferred and will replace invasive prenatal confirmation testing.

UPDATED PGDIS RECOMMENDATIONS FOR REPLACEMENT OF MOSAIC EMBRYOS

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Objective:
To review current data on the developmental potential of mosaic embryos and update previous guidelines and recommendations for transfer of mosaic embryos.

Background:
From PGT studies of blastocyst biopsies using next generation sequencing (NGS), the average frequency of embryonic mosaicism is around 10% (range 5% - 30%). Mosaicism is generated by mitotic nondisjunction errors that can affect any chromosome and manifests as either diploid mosaic, aneuploid mosaic or complex aneuploid mosaic embryos. The level of mosaicism, defined from analysis of a trophectoderm (TE) biopsy, is generally related to the timing of mitotic errors during preimplantation development. Currently, there are disputes about the biological relevance of mosaicism levels measured in a blastocyst TE biopsy by NGS. Thus, in situations where there is no available euploid embryo for transfer, even with current guidelines published by PGDIS, confusion still reigns in many clinics on whether to transfer diploid mosaic embryos.

Recent observations:
A number of studies have now been performed to follow the outcome of transferring diploid mosaic embryos detected with either whole chromosome or segmental abnormalities involving one or more chromosomes. Compared to euploid embryos, diploid mosaics with 20-40% levels of trisomy or monosomy have slightly reduced implantation potential and, those that do successfully implant are capable of developing to live births without signs of chromosome disease syndromes. Re-analysis of mosaic embryos indicates that while the majority of second TE biopsies are concordant for the type of mosaicism originally detected by PGT, there can be differences in levels of mosaicism in the TE compartment, and in some cases, second biopsies appear euploid. In general, the mosaicism found in TE biopsies is not reflected in the ICM lineage. These studies suggest that the level of mosaicism determined by NGS and reported to the clinician from a PGT cycle may not always be indicative of the entire blastocyst. More recently, there is also growing evidence from sequencing data that artificial segmental mosaicism can occur due to biased chromosome representation in the WGA and library construction steps. Thus, biological and technical factors may occasionally falsely contribute to a diagnosis of embryonic mosaicism.

Recommendations:
Clinics that routinely observe high levels of mosaicism should systemically review IVF and PGT protocols. In the absence of euploid embryos for transfer, genetic counseling should be first offered to explore the possibility of transferring a diploid mosaic embryo or initiating another PGT cycle. While outcomes are slightly compromised, diploid mosaic embryos with 20-50% mosaicism can be transferred regardless of the chromosomal abnormality involved. Transfer of embryos with 50-80% mosaicism should be considered with more caution, in particular, mosaic embryos associated with (i) trisomies of chromosome 21, 18 and 13 capable of developing to live births, (ii) trisomies causative of intrauterine growth restriction or (iii) segmentals causative of known chromosome disease syndromes. Following an ongoing pregnancy achieved with a diploid mosaic embryo, prenatal diagnosis by amniocentesis is highly recommended.

GENOME EDITING OF A HUMAN EMBRYO – COULD CORRECTION REPLACE SCREENING?

Helen O'Neill

Genome editing (CRISPR-Cas9 or Base Editing) is a revolutionary tool which allows DNA or RNA to be altered at directed loci in the genome with ease and efficiency. The technology, which is derived from and mimics the immune system of bacteria, offers unprecedented ability to alter, correct or insert genes into a genome. The technology has been adopted globally in the research setting and offers limitless potential for personalised medicine; indeed, somatic cell therapies are already benefiting from this technology. The advances in genome editing coupled with assisted reproductive technologies and genomics lend themselves to germline therapy and the correction of genetic disorders in embryos. Could correction replace screening?

In order for this to reach the clinic, significant and rigorous testing must be carried out ensure that edits are controllable, reproducible and that mosaicism is avoided. Our research focuses on the delivery of components of CRISPR, assessing the accuracy of edits and measuring mosaicism in edited murine preimplantation embryos.

CLINICAL IMPACT OF MATERNAL-EMBRYONIC COMMUNICATION AT IMPLANTATION

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The endometrium is a hormonally regulated organ that is non-adhesive to embryos throughout most of the menstrual cycle in humans. Endometrial receptivity refers to a hormone-limited period in which the endometrial tissue acquires a functional and transient ovarian steroid-dependent status allowing blastocyst adhesion. Functional genomic studies of human endometrium in natural cycles have demonstrated that endometrial receptivity is an active process involving up- and down-regulation of hundreds of genes (1).

Personalized medicine is a well-accepted concept in reproductive medicine except for the endometrial factor that is still neglected. Our group has developed the endometrial receptivity array (ERA) (2), a customized array of 238 genes now performed using Next Generation Sequencing (NGS) coupled to a computational predictor capable of diagnosing the window of endometrial receptivity regardless of its histological appearance (2). The accuracy of the diagnostic tool ERA has been demonstrated to be superior to endometrial histology and results are completely reproducible 29 to 40 months later (3).

The aim of this presentation is to demonstrate the diagnostic and therapeutic efficiency of ERA in patients with implantation failure (IF), through personalization of the day of embryo transfer (pET) (4-8). We are conducting an international RCT to investigate the reproductive outcome of infertile women under 38 year (BMI of 18.5-30 and AFC>8) s in their first IVF/ICSI cycle with elective blastocyst transfer randomly allocated to be performed in a fresh cycle, after freezing all embryos or after identification of the personalized WOI with the ERA test (pET). According to a preliminary analysis after recruiting 356 patients of the 546 planned, the pET group had a higher pregnancy rate per ET, and a trend to a higher implantation rate and ongoing pregnancy rate (9).

Finally, the investigation of endometrial bacterial communities has revealed that the endometrial cavity is not sterile. The presence of a Non-Lactobacillus-dominated microbiota (NLD)(<90% Lactobacilli)(10) or pathogens responsible for chronic endometritis (11) in infertile patients are associated with decrease reproductive outcome in terms of implantation, pregnancy and live birth rates.

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NON-INVASIVE PGT-A WITH THE ANALYSIS OF EMBRYONIC CELL FREE DNA IN SPENT BLASTOCYST MEDIA

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The success of assisted reproduction treatments is based on the selection of the best embryos for transfer, those with the highest implantation potential and ongoing pregnancy rates: euploids. The most reliable method to assess the chromosomal status of preimplantation embryos is preimplantation genetic testing for aneuploidies (PGT-A). Other options such as morphology and morphokinetics are not good candidates to replace PGT-A techniques as their correlation with embryo ploidy is weak. However, biopsy-based approaches on PGT-A entail both technical and economic challenges as embryo manipulation is needed and it could affect viability. To avoid those limitations, 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.

In the last years there have been different attempts to overcome trophectoderm biopsy to diagnose the chromosomal content of the embryos. Some groups started with the analysis of blastocoel fluid obtained by aspiration with a thin micropipette as a less invasive approach than TE biopsy. Later on, some groups proposed a "true" non-invasive approach consisting in the study of the spent culture media to analyse the embryonic cell free DNA (cfDNA) released by the embryo during the latest stages of preimplantation development. After the first publications, several studies have compared the results of PGT-A in TE biopsies with the results of the spent culture media, to establish the concordance rates among both approaches. In these studies, the percentages of informative samples vary widely, likely reflecting the existence of mosaicism and/or presence of DNA from granulosa, cumulus cells or polar bodies in the spent blastocyst media (SBM) urging the need for technical improvements before this new non-invasive technology can be clinically applied.

We have conducted a pilot study comparing aneuploidy testing using a non-invasive approach to trophectoderm biopsy, advancing one step towards understanding the potential value of niPGT-A. With the incorporation of a non-invasive approach, we aimed to address the two main limitations of current PGT-A, namely invasiveness and diagnosis of mosaicism, as only 5-8 cells are retrieved from the whole blastocyst in a TE biopsy. Regarding clinical outcomes, we have followed-up data on a subset of patients after SET performed according to TE biopsy results and compared the clinical outcome retrospectively according to cfDNA results. Interestingly, ongoing implantation rates were three-times higher when both TE and cfDNA were euploid, than in euploid TE paired with aneuploid cfDNA indicating that embryonic cfDNA might open a new avenue for the understanding of embryo ploidy. The origin of embryonic cfDNA is still unclear. Also, examining the mechanisms implicated in the secretion of cfDNA to the media is challenging.

ADVANCES IN IN-VIVO FERTILIZED AND MATURED HUMAN EMBRYOS AND CHARACTERIZATION BY PRE-IMPLANTATION GENETIC TESTING (PGT-A)

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Keywords:
in vivo, uterine lavage, blastocyst, IVF, PGT

Introduction:
Early work performed by Buster et al. (1985) demonstrated the recovery of embryos from the uteri using uterine lavage. The advancement in embryo cryopreservation, blastocyst culture and the development of comprehensive methods of PGT-A have refocused attention on uterine lavage as a possible nonsurgical minimally invasive approach for fertile individuals who desire PGT-A without utilization of in vitro fertilization (IVF).

Materials and Methods:
Gonadotropin induced ovarian stimulation and insemination were performed followed by uterine lavage to recover the in vivo conceived embryos. Recovered embryos were characterized morphologically using the Gardner Scale, underwent trophectoderm (TE) biopsy, were then vitrified and stored in liquid nitrogen. Biopsies were analyzed using next-generation sequencing (NGS) technique. Results were compared with in vitro conceived embryos from other cycles of the same patients.

Results:
Uterine lavage performed after insemination successfully recovered blastocysts providing a reliable and consistent means to capture in vivo conceived embryos. Results to date from this ongoing study (134 of 500 planned lavage cycles completed) show consistent improvement in successive cohorts with respect to the ability of the lavage system to recover embryos. Embryos of many stages were recovered in 42% (56/134) of the cycles, out of which, 71% (96/136) embryos were blastocyst stage embryos representing a most advanced stage of embryogenesis.

The 63% of in vivo conceived embryos were euploid or low-mosaic (<40% abnormal), which was similar to that of IVF conceived embryos (64%). High-mosaicism rate (11% vs 9%), complex abnormal rates (8% vs 10%) and aneuploidy rates (19% vs 17%) were also similar. Three blastocysts were not identified and left out of the analysis. A 73.7% (70/95, 1 blastocyst not graded) of recovered in vivo embryos were of good quality as determined by the Gardner Scale of Morphology compared to 178 of controls.

Conclusions:
Although a nascent technology, studies indicate that Uterine Lavage may have the potential to provide a new tool to acquire genetic information on in vivo conceived embryos. The procedure has the potential to provide a low cost, minimally invasive, reproducible and effective way to acquire in-vivo conceived embryos.

Uterine Lavage provides an alternative option to IVF for fertile couples who want to be proactive in the health of their future children. It offers a platform for genetic screening (PGT-A) prior to the establishment of pregnancy as well as the diagnosis and identification of genetic indications. From a research perspective, the study of in-vivo conceived embryos when compared to IVF-derived embryos may provide new scientific insights into embryo science by characterizing any difference between embryos conceived within and outside the uterus.

	in vivo		IVF	
	Blastocysts		Blastocysts	
Euploid	50	54%	83	51%
Low Grade Mosaic (≤40%)	8	9%	22	13%
High Grade Mosaic (>40%)	10	11%	14	9%
Aneuploid	18	19%	28	17%
Complex Abnormal	7	8%	16	10%
Total	93	100%	163	100%

OC-01

SUCCESSFUL HEMATOPOIETIC STEM CELL TRANSPLANTATION IN 62 CHILDREN FROM HEALTHY SIBLINGS CONCEIVED AFTER PREIMPLANTATION HLA MATCHING

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hematopoietic stem cell transplantation (HSCT), human leukocyte antigen (HLA) matching, inherited and acquired hematological disorders, in vitro fertilization (IVF), preimplantation genetic diagnosis (PGD)

Introduction:
The technique of PGD, coupled with HLA matching, emerged as a therapeutic tool in 2001, when it was first used for Fanconi anemia to select mutation-free and HLA identical embryos generated through IVF techniques (Verlinsky et al., 2001). In addition to allowing couples the opportunity of having an unaffected child, the technique also allows a potential donor for stem cell transplantation for the affected sibling to be selected (Grewal et al., 2004). HLA matching without mutation analysis has also been used for acquired diseases, such as acute myeloid leukemia, acute lymphoblastic leukemia, and chronic myeloid leukemia (Goussetis et al., 2011; Rechitsky et al., 2004).

Material and methods:
Between 2003 and 2018, 312 referred couples, with at least one child in need of HSCT, underwent PGD for HLA matching at our clinic. The referred couples applied mainly for hemoglobinopathies, hematological malignant disorders, bone marrow failure disorders, metabolic diseases and immunodeficiencies. Although the indications were diverse, the major indication for preimplantation HLA matching was beta-thalassemia. The genetic diagnosis of the disorders in children had been completed before their application to our center. A preclinical set-up study was first conducted. Before preimplantation HLA matching, HLA haplotype analysis was carried out on peripheral blood samples of the mother, father and child of each family. When available, samples from other family members were obtained, such as healthy siblings or grandparents.

Results:
312 couples underwent 626 PGD for HLA matching cycles (122 HLA only and 504 single gene disorder and HLA typing cycles). Each couple has an average of 2.04 PGD cycles. A total of 114 live births were reported (128 babies born). 66 allogenic HSCT were performed, of which 62 were successful. In 57 patients, HSCT was not performed: 10 patients are awaiting HSCT, 5 children passed away while awaiting HSCT, for 15 children HSCT is not planned yet, 3 children had a HSCT from an unrelated donor, 3 children born were HLA non-identical. At the time the abstract was written detailed clinical information for 14 children were still being gathered from hematologist and 7 pregnancies were still ongoing.

Conclusions:
The follow up of families with affected children and siblings conceived after PGD is crucial. In the present study, full recovery was achieved after successful HSCT in 62 affected sib-lings. World-wide awareness should be increased, and the feasibility of this technique should be discussed with families as an alternative option. Preimplantation HLA matching, therefore, provides a realistic option for couples in the treatment of an affected sibling when no HLA-matched donor is available.

OC-02

PATHOGENIC DNA VARIATION WITHIN ACMG SECONDARY FINDINGS GENES IN 18,000 HEALTHY INDIVIDUALS USING CLINICAL EXOME SEQUENCING FOR CARRIER SCREENING

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Expanded carrier screening, next generation sequencing, secondary findings, clinically actionable genes, assisted reproduction.

Introduction:
Expanded carrier screening (ECS) programs aim to reduce the burden of recessive single-gene disorders by identifying the genetic risk for the offspring of individuals in reproductive age. ECS programs using Next Generation Sequencing (NGS) technology can get additional DNA variation in genes that may impact on individual's health. A significant group of clinically actionable genes are the secondary findings (SF) genes defined by the ACMG. Here, we describe the impact of pathogenic DNA variation in the SF genes providing evidence of additional useful clinical information for patients' health management.

Material and methods:
Retrospective analysis of sequencing data of 18,364 individuals that underwent ECS for ART purposes from September 2015 to December 2018. Females represented the 51.7% (9,488) of tested individuals whereas 8,876 males accounted for the remaining 48.3%. Distribution between patients and gamete donors was 59.6% (10,943) vs 40.4% (7,421) respectively. DNA samples were sequenced in a NextSeq 500 system (Illumina) using a clinical exome kit evaluating 4,800 genes (TruSight One sequencing panel; Illumina). Anonymized sequencing data were processed bioinformatically for a retrospective analysis focused on the 59 genes included on the ACMG recommendations for reporting of secondary findings (ACMG SFv2.0) (Kalia et al., 2016).

Results:
Based on Clinvar (Landrum et al., 2016) interpretation criteria, at least one pathogenic/likely-pathogenic variant associated to SF recommended for return was identified in 2% of the tested individuals (n=369). The distribution of positives findings between genders was: 176 males (2% of 8,876) and 193 for females (2% of 9,488). Likewise, the distribution of positives findings between ART patients and gamete donors was: 218 positive patients (2% of 10,943), and 151 for donors (2% of 7,421). In the retrospective analysis, a total of 238 unique pathogenic variants were detected. At least one variant was found in 33 (30 AD; 1 AR; 2 XL genes) of the 59 ACMG SF gene list. The most frequent pathogenic finding was the splice acceptor variant NM_000238.3:c.1946-2A>C in the KCNH2 gene (n=13), associated to QT long syndrome. The BRCA1 and BRCA2 genes were the most frequently positive called genes, with 75 unique pathogenic variants (31.5% of 238). Lynch syndrome associated genes were the second more frequently called positive genes, with 35 unique pathogenic variants distributed among the 4 analysed genes.

Conclusions:
The analysis of the ACMG SF genes provided evidence of pathogenicity for about 2% of the tested individuals with around 30% of positive cases associated to BRCA1 and 2 genes. Based on these results, analysis of SF genes can be optionally offered along with ECS, and patients willing to know this potentially useful information can have a benefit from it.

OC-03

SELECTIVE TRANSFER OF EUPLOID NONCARRIER EMBRYOS WITH THE USE OF LONG-READ SEQUENCING IN PREIMPLANTATION GENETIC TESTING FOR RECIPROCAL TRANSLOCATION

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PGT-SR, nanopore sequencing, long-read, reciprocal translocation

Introduction:
Balanced reciprocal translocation is one of the most common chromosomal abnormalities in humans. Carriers are usually phenotypically normal but are at an increased risk of infertility, recurrent miscarriage or having affected children. Preimplantation genetic testing for chromosomal structural rearrangement (PGT-SR) offers a way to screen against embryos with unbalanced translocation during in vitro fertilization (IVF) treatment cycles. In this case report, we demonstrated a new approch to discriminate carrier from noncarrier embryos. First, high-resolution breakpoint mapping was performed using long-read sequencing, followed by breakpoint PCR. In the treatment cycle, PGT-SR was performed by NGS and euploid noncarrier embryos were selected using breakpoint PCR.

Material & methods:
a) Long-read sequencing
A couple requested PGT-SR because the wife, aged 33 years, was known to be a carrier of balanced translocation [46,XX,t(7:13)(p13;q12.3)]. A sequencing library (1D) was prepared on the genomic DNA of the wife using the SQK-LSK-108 kit (Oxford Nanopore, UK) according to the manufacturers's protocol. 1D sequencing was performed on a MinION flow cell (R9.4, Oxford Nanopore) with a 48-hour protocol on MinKNOW (2.2.12). Local base calling was performed by Albacore 2.3.1. After sequence alignemnt, breakpoints were determined by NanoSV 1.2.0.
b) Breakpoint PCR
Based on the predicted breakpoints that correlated with cytogenetic report, breakpoint PCR primers were designed and validated using genomic DNA of couple and the sequences flanking the breakpoints were confirmed by Sanger sequencing.
c) PGT-SR
Whole genomic amplification (WGA, REPLI-g Single Cell kit) was performed followed by PGT-SR by NGS (VeriSeq-PGS, Illumina). Breakpoint PCR primers validated in (b) were used to discriminate carrier from noncarrier embryos.

Results:
Nanopore sequencing obtained an average read length of 7000 bases. The total no. of based aligned to human genome was 16 Giga bases. Breakpoints were accurately predicted on chromosomes 7 and 13. Eighteen oocytes were retrieved and 15 were fertilized. Trophectoderm biopsy was performed on 9 blastocyts. After PGT-SR, there were 2 euploid embryos and 2 mosaic embryos. Four embryos were unbalanced carriers and one embryo with no result due to failure in WGA. Breakpoint PCR was used to discriminate carrier from noncarrier embryos. Both euploid embryos and one mosaic embryo were noncarriers while the other mosaic embryo was a carrier. One euploid noncarrier blastocytst was replaced in FET cycle. The patient is pregnant and has been referred to prenatal clinic for follow up (10-week at the time of abstract submission).

Conclusion:
Long-read sequencing enables accurate high-resolution breakpoint mapping directly on balanced reciprocal translocation carriers. In addition, nanopore sequencing required simple library preparation (90 minutes) and low capital cost, therefore the cost of preclincial test (nanopore sequencing and validation of breakpoint PCR) for the first PGT-SR cycle is approximately US$ 1000. In conclusion, we demonstrated a new method to discriminate carrier from noncarrier embryos with the use of nanopore sequencing and breakpoint PCR. This method allows patients to prioritize the transfer of euploid noncarrier embryos.

OC-04

PGT-M FOR *DE NOVO* MUTATIONS – HAPLOTYPE DETERMINATION USING MORPHOLOGICALLY POOR EMBRYOS

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PGT-M, de novo

Introduction:
Couples requesting PGT-M involving a *de novo* mutation pose a challenge when the PGT-M test used incorporates linkage analysis, as parental samples cannot be used to determine the mutant and normal allele haplotypes. In our experience, *de novo* mutations represent approximately 6-7% of clinical referrals, and thus it is important to have strategies in place to be able to offer PGT-M for these patients.
Historically, we have employed two main strategies for these couples. If the mutation in question was paternal, we would perform single sperm analysis to determine the mutant haplotype. When the mutation was maternal, we would perform single chromosome sorting to determine the mutant haplotype. We would then confirm the phase when we obtained samples from clinical embryos.
More recently, we began to question if determining phase prior to a PGT-M cycle was necessary, as we were confirming the phase as part of the cycle. We decided to evaluate if it was quicker and simpler to aim to determine phase directly from the embryos created as part of the clinical PGT-M.

Materials and methods:
Haplotype determination was performed for the cases, utilising biopsies from clinically usable embryos. In addition, in order to obtain enough sample numbers to determine phase, when there were less than 4-5 embryos biopsied, we also requested samples from embryos with poor morphological development. Karyomapping was the PGT-M test performed, in combination with a method to detect the mutation from MDA amplifications from the biopsied samples. A grandparental DNA reference was available in all cases to evaluate SNP coverage prior.

Results:
We obtained 3-7 samples from each of 14 cases to determine mutant and normal haplotypes. On 8/14 (57%) occasions, there were not enough samples from clinically usable embryos to provide us with the required sample numbers, so additional samples from embryos with poor morphological development were requested. Phase was established in all 14 cases. In all 12/14 (86%) cases the de novo mutation had a grandpaternal origin consistent with previous cases showing 89% of these de novo mutations have arisen in a sperm.

Conclusions:
This strategy proved successful in all 14 cases, allowing PGT-M to proceed without any delay. Additional samples from morphologically poor embryos were required 57% of the time, in order to obtain the necessary sample numbers to determine the mutant haplotype. This strategy can be employed to facilitate fast access to Karyomapping based PGT-M for cases involving *de novo* mutations, with a back-up strategy of using the above mentioned other methods if sample numbers are lacking.

OC-05

NOVEL APPROACH ENABLING THE SIMULTANEOUS DETECTION OF SNV AND CNV FOR PGT-M AND PGT-A USING A SINGLE-TUBE ASSAY.

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Preimplantation Genetic Testing (PGT), monogenic diseases, aneuploidies, cystic fibrosis

Introduction:
Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene, *CFTR.* An individual must inherit two defective cystic fibrosis alleles, one from each parent, to have the disease. Worldwide, over 70,000 individuals are affected by this monogenic disease. Preimplantation genetic testing (PGT) aims at selecting healthy embryos by screening for chromosome abnormalities and monogenic diseases. Often a limited number of cells or genetic material from the embryo is available to perform the detection of both aneuploidies and monogenic diseases with accuracy. To overcome this challenge, we developed a novel single tube assay enabling the simultaneous detection of both copy number variations (CNV) across the whole genome and single nucleotide variants (SNV) or small insertion/deletion (indels) located in the *CFTR* gene target region.

Material and Methods:
The assay was performed using a modified SMARTer® PicoPLEX® Gold Single Cell DNA-Seq kit (Takara Bio USA, R300669). More specifically, the assay included simultaneous whole genome amplification (WGA) and targeted enrichment of selected regions of the CFTR gene. All 23 mutations of *CFTR* recommended to be tested by the American College of Medical Genetics (ACMG) were covered. As proof of principle, we prepared libraries from either single cells or five cells using model cell lines purchased from the Coriell Institute that contain different known *CFTR* variants, such as delF508, or aneuploidies of different sizes (2.5MB to 25 MB). In addition, libraries were made from trophectoderm biopsies isolated in the clinic from human blastocysts containing known *CFTR* mutations. The resulting libraries were sequenced on a MiSEQ to achieve 1M reads per sample (2 x 75 cycles).

Results:
The percentage of sequencing reads allocated to both applications (CNV and SNV detections) was optimized to maximize the performance of the assay. To detect copy number variations, a shallow and even coverage of the genome is sufficient (~0.025x). However, to detect variants, SNPs or small indels, the targeted regions of interest required a robust and deeper coverage (500x). At a sequencing depth of 1M reads, the coverage of the genome was ensured by 95% of the reads and the targeted regions of *CFTR* were covered by ~5% of the reads. When using the five-cell model system, the 2.5 and 25MB chromosomal losses and the characterized heterozygous variants of *CFTR* were detected virtually 100% of the time (n of 18). When using single cells, we consistently detected the 25MB deletion (n of 18). Also, the expected heterozygous *CFTR* variants were detected over 80% of the time.

Conclusion:
The sequencing reads allocated to both applications was optimized to maximize the performance of both CNV and SNV detection. Over 20 samples were successfully processed on a single MiSeq run. From 5 sorted cells our new approach provided representative and uniform coverage of the genome, suitable for detection of copy number variations (CNV). From the same assay, mutations contained in the *CFTR* gene were also accurately reported. Studies with TE biopsies with known *CFTR* mutations are now in progress and will be reported at the meeting.

OC-06

PGT-A METHOD ALLOWING COMBINED PGT-M FOR DETECTION OF HETEROPLASMY AND ESTIMATION OF MTDNA MUTATION LOAD IN EMBRYO BIOPSIES.

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PGT-M, mitochondria, mtDNA, heteroplasmy, WGA

Introduction:
A large number of pathogenic mitochondrial DNA (mtDNA) mutations have been identified and are implicated in a variety of disorders. As mtDNA is maternally inherited, women with pathogenic mtDNA mutations are likely to have affected children, the severity of the phenotype depending on the heteroplasmy proportion. A systematic meta-analysis showed that there is a ≥ 95% chance of being unaffected at a mutant level of ≤ 18% (Hellebrekers et al, 2012). PGT-M can be used to identify embryos with mutation loads below this phenotypic threshold. The PG-Seq™ kit yields superior coverage of mtDNA and may allow combined PGT-A and PGT-M for mtDNA diseases from single biopsies.

Material and methods:
Two cell lines, a reference and "mutant", with mitochondrial single nucleotide variants (SNV) were selected. Six 5-cell samples from each cell line were whole genome amplified (WGA). The replicates were pooled before the cell lines were mixed in proportions ranging from 0% to 100% (mutant), in increments of 10%. Libraries were prepared for each of the pools prior to sequencing using the MiSeq instrument (Illumina). SNV sites with < 10x depth across all samples were removed, leaving 31 SNV sites. The proportion of mutant SNV at each site was calculated and analysed. The data were corrected for variance introduced by pipetting error in pooling. These corrections were only applied to samples with proportions of 40%, 50%, and 60%, and subsequent analyses were restricted to these data. To model data at different read depths we combined data across sites.

Results:
The average (± sd) mitochondrial read count for cell line samples was 5037 (± 1083). After filtering, the average (± sd) depth of coverage for the cell line samples at the SNV sites was 25.8x (± 12.5). Combining SNV data *in silico* to achieve a read depth typical of a standard PG-Seq™ kit 48 sample run using a single embryo biopsy (500,000 reads total), the observed SNV proportion was within 25% of the expected proportion in all cases. Combining SNV data to achieve a read depth of 5x a standard PG-Seq™ kit 48 sample run, the observed SNV proportion was within 11% of the expected proportion in all cases. This means that in any case where a heteroplasmy is detected at 7% or lower, including no detection, the mutant load should be below the phenotype threshold.

Conclusions:
The superior amplification of mtDNA achieved by the PG-Seq™ kit may allow combined PGT-A and PGT-M for mtDNA analysis. Combining PGT-A and PGT-M in this novel way would provide a streamlined workflow over currently available workflows. While accuracy is dependent on read depth, read depth can be increased by modulating sequencing throughput or by alternate means such as using PerkinElmer's Target Sequence Enrichment protocol. Due to variable but consistent depth of coverage across the mtDNA, some sites will be more suited for analysis by this method than others.

OC-07

IMPACT OF CHROMOSOMAL MOSAICISM IN IVF OUTCOMES: EXPERIENCE FROM TWO HUNDRED MOSAIC EMBRYOS TRANSFERRED PROSPECTIVELY

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Mosaic embryos, preimplantation genetic testing, next generation sequencing, chromosomal mosaicism,

Introduction:
Chromosomal mosaic embryos are characterized by the presence of chromosomally different cell lines within the same embryo. In a recent published study, we have demonstrated that mosaic embryos hold the potential to implant and result in the birth of healthy babies. Therefore, the transfer of these embryos is now offered as an option for women who undergo in vitro fertilization (IVF) resulting in only mosaic embryos but no euploid embryos for transfer. However, there is still few data concerning the impact of mosaicism on viability, and the classification of such embryos in relation with their reproductive potential is unclear. Here we investigated if chromosomal constitution of mosaic embryos influences the clinical outcome of in vitro fertilization treatments

Material and methods:
The transfer of mosaic embryos was offered to 200 women for whom IVF had resulted in no euploid embryos between May 2016-May 2018. Trophectoderm (TE) biopsy was performed on Day-5 of development or Day6/7 for slow growing embryos. The clinical outcome obtained after transfer of mosaic embryos with different chromosomal constitution was compared with each other and with that obtained from a control group of 500 euploid blastocysts. Comprehensive chromosome screening PGT-A was performed using high resolution next generation sequencing (NGS) methodology. TE biopsies were classified as mosaic if they had 20%-80% abnormal cells. For statistical analysis mosaic embryos were divided in four groups based on chromosomal constitution: mosaic monosomy (single and double monosomies; MM), mosaic trisomy (single and double trisomies, MT), mosaic complex aneuploidy (more than two different aneuploidies; MC) and mosaic segmental aneuploidy (single and double deletion or insertion >5Mb, MS).

Results:
MM showed significant higher birth rate compared to MT (46% vs 24%,p=0.02), MC (46% vs 23%,p=0.03) and MS (46% vs 22%,p=0.02). No significant difference was observed in clinical outcome between the groups MT, MC and MS. A comparison of the clinical outcomes with control euploid group showed no significant difference between euploid control and MM, while a significant low implantation rate (55.4% vs 37% MT, vs 31% MC, vs 23% MS,p<0.05) and live birth rate (48.4% vs 24% MT, 23% MC, vs 22% MS,p<0.05) between the euploid control and rest of mosaic groups was observed (Table). The highest biochemical pregnancy rate (31%) and early abortion rate (13%) was observed in MS, and MT, respectively.

Conclusion:
The study demonstrated that embryos with MT, MS and MC aneuploidies have lower chances of resulting with birth of healthy babies compared to embryos with MM. More interestingly, no difference has been find in clinical results between MM and euploid control group. These findings should be considered for genetic counseling.

		Chromosomal constitution			
	Euploid	MM	MT	MC	MS
Embryos	500	72	38	62	32
Transfer	500	71	38	62	32
Positive bhCG	325(65%)§	41(58%)	18(47)%	26(11%)	7(54%)
Biochemical pregnancies	48(9.6%)	6(8%)	4(11%)	7(11%)	4(31%)
Embryos implanted	277(55.4%)§	35(49%)	14(37%)	19(31%)	3(23%)
Early abortion	35(7%)	3(4%)	5(13%)	4(6%)	1(3%)
Babies Born	242(48.4%)§	33(46%)*	9(24%)	14(23%)	7(22%)

*p<0.05 MM vs MT, MC and MS
§p<0.05Euploid vs MT, MC, MS

OC-08

SEGMENTAL ANEUPLOIDIES SHOW MOSAIC PATTERN REDUCING PREDICTIVE VALUE COMPARED TO HIGH WHOLE CHROMOSOME ANEUPLOIDIES REPRESENTATIVENESS

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PGT-A, embryo selection, NGS, segmental aneuploidies, mosaicism

Introduction:
The introduction of highly sensitive Next Generation Sequencing (NGS) platforms for PGT-A applications concurred with an increased detection of segmental aneuploidies. Recent studies focused on understanding whether segmental aneuploidies derive from mitotic or meiotic events, however, definitive data are still lacking. To further contribute on the characterization of segmental aneuploidies and their biological significance, here we evaluated multifocal portions of trophectoderm (TE) tissues and Inner Cell Mass (ICM).

Material & methods:
Following technical validation on cell lines carrying segmental aneuploidies, a cohort study was performed between January 2018 and January 2019. After initial PGT-A analysis and genetic counselling, patients who wanted diagnostic confirmation for embryos carrying segmental aneuploidies >15Mb were enrolled. A first subset of 55 embryos carrying segmental alterations, either in concomitance (n=24) or not (n=31) with whole chromosome aneuploidies were warmed, allowed to re-expand and subjected to TE re-biopsy. An additional subset of 19 donated embryos was subjected to ICM isolation. Each biopsy was processed using Ion ReproSeq PGS kit and sequenced on Ion S5 platform. Sequencing data were blindly analyzed with Ion-Reporter software and compared with original diagnosis.

Results:
In the first subset of samples, regarding whole chromosome aneuploidies, comparisons between PGT-A results obtained from the first and second TE biopsy showed a concordance rate of 96.36% (n=53/55;95%CI=87.47-99.56) per sample and 99.84% (n=1263/1265;95%CI:99.43-99.98) per chromosome. Sensitivity per chromosome was 94.29% (n=33/35; 95%CI=80.84-99.30) and specificity 100% (n=1230/1230;95%CI=99.70-100.00). Regarding segmental aneuploidies, technical validation on cell lines resulted in 100% concordance (n=12/12;95%CI=73.5-100.0). Subsequently, overall PGT-A results showed that only 53.97% (n=34/63;95%CI=40.64-66.61) of segmental alterations were confirmed in the second TE biopsy. In this group of results, 30.16% (n=19/63;95%CI=19.23-43.02) of paired samples showed the same alteration, suggesting a meiotic origin. Of the remaining results, 23.8% of the alterations (n=15/63;95%IC=13.98-36.21) showed a different aneuploidy pattern. In detail, 11.11% (n=7/63;IC95%=4.59-21.56) carried the reciprocal segmental aneuploidy of the same chromosome fragment and 12.7% (n=8/63;IC95%=5.65-23.50) showed the corresponding whole chromosome aneuploidy. These findings suggest that a percentage of TE segmental alterations are present in a mosaic constitution, consistent with mitotic origin. Discordant results, defined as the absence of any other alteration on the chromosome affected by the segmental aneuploidy, were 46.03% (n=29/63; IC95%=33.39-59.06). In the second subset of samples, considering ICM samples showing segmental aneuploidies in their corresponding clinical TE, 10 out of 19 (52.63%;95%CI:28.86-75.55) showed concordant karyotype whilst in 9 of 19 samples (47.37%;95%CI:24.45-71.14) the segmental alteration was not confirmed.

Conclusions:
As opposite to whole chromosome, segmental aneuploidies are not uniformly present across different blastocyst sections for a significant proportion of cases, reducing their diagnostic predictive value in IVF/PGT-A cycles. These data are important for patient counselling in cases when the embryo carries only segmental alterations; practitioners should inform patients that those embryos might be mosaic and viable. Nevertheless, the clinical value of embryos showing segmental abnormalities is still unknown and shall be evaluated in future studies. Concomitantly, the high rate of intra-blastocyst concordance observed for whole chromosome aneuploidies confirms their high clinical predictive value and diagnostic robustness of NGS for PGT-A.

OC-09

ASSESSMENT OF ANEUPLOIDY AND MOSAICISM CONCORDANCE BETWEEN DIFFERENT TROPHECTODERM BIOPSY SITES AND THE INNER CELL MASS EVALUATED WITH NEXT-GENERATION SEQUENCING

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aneuploidy, mosaicism, concordance, trophectoderm, inner cell mass

Introduction:
It is recognized that the biopsy of 6-8 TE cells allows for high-resolution next-generation sequencing. However, concerns have been raised as to whether one biopsy sample can be representative of the whole blastocyst regarding aneuploidy and mosaicism. A recent study estimated the TE confined mosaicism rate at 14% and to be due to discordant chromosomal constitution of ICM and TE (Chuang et al., 2018). Another study showed that one clinical TE biopsy was an excellent representative of blastocyst karyotype in cases of whole chromosome aneuploidy, but not for cases with only segmental aneuploidy (Victor et al., 2019).

Material and Methods:
101 clinical biopsies were taken from a site opposite to the ICM (TE1) and the second sample was taken from thawed blastocysts previously diagnosed as being aneuploid with the informed consent of the patients (TE2). Five aneuploid blastocysts were also biopsied from within the ICM. Both samples were analyzed by the same platform, ReproSeq on Ion Torrent S5 (Thermo Fisher Scientific) next generation sequencing (NGS) to assess ploidy and mosaicism concordance. Blastocysts of good or top-quality belonging to 78 patients were subjected to TE biopsy for PGT for aneuploidy (PGT-A) with the clinical indications of advanced maternal age, repeated implantation failures or a previous history of abnormal fetal karyotype. Blastocysts were then vitrified as to our routine practice. Samples were analyzed by NGS and blastocysts diagnosed as being aneuploid were thawed, re-biopsied and re-analyzed by NGS.

Results:
A total of 101 TE biopsies with whole chromosome aneuploidies were compared with 96 TE biopsy samples taken at another site of the blastocyst and 5 biopsies of the corresponding ICM. 76 samples out of 101 (75.2%) were found to be fully concordant. 25 diagnoses (24.7%) differ between TE1 and TE2: 13 mosaicisms and 10 segmental aneuploidies were either found in TE1 and not in TE2 or vice versa, without altering the overall aneuploid diagnosis. For four blastocysts, whole chromosome aneuploidies differ between TE1 and TE2, the first diagnosis indicating a chaotic aneuploidy and the second one a simple monosomy/ trisomy or vice versa. Regarding the five ICM samples, they were entirely concordant for full chromosome aneuploidies with their corresponding TE samples. However, in one sample, the 30% mosaicism of chromosomes 1, 13 and 16 was not shared by the ICM.

Conclusions:
The high rate of concordance in two separate TE biopsies and ICM observed in this study validates the reliability of PGT-A by NGS. Blastocysts with segmental aneuploidies only may be re-evaluated if no euploids are found, as results suggest that segmental aneuploidies may not be shared among all TE cells. The study involved blastocysts initially diagnosed as being aneuploid, therefore the findings cannot be extrapolated to euploid or euploid-mosaic embryos.

OC–10

COMPARISON OF VARIOUS ANEUPLOIDY TYPES AMONG TWO TROPHECTODERM BIOPSIES AND A REST OF THE EMBRYO

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PGT-A, PG-Seq, aneuploidy, segmental aneuploidy, mosaicism

Introduction:
With the introduction of next generation sequencing (NGS), preimplantation genetic testing of aneuploidies (PGT-A) has become a powerful tool for the selection of euploid embryos, increasing the implantation rate and reducing the risk of pregnancy miscarriage. Due to the higher resolution and dynamic range offered by NGS, the phenomenon of mosaicism and segmental aneuploidies has become challenging for clinical interpretations. Numerous studies have reported live-births after transfer of mosaic embryos, however, only a few studies focused on the clinical relevance of segmental aneuploidies. In our study, we compared various aneuploidy findings among two trophectoderm biopsies and a rest of the embryo.

Material and methods:
In total, 89 embryos previously analyzed with the VeriSeq™ kit were de-vitrified and re-analyzed with the consent of the patients. A second trophectoderm biopsy (TE2) and the rest of the embryo including the inner cell mass (RE) was tubed separately. The TE2 and the RE as well as the amplification product from the original trophectoderm biopsy (TE1) were analyzed using the PG-Seq™ kit. After TE1 reanalysis, 18 biopsies were classified as euploid and the remaining 71 samples had the following aberrations, 62 whole chromosome aneuploid, 32 segmental aneuploid, 26 whole chromosome mosaic and 7 segmental mosaic. Concordance of various aneuploidy types was assessed between TE1, TE2 and RE.

Results:
From the original group of 18 embryo biopsies classified as euploid with TE1, 17 (94.4%) were concordant with TE2 and 16 (88.9%) in RE. Whole chromosome aneuploidy was concordant in 95.2% of cases between TE1-TE2, 93.5% of TE1-RE and 92.3% of TE2-RE with nonconformance generally due to changes in the affected chromosome to segmental aneuploidy or whole chromosome mosaic. In contrast, from 33 individual segmental aneuploidies detected in the original TE1, only 14 of them were observed again in TE2 and RE (42.2%) with 13 changing to euploid. When the TE1 segmental aneuploidy was also manifested in the TE2, it was almost always observed in the RE, with one exception (1/19, 5.3%). Only 1 of 26 (3.8%) TE1 originally found to be whole chromosome mosaic was repeated in the TE2 and 7 in the RE (26.9%). No segmental mosaics were concordant between TE1-TE2 or TE1-ROE and only 1/8 was concordant between TE2-ROE.

Conclusions:
Our study showed that euploid and whole chromosomal aneuploidies have a high predictive value towards the rest of the embryo. In contrast, mosaicism seems to have a low concordance rate between different parts of the embryo, which show us minimal predictive value of the mosaic findings. Interestingly, a 42.2% concordance rate between TE1 and other two parts of the embryo in the group of subchromosomal aneuploidies suggest their clinical potential for healthy live births since these errors may be of mitotic origin. Moreover, and in contrast to mosaics, second embryo biopsy helps to significantly increase the predictive value of segmental aneuploidies towards the rest of the embryo. Further clinical studies are needed, however, a second trophectoderm biopsy of embryos with segmental aneuploidies seems indicated, especially for patients where an euploid embryo is unavailable.

OC-11

HIGH CONSISTENCY IN EMBRYO ANEUPLOIDY TESTING OF UNIFORM, MOSAIC AND SEGMENTAL ANEUPLOIDIES WITH THE APPLICATION OF A VALIDATED ALGORITHM

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Mosaicism, preimplantation embryos, NGS, algorithm

Introduction:
Detection of mosaic embryos and degree of mosaicism is a hot topic in preimplantation genetic testing of aneuploidy (PGT-A). Several groups have reported healthy life births from mosaic embryos. A key point is the degree of mosaicism that can be detected in a trophectoderm biopsy and the impact of next generation sequencing (NGS) protocol and interpretation of sequencing data. The aim of this study was to determine the robustness of the diagnosis using a validated algorithm to establish the thresholds for mosaicism degree and the minimal resolution for de novo segmental aneuploidies.

Material & methods:
NGS protocol combined automatic library preparation (IonChef™ system, ThermoFisher Scientific), and multiplexing for up to 96 samples (S5-XL sequencer; ThermoFisher). Pools of 4-6 cells with uniform and segmental aneuploidies (10-23 Mb) from cell lines with known karyotypes were employed for platform validation. For mosaicism validation, different proportions of gDNA (0-100%) from cell lines with whole chromosome aneuploidies were sequenced. Quality parameters were evaluated to determine the conditions for accurate detection. Biopsies were diagnosed as mosaic, if only one or two chromosomes fitted the mosaicism thresholds, without uniform aneuploidy for any other chromosome. After validation, results of 34,147 biopsies from 9 diagnostic laboratories using the same diagnostic algorithm were analysed (October 2018-December 2018).

Results:
The minimal resolution for de novo segmental aneuploidies was established at 10 Mb, and the cut-off values for mosaicism detection at 30% and 70%. Biopsies were classified as: euploid (<30% aneuploidy); low mosaic (≥30%-<50% aneuploidy); high mosaic (≥50%-<70% aneuploidy) and aneuploid (≥70% aneuploidy). In total 33,173 biopsies were informative (97.1%). Mosaicism rate was 5.8%, 3.8% low mosaic degree and 2% high mosaic degree. Uniform aneuploidies were present in 46.9% of the biopsies and the remaining 47.3% biopsies were diagnosed as euploid (mean female age 36.8). The incidence of mosaicism was not different for the 9 diagnostic laboratories, total mosaicism rates ranged from 4.2% (mean age 38.1) to 6.7% (mean age 38.2). Low and high mosaicism rates were also comparable (low mosaic: 2.4%-4.4% and high mosaic: 1%-2.6%).
Finally, we wanted to assess the incidence of mosaicism for chromosomes considered suitable for transfer after genetic and psychological counselling according to Gratti et al (2018). The percentage of low degree mosaicism for low risk chromosomes (1, 3, 4, 5, 10, 12, 19) was 1.1% and for chromosomes with moderate risk (2, 6, 7, 9, 11, 15, 17, 20, 22) was 1.5%.

Conclusions:
High informativity rates were achieved using a high-throughout platform. The application of an internally validated algorithm for PGT-A showed high consistency among different laboratories, avoiding subjectivity and interindividual differences in the diagnosis, and minimizing the risks for overdiagnosis of mosaicism. Low mosaicism rates were identified and even lower if we consider only low mosaics for chromosomes suitable for transfer in the absence of euploid embryos and after proper genetic and psychological counselling.
Grati FR, Gallazzi G, Branca L, Maggi F, Simoni G, Yaron Y. An evidence-based scoring system for prioritizing mosaic aneuploid embryos following preimplantation genetic screening. Reprod Biomed Online. 2018 Apr;36(4):442-449.

OC-12

WHAT TO DO WHEN THERE IS NO EUPLOID EMBRYOS? TRANSFER MOSAIC EMBRYOS OR PROPOSE ANOTHER PGT-A CYCLE?

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euploidy, mosaicism, PGT-A, embryo transfer

Introduction:
Embryonic mosaicism is defined as the presence of karyotypically distinct cell lines within an embryo and can be detected with Next Generation Sequencing (NGS) at a rate between 20-80%. High incidence of mosaicism has been reported in preimplantation embryos, with the blastocyst mosaicism being between 4-24% (Harton et al., 2017). Although mosaic embryos have a chance to implant (Munne et al., 2017; Spinella et al., 2018), their pregnancy rate is lower, with an increased miscarriage rate, possible risk of intrauterine growth retardation and uniparental disomy. Therefore, low-level mosaics are preferentially selected for transfer if no euploid embryos are found.

Material and Methods:
This retrospective study was based on 1958 preimplantation genetic testing for aneuploidy (PGT-A) cycles initiated between January 2017 and December 2018 in Istanbul Memorial Hospital. At least one euploid embryo was found for 1066 cycles (54.4%) and in 892 PGT-A cycles either full aneuploid or euploid/ mosaic embryos were diagnosed. PGT-A was done by NGS ReproSeq on Ion Torrent S5 (Thermo Fisher Scientific) following trophectoderm biopsy. Among 168 cycles at least one mosaic embryo was found, and 37 mosaic embryo transfer cycles were achieved (mean female age: 36.0). 42 patients refused the transfer of mosaic embryos and went through another PGT-A cycle (mean female age: 37.1). Student's t-test and Mann-Whitney U test were applied for continuous variables and Chi-square test for categorical group comparisons.

Results:
Among the 42 patients who have chosen to go for a second PGT-A cycle, 31 had at least one euploid embryo diagnosed (73.6%) and 28 couples came for a frozen-thawed embryo transfer cycle at the time the abstract was written. The mean mosaicism rate of the transferred embryos was 29% with a range of 20% to 40%. The biochemical pregnancy rate was 64.8% and 78.5% in the mosaic embryo transfer group and in the second PGT-A cycle group, respectively (p=0.23). The biochemical miscarriage rate was similar for both groups (16.7% vs. 13.6%, respectively). The clinical pregnancy rate was 54.1% and 67.9% for mosaic embryos and for the transfer of euploid embryos found in a second PGT-A cycle, respectively (p=0.26). The clinical miscarriage rate was 10% and 15.8% in the mosaic embryo transfer group and in the second PGT-A cycle group, respectively (p=0.71). The ongoing pregnancy rate was 48.6% and 57.1% for mosaics and second cycle's euploids, respectively (p=0.50).

Conclusions:
As no statistically significant differences were found and considering the high rate of at least one euploid embryo in the second PGT-A cycle (73.6%), couples should be encouraged to start a new PGT-A cycle. For advanced maternal age patients (≥42) and/ or patients with a diminished ovarian reserve a second PGT-A cycle may not be a realistic option, thus mosaic embryo transfers may still be feasible. The limitations of our study include the fact that this was a retrospective analysis. Cohort sizes are undeniably small which prevents us to obtain statistically significant results and draw strong conclusions. Prospective studies are warranted.

OC-13

CORRELATION BETWEEN ANEUPLOIDY, MOSAICISM AND MORPHOKINETIC DEVELOPMENT IN 1550 BIOPSIED BLASTOCYSTS

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Euploid, aneuploid, mosaic, morphokinetics

Introduction:
Despite the high number of recent studies, conflicting data are reported, and there is still considerable disagreement regarding which morphokinetic parameters are useful to predict blastocyst formation, implantation potential and ploidy status of the embryo. The first attempt to develop a model predicting embryo implantation used the time of division to 5 cells, the time between division from 3 to 4 cells and the time between division from 2 to 3 cells (Meseguer et al., 2011). More recently, the aneuploidy status of embryos was related to the start of blastulation and the formation of a full blastocyst. The aneuploidy risk classification built proved beneficial in a correlation with live birth when applied to non-biopsied embryos (Campbell et al., 2013).

Material and Methods:
This retrospective study was based on 1550 blastocysts which were subjected to preimplantation genetic testing for aneuploidy (PGT-A) between August 2011 and December 2018 in Istanbul Memorial Hospital (n=659 euploids; n=831 aneuploids; n=60 mosaics). Incubation was performed in time-lapse incubators (EmbryoScope™). All relevant events (fertilization, cleavages, morula and blastocyst formation) were checked daily, and time of cleavage to two-cell embryo (t2) and subsequent divisions t3, t4, t5, t6, t7, t8, t9+, tM, tSB, tB and tEB were recorded in the EmbryoViewer® workstation. PGT-A was done by NGS ReproSeq on Ion Torrent S5 (Thermo Fisher Scientific) or aCGH (Illumina) following trophectoderm biopsy. Mann-Whitney U and Kruskal Wallis tests were used in this study.

Results:
When euploid embryos were compared with aneuploids, the time to reach the blastocyst stage was 3 hours earlier in euploids (tB=104.60h vs. 107.64h; $p<0.0001$). Interestingly, euploids and mosaics were found to have a similar morphokinetic behavior; for example, t2 was 25.6h in euploids and mosaics, and 26.3h in aneuploids ($p=0.006$). Aneuploids were then subdivided into five groups: 2-chromosome aneuploidies, complex aneuploidies, monosomies, trisomies and partial aneuploidies. The sub-analysis done for the time of the first cleavage t2 was statistically significant ($p=0.021$) and showed that euploids were different from 2-chromosome aneuploids and from complex aneuploids; complex aneuploids were different from mosaics which were different from partial aneuploids; two-chromosome aneuploids were different from mosaics and from trisomies. A second subgroup analysis was done for the time to achieve the 8-cell stage (t8) and showed that euploids are statistically significantly different from all aneuploid subcategories but not from mosaics ($p=0.0017$).

Conclusions:
The mosaic embryos' morphokinetic parameters were similar with the euploid blastocysts. Observed time-lapse differences between euploid and aneuploid blastocysts suggests that the embryos are being affected by the chromosomal constitution throughout the embryo development. Even though the variances at the initial cleavages are as short as half an hour, this increases to a three hour at the blastocyst stage. The study presented above can be used to prioritize the embryos according to the morphokinetic variables to biopsy and diagnose the embryos with highest chance of being euploid thus reducing the costs especially for couples who have more than one blastocyst.

OC-14

MOSAIC EMBRYO TRANSFERS – A COMPILED ANALYSIS OF PUBLISHED DATA

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Mosaic, Clinical Outcomes

Introduction:
Modern PGT-A methods make it possible to identify mixes of aneuploid and euploid cells in trophectoderm biopsies. In such instances the source embryos are classified as mosaic. IVF clinics must decide whether those samples should be considered for transfer. Different groups have published their observations on mosaic embryo transfers. We provide a comprehensive review of those reports and perform a combined evaluation of the data with the goal of achieving a higher-powered analysis.

Materials and Methods:
We retrieved information on mosaic embryo transfers from the published literature, and analyzed the compiled data.

Results:
Analysis of 312 mosaic embryo transfers indicate successful clinical outcomes, albeit with inferior implantation rates and incidences of ongoing pregnancy/birth (OP/B) compared to euploid embryos (45.8% vs 62.6% implantation rates, 35.3% vs 55.2% OP/B rates, n=312 mosaic embryos and n=1774 control group, $P < 0.01$ for both outcomes). The amassed data reveals the parameters of mosaicism that significantly influence clinical outcome (See tables). Level of mosaicism, which is the estimate of percent aneuploid cells in the biopsy sample, is insignificant when using 40% as the cutoff between low and high groups. A trend correlating poorer clinical outcome with high levels becomes apparent when using 50% as the cutoff, but is nonetheless statistically insignificant. Maternal age does not affect outcomes in a significant way, and comparison of mosaic single whole chromosome monosomies (losses) versus trisomies (gains) shows statistically insignificant differences in clinical outcomes. The type of mosaicism has a significant effect on outcome. While segmental mosaics show similar clinical outcomes compared to mosaics with one or two affected chromosomes, these groups result in significantly more favorable outcomes than complex mosaics in which more than two chromosomes are affected.

Conclusions:
Compiled data from the different published studies on the subject unequivocally show that mosaic embryos should be considered for transfer when no euploid embryos are available. Complex mosaic samples should be deprioritized, but can still result in implantation and births. Additional reports will be needed to definitively conclude whether the noted trend between percent aneuploidy and inferior outcomes is real or not. In summary, this compiled data analysis can be a resource for mosaic embryo selection in the clinic.

General	Mosaic Embryos Transferred (n)	Control Group (n)	Mosaic Embryos Implantation (%)	Control Group Implantation (%)	*P*	Mosaic Embryos OP/B (%)	Control Group OP/B (%)	*P*
	312	**1774**	**45.8%**	**62.6%**	**<0.0001**	**35.3%**	**55.2%**	**<0.0001**
Mosaic Levels 40	≤40 (n)	>40 (n)	≤40 Implantation (%)	>40 Implantation (%)	*P*	≤40 OP/B (%)	>40 OP/B (%)	*P*
	223	**98**	**47.5%**	**37.8%**	**0.114**	**37.7%**	**28.6%**	**0.128**
Mosaic Levels 50	<50 (n)	≥50 (n)	<50 Implantation (%)	≥50 Implantation (%)	*P*	<50 OP/B (%)	≥50 OP/B (%)	*P*
	240	**81**	**47.5%**	**35.8%**	**0.0715**	**37.9%**	**25.9%**	**0.0591**
Age	≤34 (n)	>34 (n)	≤34 Implantation (%)	>34 Implantation (%)	*P*	≤34 OP/B (%)	>34 OP/B (%)	*P*
	85	**157**	**51.8%**	**42.0%**	**0.1764**	**38.8%**	**33.1%**	**0.3994**
Losses vs Gains	Losses (n)	Gains (n)	Losses Implantation (%)	Gains Implantation (%)	*P*	Losses OP/B (%)	Gains OP/B (%)	*P*
	80	**47**	**52.5%**	**48.9%**	**0.7169**	**37.5%**	**38.3%**	**1.00**

Type	Segmental (n)	1 or 2 Whole Chr (n)	Complex (>2)	Segmental Implan-tation (%)	1 or 2 Whole Chr Implan-tation (%)	Complex Implan-tation (%)	*P* (Complex vs Rest)	Segmental OP/B (%)	1 or 2 Whole Chr OP/B (%)	Complex OP/B (%)	*P* (Complex vs Rest)
	87	**181**	**53**	**49.4%**	**49.2%**	**20.8%**	**0.0001**	**36.8%**	**39.8%**	**13.2%**	**<0.0001**

OC–15

THE INCIDENCE AND ORIGIN OF CHROMOSOME ANEUPLOIDIES IN HIGH QUALITY KARYOMAPPING SNP PROFILES

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aneuploidy, karyomapping, segmental abnormality, mosaicism

Introduction:
Karyomapping serves as the universal PGT-M platform based on Mendelian inheritance of SNP markers which assist in identifying haplotypes linked to a mutated gene. Moreover, karyomapping enables detection of aneuploidies by interrogating genome-wide SNP data in embryo samples and assists in a selection of euploid embryos for a transfer. We have found out that high quality SNP profiles, characterized by overall call rate ≥95%, might be reliably used for the identification of mitotic chromosome errors, aneuploidies in mosaic or segmental chromosome aberrations. Since karyomapping utilizes parental DNA samples, the origin of identified aneuploidies might be tracked in embryo samples. Our study describes the incidence and origin of aneuploidy, aiming at segmental chromosomes errors and mosaic aneuploidies which represents hot topic in respect to their clinical significance.

Material & methods:
SNP data obtained by HumanKaryomap-12 BeadChips, Illumina SNP arrays were analysed in 2085 trophectoderm (TE) samples derived from 319 couples requesting PGT-M. Mean maternal age was 32.4 ± 4.5. Based on the karyomapping SNP data QC metrics, mean and median call rate was 90.4% and 95%, respectivelly. In total, 936 embryos fulfilled criteria of high quality SNP profiles (call rate ≥95%). A subset of twenty TE samples detected with mitotic trisomies, mosaic aneuploidies and segmental chromosome abnormalities by karyomapping were reanalysed using PG-Seq™ kit, which is the fully validated PGT-A platform in our laboratory. We have observed full concordance between both platforms.

Results:
Analysing 936 high quality TE samples, we have observed that 617 (65.9%) of them were euploid. The remaining 319 TE samples were detected with the following aberrations: a) only aneuploid 234 (73.4%) samples; b) only mosaic 43 (13.5%) samples; c) aneuploid + mosaic 42 (13.2%) samples. In other words, overall mosaicism rate in 936 TE samples was 9.1% (85/936) in our study. This number tend to be underestimated, since we did not report low level mosaicism due to a lack of proper sensitivity validation of the karyomapping in last years. We have detected segmental aneuploidies in 12.6% (118/936) of TE samples, 56 (47.4%) of them were present in mosaic form. Regarding the origin of aneuploidies, 87% (220/253) of whole chromosome aneuploidies affected maternal chromosomes, the ratio difference was even more highlighted in whole chromosome trisomies (93%; 91 out of 98). In contrast, segmental chromosome abnormalities affected more frequently paternal chromosomes (61,3%; 38 out of 62). Notably, this trend towards paternally related segmental aneuploidies were also followed in segmental chromosome abnormalities in mosaic (60,7%; 34 out of 56).

Conclusions:
1. According to our validation, high quality SNP profiles (overall SNP call rate ≥95%) enables detection of mitotic trisomies, mosaicism at 25% sensitivity level and segmental chromosome abnormalities (in 5Mb resolution).
2. Meiotic whole chromosome trisomies prevail over mitotic whole chromosome trisomies (in full or mosaic state) in the ratio 4:1.
3. Segmental chromosome abnormalities (in full or mosaic state) affects more frequently paternal chromosomes (P value <0.01).
4. Whole chromosome aneuploidies in mosaic affect equally maternal and paternal chromosomes.

OC-16

INTRACYTOPLASMIC SPERM INJECTION IS NOT NECESSARY AS A PREVENTIVE MEASURE AGAINST PATERNAL CELL CONTAMINATION IN PREIMPLANTATION GENETIC TESTING

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PGT, ICSI, IVF, contamination, insemination

Introduction:
Conflicting information exists as to which method of insemination to use for PGT patients. Recommendations from some professional bodies advise the use of intracytoplasmic sperm injection (ICSI) for insemination for all amplification based testing to reduce the risk of paternal contamination from extraneous sperm bound to the zona pellucida, or non decondensed sperm within blastomeres. The vast majority of PGT is now performed via amplification based techniques, beginning with whole genome amplification of the submitted samples. Therefore, it is important to examine the need for this additional and expensive intervention for such a large number of patients that could undergo and achieve fertilisation via conventional in vitro fertilisation (IVF).

Materials and Methods:
Semen samples were obtained from 5 male patients consenting to research. The semen sample was prepared as per the IVF laboratory standard operating procedure for sperm preparation for IVF/ICSI insemination. From each sample 1, 2, 4, 8 and 10 sperm were collected in PCR tubes and submitted to the genetic testing laboratory as blinded research samples. The tubed sperm samples underwent whole genome amplification via SurePlex, and DNA amplification was assessed via gel electrophoresis, in line with PGT sample processing.

Results:
All results were as expected for internal positive and negative controls. However, none of the 25 tubed sperm samples submitted showed evidence of DNA amplification.

Conclusions:
Sperm DNA failed to amplify under the conditions used to process polar body, blastomere and trophectoderm samples in PGT. DNA can be amplified from individual sperm, but requires additional sperm DNA decondensation steps. Therefore, paternal cell contamination resulting from using IVF as the insemination method is a negligible risk and ICSI, in the absence of male factor infertility, is an unnecessary intervention for PGT patients.

OC-17

RECOMBINANT VERSUS URINARY GONADOTROPHINS: A PILOT STUDY TO EVALUATE PLOIDY STATUS OF EMBRYOS DERIVED FROM IVF.

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recombinant, urinary, euploidy, aneuploidy

Introduction:
The efficacy between both recombinant and urinary gonadotrophins in ovarian stimulation have always been actively compared in different perspectives as they are biochemically different. Urinary gonadotrophin is said to have a higher biological activity which may improve the pregnancy outcome. Besides, urinary gonadotrophin contains LH which is usually added to patients with poor prognosis to obtain better quality oocytes. Yet in other studies, better results were obtained with recombinant gonadotrophins. To date the quality of embryos produced from treatment with either gonadotrophins has not been compared. This pilot study aims to evaluate the ploidy status embryos derived from stimulation with recombinant and/or urinary gonadotrophins.

Methods & Materials:
A total of 345 Preimplantation Genetic Testing for Aneuploidy (PGT-A) cases from Sunfert International Fertility Centre in 2018 were retrospectively analysed. Oocyte donor cases were excluded from this study as some of them were stimulated by external IVF centres. All cases were classified into four groups according to the type of prescribed gonadotrophins given throughout the controlled ovulation stimulation: **Monotherapy recombinant (MR)** such as Gonal F® and Puregon® (n=42); **Monotherapy Urinary (MU)** such as Menopur® and Humog® (n=43); **Combined Recombinant (CR)** which is a combination of Gonal F® and Pergoveris® (n=81); and **Combined Recombinant Urinary (CRU)** such as the addition of Menopur® or Humog® to either Gonal F® or Puregon® (n=179). Intracytoplasmic Sperm Injection (ICSI) was carried out for MII oocytes upon egg retrieval. Fertilized (2PN) oocytes were further cultured to blastocysts (n=1067) for biopsy. Ploidy screening was performed using Next-Generation Sequencing (Veriseq® protocol, Illumina).

Results:
Analysis of fertilisation rates did not show significant difference within the groups of monotherapy (68.8% and 69.0%, p=0.94) and combined therapy (63.8% and 64.8%, p=0.66). The monotherapy groups however have a trend towards higher blastulation rate (BR) and blastocyst utilisation rate (BUR) rates compared to combined therapy groups, with the **MU** having the highest BR (76.4%) and BUR (57.4%).
In terms of embryonic ploidy status, both groups of monotherapy showed a trend towards higher euploid rate and lower aneuploid rate compared to the combined therapy groups. Within the monotherapy groups however, the **MU** group seemed to do better than **MR** as it has a similar euploid rate (51.2% vs 50.3%) even though the mean age is higher for this group (36.5 vs 32.0). All groups, both combined and monotherapy have similar mosaic rate. Interestingly, **CR** group has the lowest euploid (42.6%) and highest aneuploid rate (47.1%) compared to all other groups, and this deserves further investigations.

Conclusion:
In this small pilot study, we have demonstrated that stimulation with different gonadotrophins used either solely or in combination has a possible impact on the ploidy (but not mosaic) status of the resulting blastocysts. This may however be impacted by different stimulation policies of individual centres. In this study, adding LH into the stimulation protocol seemed to improve on the euploid rate, especially in older women but this seemed to be true in urinary gonadotrophins only. Further studies are necessary to confirm this.

OC-18

THE INSIGHTS OF EMBRYO MOSAICISM IN IVF CYCLES

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PGT-A, mosaicism, blastocyst, biopsy

Introduction:
The development of new methodologies for PGT-A has led to the detection of a certain percentage of mosaic embryos. It has been postulated that this could be a normal feature in human embryos. To date, the real incidence of mosaicism in blastocysts is unknown as illustrated by the very variable incidences reported between groups. Certain factors could influence mosaicism occurrence such as patients' characteristics, ovarian stimulation and in vitro culture conditions. Biopsy sample manipulation could also induce artefactual mosaicism. Moreover, different diagnostic platforms, algorithms and detection limits are used by different groups, thus making comparisons difficult.

Material & methods:
The cytogenetic results of 1339 blastocysts obtained from PGT-A cycles performed in our centre from September 2017 to January 2019 were retrospectively analysed. Embryos were biopsied at the blastocyst stage between day 5 and 7 of development and genetic analysis was performed by next-generation sequencing (NGS) according to VeriSeq™-PGS protocols (Illumina). Embryos were diagnosed as euploid, aneuploid or mosaic. An embryo was classified as mosaic when the percentage of affected cells ranged from 30-70%.
Both the incidence of mosaicism in this cohort and the specific chromosomes affected were studied.
Mosaicism was studied versus patients' factors (age, indication for PGT-A, ovarian reserve), embryonic factors (blastocyst development and quality), potential iatrogenic factors (ovarian stimulation treatment and the use of two different single-step culture media), and the biopsy operator as a proposed source of artefactual mosaicism.
A logistic mixed multivariable model was applied to estimate the odds for every endpoint. Patient and biopsy procedure were treated as random factor to control the correlated observations effect.

Results:
The overall incidence of mosaic embryos was 13.0% (7.5% of euploid-aneuploid mosaic embryos and 5.5% of aneuploid-aneuploid mosaic embryos). Most mosaic embryos showed just one chromosome affected (85.5%).
No chromosomes were especially associated with mosaic aneuploidy. However, segmental mosaicism was more common in larger chromosomes.
No association could be stablished between embryo mosaicism and the factors studied. Neither maternal nor paternal ages showed any association with mosaicism (ORa=0.96(0.88-1.04); ORa=1.04(0.99-1.09)). The antral follicle count did not show any correlation either (ORa=0.987(0.95-1.02)) nor the indication for PGT-A. In terms of embryo development, no associations were found either: ORa for blastocyst formation in day 5 vs day 7 was 0.72(0.31-1.68); ORa for blastocyst formation in day 6 vs day 7 was 0.53(0.23-1.22); ORa for excellent and good quality embryos vs fair and poor quality embryos was 0.86(0.54-1.36). Regarding the studied factors that were potentially associated with iatrogenic mosaicism, neither type of stimulation (agonists vs antagonists), nor the use of two different single-step media showed an association. No impact on the incidence on mosaicism has been related to a given operator.

Conclusions:
There is no preferential distribution of mosaicism among the chromosomes although large chromosomes seem to be more sensitive to segmental mosaicism.
None of the studied potential sources of intrinsic, iatrogenic, or artefactual mosaicism showed an association with such feature.

P-01

EVALUATION AND IMPLEMENTATION OF ONEPGT FOR MONOGENIC CONDITIONS

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SNPs, NGS, STR, PGT-M, haplotyping

Introduction:
Preimplantation genetic testing for monogenic disorders (PGT-M) offers couples who carry a known inherited genetic condition the chance of having children free of the disorder. Currently, a bespoke genetic test is developed for each new condition which can take up to 6 months to implement; over 300 gene specific tests have been produced at our centre.
OnePGT (Agilent) utilises genome-wide Next Generation Sequencing (NGS), allowing most genetic conditions to be tracked within families and embryos. Availability of a commercially developed genetic test eliminates the time involved with new test development. Evaluation of OnePGT for the testing of embryos for familial monogenic conditions has been undertaken.

Material & methods:
Whole genome amplified (WGA) biopsy DNA was available for 33 embryos from 13 couples who previously underwent PGT-M; all embryo biopsies consisted of ~5 trophectoderm cells. The genetic status of the embryos had been determined using an ISO15189 accredited STR haplotyping approach. 13 single gene disorders were tested including 9 autosomal dominant, 2 autosomal recessive and 2 X-linked.
The embryo samples and respective reference family gDNA samples were processed using the OnePGT workflow, sequenced on a NextSeq550 and the data analysed using Alissa software for SNP haplotyping of the monogenic disorder, in conjunction with targeted assessment of copy number of the chromosome on which the gene was located, to aid interpretation of the monogenic results in embryos.

Results:
OnePGT results were all in concordance with the outcomes from the STR results (33/33). The automated PGT-M call rate was 28/33 whilst 5 required manual inspection. Of these, 3 were because there was less than 1Mb between the genes to the end of the chromosome, 1 was due to the proximity of the gene to a chromosome crossover breakpoint and 1 was due to a reduced number of concordant SNPs which was caused by the low-level presence of both maternal haplotypes in the embryo.
In one case, an affected embryo was successfully used as the reference for phasing for the couple and in another, the availability of chromosome copy number identified an embryo as having uniparental Inheritance.

Conclusions:
The NGS approach of OnePGT has been successfully evaluated for determining the genetic status of embryos for monogenic disease. Due to the sequencing costs involved with NGS, it may not be merited when there is an established ‘in-house’ test. On balance, taking all laboratory aspects into consideration, OnePGT is a contender to evaluate new disorders. It is an anxious time for couples as they wait to know whether they have embryos suitable for replacement. Implementing OnePGT will eliminate test development and expedite genetic testing for couples requesting PGT-M for rare monogenic diseases.
Declaration of Interest: The OnePGT kits were provided by Agilent as part of an early access programme

P-02

CLINICAL COMPARISON OF TWO PGT-A PLATFORMS UTILIZING DIFFERENT THRESHOLDS TO DETERMINE PLOIDY STATUS

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Mosaicism, NGS, aneuploidy, PGT-A

Introduction:
Unlike previous technologies that lack the diagnostic performance to reliably detect mosaicism for preimplantation genetic testing for aneuploidy (PGT-A), next generation sequencing (NGS) based platforms now allow for the detection of lower-level mosaicism. Due to this increased sensitivity, thresholds need to be put in place to classify embryos as euploid, aneuploid, or mosaic. The lower cutoff for mosaic samples ranges from 20 % to 30 %, and the upper cutoff for mosaic samples ranges from 70%-80% in most commercial PGT laboratories in the field today. These varying cutoffs will lead to varying percentages of mosaic embryos reported, suggested to be somewhere between < 5% to > 20 %. A more stringent threshold could result in greater pregnancy rates. Here we compare clinical outcomes and embryo reporting between two different PGT-A reference laboratories that use different testing platforms and different mosaic cutoffs.

Materials and Methods:
Retrospective analysis of PGT-A results (n=623) and frozen embryo transfer (FET) cycles (n=99) using embryos diagnosed by either Cooper Genomics or Igenomix between January 2018 - January 2019. Cooper Genomics uses Illumina's MiSeq platform with Bluefuse software and thresholds of 20% to 80 % (lower to upper cutoff) while Igenomix uses ThermoFisher's Ion Torrent platform with Ion Reporter software and thresholds of 30% to 70 %. Patients with a history of recurrent miscarriage were excluded from this study.

Results:
Fifty six (56) cycles included embryos analyzed by Cooper Genomics and 43 cycles included embryos analyzed by Igenomix as a PGT-A reference laboratory. There was no differences in maternal age or number of embryos replaced per transfer between the two cohorts. IVF outcome was similar between the two groups: The pregnancy (+FHB) observed in cycles that transferred embryos diagnosed as euploid by Cooper Genomics was 67.9% (38/56) compared to 69.8% (30/43) with Igenomix. Miscarriage rate was also similar, 8.9% (5/56) and 6.9% (3/43) respectively. Cooper Genomics reported a much higher rate of mosaicism (17.0%) compared to Igenomix (4.9%) ($p < 0.001$). A trend towards a higher euploidy rate was observed in the Igenomix cohort (37.6%) compared to Cooper Genomics (32.7%).

Conclusion:
Retrospective analysis of PGT-A+FET cycles revealed there was no differences in pregnancy or miscarriage rates indicating a similar diagnostic efficacy achieved by the two PGT-A platforms. Although the embryos diagnosed as euploid by the two platforms appeared to have the same potential to generate a pregnancy, the methodology and higher threshold utilized by Igenomix resulted in significantly less mosaic embryos and a slightly greater percentage of euploid embryos available for transfer. It is assumed that cumulative pregnancy rates between the two reference laboratories would vary based on the finding that there were more euploid embryos available from Igenomix. Results from subsequent FET cycles will be collected and further examined. NGS has now created three categories of embryos that exist after PGT-A, the interpretation of the millions of data points generated by this technology still remains a challenge and it is important that the diagnostic methodology used does not overestimate the degree of mosaicism.

P-03

RAMAN PROFILING OF EMBRYO CULTURE MEDIUM TO IDENTIFY ANEUPLOID AND EUPLOID EMBRYOS

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Raman micro spectroscopy, machine learning, chromosome abnormality, IVF, preimplantation genetic testing for aneuploidy (PGT-A)

Introduction:
In this case, we are aimed to develop and validate Raman metabolic footprint analysis to determine chromosome euploidy and aneuploidy in embryos fertilized in vitro.

Material & methods:
To establish the analysis protocol, spent embryo culture medium samples with known genetic outcomes from 87 human embryos were collected and measured with the use of Raman spectroscopy. Individual Raman spectra were analyzed to find biologic components contributing to either euploidy or aneuploidy. To validate the protocol via machine-learning algorithms, an additional 1,107 Raman spectra from 123 embryo culture media (61 euploidy and 62 aneuploidy) were analyzed.
During the test, 10 µL culture media was carefully moved to a special-designed sample cell with a gold coating after thawed and centrifuged in order to avoid the interference of oil. All spectra were preprocessed by subtracting the dark signal background from each spectrum. Spectral normalization was done by vector normalization of the fingerprint region from 600 to 1,800 cm^{-1}. Then, machine-learning models were implemented to classify embryo euploidy or aneuploidy based on original Raman spectra of embryo culture media. A total of 1,017 spectra (549 for euploidy and 558 for aneuploidy) was split into the training set and the testing set in a ratio of 8:2.

Results:
Mean-centered Raman spectra and principal component analysis showed differences in the footprints of euploid and aneuploid embryos growing in culture medium. Significant differences in Raman bands associated with small RNAs and lipids were also observed. Stacking classification based on k-nearest-neighbor, random forests, and extreme-gradient-boosting algorithms achieved an overall accuracy of 95.9% in correctly assigning either euploidy or aneuploidy based on Raman spectra, which was validated by PGT-A sequencing results.

Conclusions:
This study suggests that chromosomal abnormalities in embryos should lead to changes of metabolic footprints in embryo growth medium that can be detected by Raman spectroscopy. The ploidy status of embryos was analyzed by means of Raman based footprint profiling of spent culture media and was consistent with PGT-A testing performed by next-generation sequencing.

P-04

VALIDATION STUDY OF A NGS-BASED PGT-A WORKFLOW FOR MOSAICISM AND SEGMENTAL COPY NUMBER VARIATION

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PGT-A, mosaic calling algorithm, segmental copy number variation

Introduction:
Recently, with the introduction of Next Generation Sequencing (NGS), preimplantation genetics test for aneuploidy (PGT-A) has been greatly advanced. While copy number variation (CNV) calling of full chromosome aberrations has been well developed by many NGS-based PGT-A workflows, the calling algorithms for mosaicism and segmental CNVs are still not well established. In this study, we assess the specificity and accuracy of a newly developing PGT-A workflow for calling mosaicism and segmental CNV.

Material & methods:
Thirteen Coriell cell lines with known karyotypes were analyzed in this study. To test the accuracy of mosaic calling, 5 cells were precisely picked under dissection microscope and processed using the protocol for the Ion ReproSeq™ PGS Kits for the Ion GeneStudio S5™ System (Thermo Fisher Scientific). Mosaic samples with mosaicism of 0%, 20%, 40%, 60%, 80% and 100% were generated by combining defined number of cells from two cell lines. For evaluating full and segmental chromosome CNV calling, 4-5 cells of each cell lines were sampled. The sequence data was analyzed using decimal ploidy value copy number calling (mosaic) algorithm in Ion Reporter v5.10. This algorithm was combined in a workflow with either default (2Mb), 1Mb or 0.5Mb tile size reference baseline. In total, 144 samples were analyzed with 4 replications for each mosaic sample and 3 replications for all the other samples.

Results:
To evaluate specificity and sensitivity of mosaic calling, two sets of mosaic samples were generated by mixing (i) 46,XY with 47,XX,+21, and (ii) 46,XY,del(4p) with 47,XY,+13 cell lines. Mosaic profiles of Chr13 closely matched expected values at all tested combinations, and in contrast, those of Chr21 showed expected mosaic values at 60% and 80% but not 20% and 40% groups. Meanwhile, mosaic calling workflow with 0.5Mb baseline could not detect 20% segmental mosaic loss of del(4p)27Mb, and displayed 9%, 15% and 17 % overestimation of mosaicism at 40%, 60% and 80% groups, respectively.
By choosing different tile size reference baselines while keeping other parameters at default values, we tested the ability of the workflow to detect full and segmental chromosome CNV. With default baseline, trisomy of Chr13, Chr18, Chr21 and ChrX, monosomy of Chr21, dup(3q)99.1Mb, del(5p)32Mb, del(4p)27Mb and dup(6p)20.97Mb could be correctly called. With 0.5Mb baseline, dup(22q)14.75Mb and dup(9p)11Mb but not dup(16p) 3.6Mb, del(5q) 2.3Mb, dup(6p) 2.19Mb, del(9p) 1.81Mb and del(14q) 1.18 Mb, could be consistently reported with expected size and breakpoints. Of note, the workflow with 0.5Mb baseline identified some unexpected segmental CNV events of various sizes and locations, such as dup(1q)4.6Mb, del(15q)2.6Mb, del(4p)1.4Mb, del(17q)1.4Mb, dup(2q)0.9Mb, del(22q)0.8Mb and del(15q)0.7Mb.

Conclusion:
Accuracy and sensitivity of both mosaic calling and segmental CNV callings may vary among different chromosomes, different sizes and regions of the chromosomes. Segmental mosaic calling is less sensitive and accurate than full chromosome mosaic calling. Choosing higher resolution baseline could enhance sensitivity of small segmental CNV calling but reduce specificity. Further modification and optimization of the calling algorithm for mosaicism and small segmental CNV events are needed. This study provides useful information for establishing NGS-based PGT-A guidelines.

P-05

EUPLOID BLASTOCYSTS HAVE SIMILAR CLINICAL OUTCOME REGARDLESS OF THEIR D5 KIDSCORE™

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D5 KIDScore™, euploid, clinical outcome, implantation, clinical pregnancy

Introduction:
Time-lapse monitoring (TLM) uses morphokinetics assessment and annotation models such as KIDScore™ to help predict the implantation potential of a blastocyst. Meanwhile, preimplantation Genetic Testing for Aneuploidy (PGT-A) determines the chromosomal status of an embryo, allowing the identification of embryos with the correct number of chromosomes. Theoretically, combining both of these advanced technologies in IVF may enable the selection of a competent blastocyst for transfer, thus maximising the chance of a successful pregnancy. To the best of our knowledge, the significance of KIDScore™ annotation model in euploid blastocysts has not been studied. This is a retrospective study to assess the clinical outcomes of elective frozen embryo transfer (eFET) of euploid blastocysts in various D5 KIDScore™ groups in Alpha Fertility Centre, Malaysia, between January to November 2018.

Material and Methods:
Two hundred and twenty-one (221) patients aged 18 – 45 years (mean age 30.8 years) had their embryos cultured to blastocyst stage in the Embryoscope™ incubator (Vitrolife, Sweden) and screened using Next Generation Sequencing (Ion Torrent, USA). In order to accurately determine the D5 KIDScore™ of the euploid blastocysts transferred, only blastocysts intended for single frozen embryo transfer were included in this study. All the 221 blastocysts transferred were categorised according to D5 KIDScore™ ranged between 1.0 – 4.0 (Group A, n = 4), 4.1 – 5.0 (Group B, n = 4), 5.1 – 6.0 (Group C, n = 15), 6.1 – 7.0 (Group D, n = 49), 7.1 – 8.0 (Group E, n = 83), 8.1 – 9.0 (Group F, n = 53), and 9.1 – 9.9 (Group G, n = 13) respectively. Clinical pregnancy and number of gestational sacs were determined by ultrasound.

Results:
The clinical pregnancy rates and implantation rates for Group A, B, C, D, E, F and G were 25.0%, 75.0%, 66.7%, 46.9%, 60.2%, 60.4% and 53.8% respectively. The miscarriage rates for Group A, B, C, D, E, F and G were 0.0%, 33.3%, 20.0%, 4.3%, 6.0%, 12.5% and 14.3% respectively. There was no significant difference in clinical pregnancy rate, implantation rate and miscarriage rate between all groups ($p>0.05$).

Conclusions:
Our study shows no significant difference in clinical pregnancy rate, implantation rate and miscarriage rate across all D5 KIDScore™ groups for euploid blastocysts. Euploid blastocysts with lower D5 KIDScore™ gives comparable clinical outcomes of that to blastocyst with higher D5 KIDScore™ and should be considered in the selection for transfer. D5 KIDScore™ may serve as a guide for selection of blastocyst with high implantation potential. However, PGT-A still remains the more reliable method compared to solely relying on D5 KIDScore™ in TLM for blastocyst selection. Nevertheless, further studies with a larger sample size should be carried out to ascertain the significance of D5 KIDScore™ in blastocyst that was screened euploid using PGT-A.

P-06

IDENTITY-BY-STATE BASED COMPREHENSIVE PGT: ADVANTAGES AND CHALLENGES

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haplarithmisis, identity-by-state, PGT-M, PGT-A

Introduction:
Genome-wide haplotyping strategies, such as karyomapping, haplarithmisis and OnePGT, have paved the way for comprehensive PGT, leveraging PGT-M, PGT-A and PGT-SR in a single workflow. Nevertheless, family-specific challenges can complicate the implementation of current methods. First, to enable haplotype reconstruction, genotyping the prospective parents and direct family members, i.e. offspring of the couple or their parent(s), is required. However, if these references are not available or suitable for phasing, such approaches cannot be used, prompting investigation towards alternative phasing modules. Additionally, the advancement of current technologies leads to the identification of an increasing number of variants of unknown significance. Development of novel analysis approaches can assist further evaluation and interpretation of such variants, based on the familial segregation of the variant, in order to assess if PGT is an appropriate clinical option.

Material & methods:
Twelve couples i) burdened with a known hereditary genetic disorder participating in the genome-wide haplotyping-based PGT-M program and ii) with at least one parental sibling from the parental side carrying the genetic disorder available, were recruited for the development of the new method. Genotyping data from both prospective parents and parental sibling(s) were used to trace the shared and/or different allele and impute the disease-carrying allele based on identity-by-state (IBS) information. Concurrent haplotyping and copy number typing of embryo biopsies was then performed using an adapted version of siCHILD/haplarithmisis.

Results:
Six out of 12 (50%) PGT-M couples could be phased using parental sibling(s). This is in line with the theoretical expectations of allele-sharing between sibling-pairs. Genome-wide haplotypes and copy-number profiles generated for each embryo using the new phasing approach were consequently compared to the clinical results, showing 100% concordancy.
Clinical implementation of the method has resulted in the analysis of 7 families so far. Additionally, IBS analysis has enabled the inclusion of two special cases in the PGT program. In the first, PGT-M was offered for an X-linked disorder. The female partner had karyotype 47,XXX with presence of both maternal X chromosomes, so that no distinction could be made between the affected and unaffected allele. In the second, IBS analysis revealed the *de novo* and consequently pathogenic nature of an autosomal dominant variant in the maternal grandmother, allowing PGT.

Conclusions:
IBS analysis in the context of PGT can be proven to be advantageous on several levels. First, it allows offering genome-wide linkage analysis-based comprehensive PGT to families lacking standard phasing reference(s). A rapid pre-PGT work-up can define whether the couple can benefit from this technology. Importantly, by including more than one extended family member, the chance of obtaining informative results in the interrogated locus increases. Furthermore, by following the familial segregation of the variant of interest using IBS information, more evidence can be obtained regarding its pathogenicity. This is important for making the decision if the specific indication is valid for PGT.

P-07

GENETIC TESTING FOR COPY NUMBER VARIATIONS IN FIRST POLAR BODIES AND OOCYTES BY SINGLE-CELL NEXT-GENERATION SEQUENCING

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NGS, polar body, aneuploidy, meiotic errors

Introduction:
Female meiotic errors are the main cause of whole chromosomal aneuploidy in human embryos and significantly contribute to early embryo loss. Meiotic chromosomal malsegregation occurs during divisions of the maturing oocyte in oogenesis concurrently with extrusion of two polar bodies: polar body first (PB1) and polar body second (PB2). Based on meiosis mechanism, genome of a single oocyte can be deduced by analyzing its sibling polar bodies. PB genetic testing can identify abnormalities of maternal meiotic origin and may be the only form of preimplantation genetic testing for aneuploidies (PGT-A) accepted by patients who object embryo biopsy for ideological reasons. In the majority of previous studies, array comparative genomic hybridization (aCGH) was a method of choice for comprehensive chromosome screening (CCS) in polar bodies. Here, we test the use of single-cell next-generation sequencing (NGS) method in polar body CCS for detecting copy number variations (CNVs) in PB1 genome and validate methods for whole genome amplification (WGA) of PB1.

Material and methods:
In this study, single-cell NGS technology for CCS was used to asses ploidy status of oocytes and their sibling PB1. A total of 120 single cells (60 first polar bodies and 60 oocytes) were collected from in vitro fertilization (IVF) couples and subjected for WGA using four different kits based on three distinct amplification systems: (i) polymerase chain reaction (PCR-based WGA), (ii) multiple displacement amplification (MDA) or (iii) multiple annealing and looping-based amplification cycles (MALBAC). WGA products were processed and analyzed with VeriSeq PGS MiSeq kit on MiSeq sequencing platform (Illumina) and random samples were validated with the aCGH method. Amplification uniformity and accuracy for CNV detection of the four tested kits were evaluated.

Results:
Ploidy status was determined for 112 of 120 (93,3%) samples (84/112 based on direct analysis and 28/112 based on indirect analysis of the well-amplified sibling PB1 or oocyte). Estimated aneuploidy rate for the studied group which resembles the total oocyte abnormality resulting from meiosis I errors rate was 33% (37/112 samples). In the group of well-amplified PB1-oocyte pairs (33/60) a 97% concordance between the chromosomal status of PB1 and the corresponding oocyte was observed. Both whole chromosome nondisjunction (6 of 37 aneuploid samples) and abnormal chromatid pre-division and segregation were detected (31 of 37 aneuploid samples). The result is concordant with previous studies - abnormal chromatid segregation was the dominant aneuploidy-causing mechanism. Differences in WGA kits performance including amplification uniformity, aneuploidy calling potential and a number of interpretable NGS results were evaluated and the most optimal kit for PB1 genome WGA was pre-selected. Results from aCGH validation of the randomly selected samples showed full consistency with the NGS results.

Conclusions:
The NGS-based method used for PB1 genome analysis showed a high predictive potential of PB1 in deducing ploidy status of the corresponding oocyte and is a promising method for PGT-A of oocytes. The study also contributes to a better understanding of the chromosome segregation patterns and maternal meiosis errors.
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P-08

COMPARISON OF NATURAL CYCLES VERSUS HORMONE REPLACEMENT TREATMENT FOR 2398 FROZEN-THAWED EMBRYO TRANSFER CYCLES FOR PREIMPLANTATION GENETIC TESTING PATIENTS

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frozen-thawed embryo transfer, preimplantation genetic testing, natural cycle, hormone replacement treatment

Introduction:
Multiple studies have been performed to evaluate the efficiencies of various endometrial preparation protocols. However, to date, the most appropriate method of endometrial preparation for frozen-thawed embryo transfer (FET) remains debated. To our knowledge, there are no studies comparing FET cycle endometrial preparation protocol for PGT patients, and this study is the first study to compare FET cycle endometrial preparation protocols for PGT patients.

Material & methods:
This retrospective analysis included 2398 FET cycles for 1497 preimplantation genetic testing (PGT) patients with regular menstrual cycles who underwent frozen-thawed single blastocyst transfer from January 2014 to December 2017. The patients were divided into two groups according to the method of endometrial preparation for FET: 586 cycles were natural cycles(NC), and 1812 cycles were hormone replacement treatment (HRT). Reproduction-related clinical outcomes of the two groups were compared.

Results:
The NC group showed a higher clinical pregnancy rate (54.78% vs 51.66%, P=0.188) and live birth rate (45.22% vs 43.21%, P=0.394) than did the HRT group, but these differences were not statistically significant. Moreover, endometrial thickness was significantly greater in the NC group (9.91±2.05 mm vs 8.95±1.53 mm, P<0.001). Furthermore, the ectopic pregnancy rate was significantly higher in the HRT group (0% vs 1.5%, P=0.028). The cesarean section rate (64.75% vs 52.45%, P<0.001) and pregnancy complication rate (21.47% vs 15.58%, P=0.023) were also significantly higher in the HRT group.

Conclusion:
Our study shows a trend towards higher pregnancy rate and live birth rate for NC compared to HRT. Large randomized controlled trials are warranted to confirm this trend. Additionally, NC is safer than HRT, avoiding ectopic pregnancy and adverse obstetric outcomes.

Key words:
frozen-thawed embryo transfer, preimplantation genetic testing, natural cycle, hormone replacement treatment

P-09

PREVALENCE OF SEGMENTAL ABNORMALITIES IN PGD-A

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PGT-A, NGS, Segmental variations

Introduction:
Prevalence and types of segmental abnormalities varies in different studies, requiring accumulation of more data for understanding their clinical significance, especially reported as mosaicism.

Material and Methods:
A total of 5,869 NGS-based PGD-A cases were studied, involving testing of 29,376 trophectoderm samples and included the analysis of proportion of deletions and duplications in all 24 chromosomes as small as 3 MB and as large as 139 MB detected by NCS.

Results:
Of 56.9% aneuploidy embryos, 14.4% were with deletions and duplications, of which 62% were with a deletion, 30% with duplication, and 8% representing multiple sub-chromosomal variations. Mosaicism was detected in 59.3% of these variations. The larger chromosomes contained a higher proportion of deletions and duplications, with the exception for chromosome 6, while majority of duplications were observed in chromosome 20 and Y, and deletions mainly in chromosomes 2, 12, 17 and 22.

Conclusion:
The observed segmental abnormalities represent de novo findings of mitotic origin, with the majority being mosaics, showing no maternal age dependence. Reproducibility in the follow up testing and prevalence in pregnancy losses will be useful for consideration on their biological and clinical significance.

P-10

DIFFERENCES IN MRNA EXPRESSION OF SELENOPROTEINS IN DOMESTIC CAT OOCYTES AND CUMULUS CELLS DURING IN VITRO MATURATION

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oxidative stress, antioxidant enzymes, feline oocytes, selenoproteins

Introduction:
Reactive oxygen species (ROS) is one of the major factors that has a direct negative impact on oocyte quality. The presence of antioxidant enzymes including selenoproteins across different mammalian species suggests that defence mechanisms are conserved and are likely to be important for the final steps of in vitro oocyte maturation. Domestic cat serves as a good animal model for future research in conservation breeding of endangered feline species, but also it is a valuable model for studying developmental pathways that can be affected by in vitro culture and maturation of oocytes for many mammalian species including human.
Competence of in vitro development of cat oocytes is low in comparison with other model organisms. A major factor affecting the viability of cells during in vitro culture is an increased oxidative stress. Oxidative modifications could be responsible for oocyte incompetence for in vitro maturation (IVM). The objective of this study was to investigate the relative mRNA expression pattern of three genes - *Sep15* (selenoprotein 15), *Gpx1* (glutathione peroxidase 1) and *Sephs2* (selenophosphate synthetase 2), each involved in protection against free radicals, between oocyte and the surrounding cumulus cells during in vitro maturation.

Material and methods:
Ovaries from healthy, feral, 8-12 months old female domestic cats (n=10), were obtained by ovariectomy in local veterinary clinic. After 48h of culture in maturation media oocytes were collected at different stages (GV n= 40, MII n=75) and pooled in groups. Total RNA was isolated and level of mRNA expression was tested by qPCR method.

Results:
Results revealed that all three transcripts were detected, independently of the maturation stage. Nevertheless, significantly higher level of *Gpx1* and *Sephs2* expression was observed in oocytes, compared with their surrounding cumulus cells. Particularly higher level of *Gpx1* mRNA expression was observed in MII oocytes ($P<0.05$), but no quantitative differences in the *Sep15* mRNA expression was detected between GV and MII oocytes ($P>0.05$).

Conclusions:
In conclusion, our results indicate that *Gpx1* and *Sephs2* can be involved in in vitro maturation of oocytes and may be a part of anti-oxidant pathways induced by oxidative stress. Further studies should be conducted on the oxidative response pathways in relation to developmental potential of oocytes matured in vitro and conservation of the mechanism in human.
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P–11

TRANSCRIPTOME ANALYSIS AND TIME-LAPSE OBSERVATION OF A CASE WITH REPEATED MULTIPLE PRONUCLEI AFTER ICSI

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transcriptome, time-lapse, multiple pronuclei

Introduction:
Pronucleus (PN) formation is a process by which the parental chromosome complements unite and engender the zygotic genome. During fertilization, the maternal PN is pulled toward the male counterpart, and the 2PN are juxtaposed and positioned in the central or paracentral region of the cytoplasm (Terada et al., 2010). More than 2PN can sometimes be observed, which is generally assumed to be due to either a polyspermic penetration or to the failure of a second polar body extrusion, especially in the case of 3PN (Rosenbusch et al., 2010). However, for oocytes with >3PN, the reason of their incidence remains unclear. The aim of the study was to identify the cause of repeated multipronuclei (MPN) formation in zygotes of a patient after intracytoplasmic sperm injection (ICSI).

Material and Methods:
This is a case study of a patient with repeated MPN formation after five ICSI cycles (four in other clinics and one in Istanbul Memorial Hospital). Time-lapse monitoring of PN formation was carried out. Embryos developed from seven MPN zygotes of the sixth cycle were analyzed by FISH with the informed consent of the patient. Six MPN day-3 embryos and two surplus day-3 embryos for which the couples did not accept freezing were collected with informed consent from the patient and two couples, respectively. They were analyzed by whole genome expression analysis (Clariom™ D Assay, ThermoFisher Scientific).

Results:
The time-lapse evaluation of the patient's 17 oocytes showed 9 MPN zygotes, one 3PN zygote, two unfertilized oocytes and three normally fertilized zygotes. The paternal pronuclei was of normal size and 7-9 micropronuclei appeared beneath the second polar body and migrated to the center of the cytoplasm. The ensuing cleavages were unequal and gave rise to a high rate of fragmentation. All MPN and the 3PN embryos arrested their development on day-3. Seven MPN embryos were analyzed by FISH and 1-20 micronuclei were observed in each blastomere. Six embryos were diagnosed as being chaotic aneuploid and one as being monosomy18 and nullisomy13.
Transcriptomes of six MPN arrested day-3 embryos and two surplus 2PN good quality day-3 embryos were compared. Three pathways involving the AXIN1 gene were found to be significantly affected: Wnt signaling pathway and pluripotency, embryonic stem cell pluripotency pathways and the apoptosis-related network due to altered Notch3 in ovarian cancer. The chemokine signaling pathway (CCL19, FGR, PREX1 genes) known to be involved in the regulation of the actin cytoskeleton, which has a role in cell division, vesicle and organelle movement and interestingly documented to be disrupted in patients with premature ovarian failure was also found to be involved.

Conclusions:
Several candidate coding genes involved in cell cycle, pluripotency of stem cells and actin cytoskeleton pathways have been identified as a new cause of MPN formation and infertility. However, transcriptional data from six MPN embryos can only provide clues for further investigation. Future studies, including whole exon sequencing of candidate genes of the patient, her family and other patients with MPN zygotes might help in identifying the exact mechanism of this phenomenon.

P-12

EMBRYOS FROM THE SAME COHORT SHOW HIGH VARIABILITY IN THE MTDNA LEVELS

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mtDNA, PGT-A, variability

Introduction:
Mitochondria play a vital role in cell function. Recently, there has been an increasing research on mtDNA as biomarker of embryo implantation. Reports showed that high levels of mtDNA in blastocyst are associated with low implantation potential. Based on the available evidence, it is unclear whether high-levels of mtDNA is a constant feature in some group of women or it depends on each embryo, showing variability among sibling embryos from the same cohort. The aim of this study was to investigate the variability in the levels of mtDNA among sibling euploid embryos from the same cohort.

Material and Methods:
A prospective non-selection study was performed from January 2018 to December 2018. We included 249 blastocysts biopsies from 89 couples who attended our clinic for PGT-A with oocyte donation and with at least two of their biopsied embryos diagnosed as euploid non-mosaic. The aneuploid testing was performed by NGS-Veriseq® (Illumina). The mtDNA analysis was performed once the embryo diagnosis was known using MitoSeek. Sequencing reads mapping to the mtDNA genome were extracted from bam files. The relative measure of mtDNA copy number was calculated by dividing the mtDNA reads by the nuclearDNA and subjected to mathematical correction factor according to the embryo genome. Given that the mtDNA content is dependent upon the number of cell division that preceded the biopsy, the mtDNA content was also corrected by a factor according to the day when the embryos were biopsied. Statistical analysis was performed using SPSSv20.0.

Results:
In order to avoid confusion related to the oocyte quality we included only egg donation cycles, therefore the average maternal age at time of oocyte retrieval was 26$\pm$ 4 years (range: 19.0–33.0). Overall, the mean average of mtDNA per genome was 0.0010$\pm$0.0008 (range: 0.0002-0.0039). We analysed the mtDNA within an embryo cohort. To evaluate the extent of the variability in the mtDNA among embryos from the same cohort we calculate the coefficient of variation, being the average 28.77%. Furthermore, each embryo was classified according to the mtDNA 10th-percentile which belongs. The mean difference in the 10th-percentile between the embryo with the higher and the lower mtDNA within a cohort was 30. This difference was not related with the age of the egg donor neither the male factor ($p>0.05$). Only 13.5% of patients showed no difference in the mtDNA content within the embryos from the same cohort.

Conclusion:
Our results suggest high variability in the mtDNA content within euploid embryos from the same cohort. MtDNA could be used to predict reproductive potential amongst chromosomally normal embryos however the utility of quantifying mtDNA levels depend on the existence of variability among embryos from the same cohort. This study reports that mtDNA content is not a marker of prognosis amongst patients because it is variable among the embryos from the same cohort, thus mtDNA could be used as an additional marker of embryo competence and a tool for prioritize the euploid embryo to be transferred.

P-13

THE CHROMOSOMAL SET ANALYSIS OF BLASTOCYSTS OBTAINED FROM MODIFIED DONOR OOCYTES BY THE PATIENT'S 1ST POLAR BODY TRANSFER

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the first polar body transfer, euploidy, aneuploidy, trisomy, monosomy

Introduction:
Transfer of the patient's 1st polar body (PB) to donor oocyte cytoplasm has proven to be an effective method to help patients with low ovarian reserve and poor response. This technique allows doubling the number of oocytes suitable for fertilization, while maintaining the biological relationship of patient with the future child. The use of transfer in our practice has shown the ability of modified oocytes to form high morphological quality blastocysts and their euploidy have been evaluated in this study.

Materials and Methods:
The study was performed in the Medical Center IGR from March 2017 to December 2018 and involved 330 cells: 168 oocytes (group A) obtained from 39 patients (mean age 40.7±5.3 years) and 162 oocytes (group B) that were received from 28 donors (mean age 28.2±2.4). Donor oocytes have been pre-enucleated and modified by the transfer of patients' 1stPB with further fertilization. The procedure was made using Nikon Ti Eclipse (Japan) inverted microscope, Saturn 3 laser console (UK). Preimplantation genetic testing for aneuploidy (PGT-A) was performed using trophectoderm (TE) biopsy on the post-fertilization hours 120 or 144. Samples were diagnosed using Ion S5 by Thermo Fisher Scientific(USA). The samples' ploidy was verified by the FISH. We evaluated the number of euploid and aneuploid blastocysts from maternal and modified oocytes by the 1st PB transfer. Statistical analysis was carried out using Shapiro-Wilk test for normality and Chi-square test.

Results:
In the group A there were 50 blastocysts (29.8%) that formed from 168 original patients' oocytes and 29 blastocysts (17.9%) developed from 162 modified oocytes (group B). 45 blastocysts from group A and 27 blastocysts from group B were biopsied for PGT-A. The number of euploid embryos was 11 (24.4%) and 6 (22.2%) in the group A and group B, respectively, without statistically significant difference (SSD). Among the aneuploidies, single (only one chromosome was involved in nondisjunction) and complex (several chromosomes involved in nondisjunction) chromosomal anomalies were encountered. In the group A 13 (38.2%) samples carried single chromosomal disorder and 21 (61.8%) samples had complex quantitative abnormalities, in the group B these numbers reached 9 (47.4%).and 10 (52.6%) samples, respectively. Among the aneuploid embryos 79 chromosome abnormalities were found in A group samples and 38 numerical aberration in B group samples.. The following types of chromosomal abnormalities were detected in groups: autosome trisomy (50/63.3% vs. 25/65.8% samples), autosome monosomy (23/29.1% vs.6/15.8% samples), numerical violation of sex chromosomes (6/7.6% vs. 4/10.5% samples), polyploidy (0 vs. 2/5.3% samples), mosaicism (1/1.3% vs. 0 samples) in the group A and the group B, respectively. No SSD was found between the groups ($p>0.05$).

Conclusions:
The results of our study demonstrated that the 1st PB transfer does not affect the chromosomal set of embryos obtained from modified oocytes in comparison with the original oocytes. This technique is useful to increase the number of female germ cells and avoid the full oocytes donation especially for patients with low ovarian reserve and for poor responders.

P-14

A FULLY AUTOMATED INSTRUMENT FOR PREIMPLANTATION GENETIC TESTING (PGT-A) LIBRARY PREPARATION

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automation, DNA sequencing, PG-Seq

Introduction:
Technology used in preimplantation genetic testing for aneuploidy (PGT-A) has rapidly evolved since its introduction in 1990. The most commonly used methods use Next Generation Sequencing (NGS) for complete genomic analysis. One example is the PG-Seq™ kit (PerkinElmer), which begins with a sample of embryo biopsy cells and ends with a full analysis of the DNA library sequence. NGS sample preparation is a laborious, hands-on, and time-intensive process. We have developed a fully automated microfluidic platform to perform library preparation for the PG-Seq kit workflow. This instrument not only reduces the “hands on” time; it also helps to standardize the DNA library preparation through removing all manual pipetting handling and ensuring precise and consistent results between sample preparations, even between NGS runs.

Materials & Methods:
The main objective of this study was to demonstrate the performance of a specifically designed automation instrument for single sample library preparation as is used in the PG-Seq kit for NGS. The NEXTFLEX® Rapid XP DNA-Seq Kit from Bioo Scientific (a PerkinElmer Company) was used to optimize this platform, following standard manufacturer’s instructions. The three main components of this assay are (1) Fragmentation, End-Repair & Adenylation, (2) Adapter Ligation, and (3) PCR Amplification. The enzymatic fragmentation of the input DNA is a crucial component of this assay as this step is necessary for the PG-Seq™ kit following whole genome amplification. The representative input DNA used in this specific study was 180 ng of Lambda DNA from New England Biolabs. This kit was also tested off-device using fully manual preparation as a control and both on-device and off-device samples were evaluated using the NanoDrop 1000 Spectrophotometer and Agilent Bioanalyzer 2100 (DNA 1000 Kit) for DNA library concentration, purity, and size analysis.

Results:
The total yield of prepared DNA library using the instrument is around 50% of the off-device, manual testing. More specifically, the off-device, manual tests resulted in 739.1 ± 44.8 ng of DNA in a 20 µL sample, while the most recent on-device testing produced 415.1 ± 54.1 ng of DNA in a 20 µL sample. The average fragment sizes in these libraries is between 300 and 400 base pairs. As performing this assay on this instrument is further optimized, it is anticipated that this library yield will increase. Additionally, this study shows a significant decrease in the amount of hands-on time needed for researchers to run the NEXTFLEX® Rapid XP DNA-Seq Kit from ~2.5 hours to less than 30 minutes required to load reagents into their appropriate wells before starting the instrument. This instrument has also been optimized for additional DNA-Seq kits and, when launched, will accommodate even more protocols offering a flexible automation system.

Conclusions:
Overall, this instrument is a hands-free device for NGS sample library preparation that automates the PG-Seq kit library preparation. In time, this technology will be combined with PG-Seq Whole Genome Amplification, providing a fully automated system that can be used for routine PGT-A.

P-15

IMPACT OF MATERNAL AGE IN THE INCIDENCE OF UNIFORM AND MOSAIC ANEUPLOIDIES

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maternal age, aneuploidy, PGT-A, NGS, mosaicism

Introduction:
Female fertility decreases with age and increases the incidence of aneuploidies mostly due to meiotic errors. The incidence of aneuploid embryos turn around 60% confirming the high abortion rates observed by the presence of chromosomal aneuploidies. Next generation sequencing (NGS) proved to be the most appropriate technology for aneuploidy testing in trophectoderm biopsies, with accurate results, high throughput and cost efficiency. However, the rates and levels of embryonic mosaicism are still a subject of discussion. It is known that they can lead to miscarriage and birth defects but the data linking advanced maternal age and mosaicism is limited. Thus, this study aims to assess the differences between the incidence of different levels of mosaicism and uniform aneuploidies in blastocyst biopsies according to maternal age to verify if they follow a similar pattern.

Material & methods:
A total of 34,387 blastocyst biopsies were sent for PGT-A analysis between October and December 2018 from private assisted reproduction centers to our laboratory. NGS technology (ThermoFisher platform) combined with diagnosis a proprietary validated algorithm was used to optimize results. Patients were stratified by age in seven groups: <30 year; 30 to 34 (<35 years); 35 to 37 years; 38 to 40 years; 41 to 42; ≥43 years; and an additional group of egg donors (ED). Results were divided in 3 groups: Euploid, Abnormal and Total Mosaic. Mosaicism results were subdivided into 2 groups, according to the degree of mosaic cells: High degree: >50% - <70% and Low degree: >30% - <50%. Data were analyzed comparing the groups and all results with Kruskal-Wallis test.

Results:
33,341 biopsies were informative with 6% (n=1997) of total mosaicism, 47% uniform aneuploidy (n= 15.670) and 47% euploid biopsies (n=15.674). Regarding the mosaicism rates, 65.3 % (n= 1305) were low mosaic degree, and 34.6% (n=692) were high mosaic degree. A significant decrease ($p<0.05$) in the rate of euploid embryos was observed with increasing maternal age (61% and 61.5% in women <30 years and ED; followed by 59.3 %; 51.4%; 38.6%; 26.9%; and 29.6% in women ≥43 years). In the mosaic category, a slight decrease rate in low mosaic embryos with advancing maternal age was detected (4.9% in women <30 years, 4.7% in ED, followed by 4.9%, 4.5%, 4.7%, 3.4%, 2.6% and 1.6% in women ≥43 years). High mosaic embryos did not show this trend (1.3% in women <30 years and 1.5% in ED, followed by 1.6%, 2.3%, 2.6%, 2.3% and 1.8% in women ≥43 years).

Conclusions:
The rate of euploid embryos decreased with advanced maternal age and aneuploidies increased, corroborating previous data from other studies. Regarding mosaicism different trends were observed for low mosaic degree, with a trend towards a decrease of low mosaic degree with maternal age, and high mosaic degree without an effect of maternal age.

P-16

CORRELATION BETWEEN NUMBER OF CHROMOSOMES ALTERATIONS AND TIME-LAPSE EMBRYO SCORE: PRELIMINARY OUTCOMES

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PGT-A, time-lapse, embryo morphokinetics, aneuploidy

Introduction:
Despite of conventional morphological evaluation is the classical method for embryo selection it clearly has limitations. Recently, time-lapse monitoring allows the embryo quality classification based on morphokinetics. Moreover, Preimplantation Genetic Test for Aneuploidy (PGT-A) is applied to investigate the genetic status of the embryos and the selection of euploid embryos for transfer is supposed to lead to improved clinical outcomes. The association of morphokinetics and PGT-A opened new opportunities to refine blastocyst selection. However, knowledge of the impact of chromosome alterations on embryo morphokinetics need to be increased. The aim of this study was to correlate embryo score from time-lapse imaging with chromosome status of the embryos evaluated by PGT-A.

Material & methods:
This is a preliminary retrospective cohort study of prospectively collected data for 81 ICSI cycles performed at a private assisted reproduction center in Brazil, between April and November 2018. A total of 260 embryos developed into blastocysts suitable for biopsy and were analyzed in this study. Embryos were cultured in the Embryoscope® time-lapse imaging chamber (Vitrolife®) and the image acquisition system was set to capture images from each embryo every 15 minutes during the entire culture interval. Embryos were scored from zero to 10 based on the timings of cell divisions with the start of ICSI until the blastocyst formation and expansion on day 5 or 6 of development. PGT-A was elective, but strongly encouraged for patient's ≥37 years old, implantation failure, recurrent miscarriage or severe male factor cases. Embryos were biopsied at a blastocyst stage and PGT-A was carried out at a global reference laboratory (Igenomix Brasil) through NGS.

Results:
The women age varied from 25 to 42 years (38.5±2.9) and 1 to 10 blastocysts were biopsied per patient (3.2±2.0). Of the 260 biopsied blastocyst, 38.7% were euploid. The embryos scores were slightly lower in aneuploid (5.9±2.1) than euploid (6.4±2.1) blastocysts with a marginally significant statistical difference (p=0.067). The number of altered chromosomes were annotated and inversely correlated to embryo score (Pearson correlation: r=-0.210; p=0.001). Then we classified the number of altered chromosomes as none (euploid), 1-2, 3-6 and more than 6 (chaotic). We only observed a significant difference between the time-lapse embryo score of chaotic (3.9±1.3) and euploid (6.4±2.1; p=0.026) blastocysts. The 1-2 (6.1±2.0) or 3-6 (5.4±2.3) alterations showed similar time-lapse embryo score to euploid embryos (p=1.000 and p=0.161, respectively). The multivariate linear regression model have confirmed the higher number of altered chromosomes lower the time-lapse embryo score (Coef=-0.149; p=0.026) adjusted for confounders as women age and time of culture.

Conclusions:
The findings of our study suggest the number of altered chromosomes affect the embryo morphokinetics, leading to a reducing time-lapse embryo score. Although there seems to be a relationship between the ploidy status and complexity of chromosome alterations with the embryo morphokinetics, time-lapse embryo score is not enough to predict chromosome alterations, and therefore, replace PGT-A. These are preliminary outcomes and further studies are being conducted to investigate the association of chromosome alterations with embryo morphokinetics and relate them with clinical outcomes.

P-17

DO PROGESTERONE VALUES AFFECT THE EUPLOID RATE OF EMBRYOS IN PGT-A PATIENTS?

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Progesterone, euploid, embryo, PGT-A

Introduction:
In about 35% of cases undergoing ovarian stimulation may occur a rise in serum progesterone in the late follicular phase. This could produce premature luteinization and affect the endometrial receptivity. However, some studies suggest that the elevated progesterone could generate damage to the oocyte affecting the embryo quality and therefore increasing the rate of aneuploidy in the generated embryos which contributes decrease the implantation and newborn live rate.

Materials and Methods:
A total of 2509 patients undergoing preimplantation genetic testing for aneuploidy screening (PGT-A) were included in this retrospective study performed between 2016 and 2018. The age of patients ranged from 22 to 49 years. Laser assisted blastocyst biopsies were done in all cases by either pulling or flicking method. Antagonist protocol was used in the majority of the cases and P4 levels were measured the day of hCG administration. Poisson regression model was used as statistical analysis where progesterone was considered as a continuous variable. Other variables such age, body mass index (BMI), estradiol and ovarian sensitivity index were included in the model. P values < 0.05 were considered statistical significant.

Results:
The increase of ovarian sensitivity index in one unit predicted the increase of euploid embryos in 1.67 times when controlling for the rest of variables. The increase in age had a negative influence decreasing the mean number of euploid embryos by 10%.
On the other hand the increase of P4 values predicted an increase in the number of euploid embryos by 7%.

Conclusions:
The increasing values of progesterone does not decrease the availability of euploid embryos for transfer therefore, based in this initial approach P4 seems a surrogate marker of higher ovarian response which is also positively associated with embryo euploidy.

P-18

EUPLOID BLASTOCYSTS IMPLANT IRRESPECTIVE OF THEIR MORPHOLOGY AFTER PREIMPLANTATION GENETIC TESTING-ANEUPLOIDY USING NEXT GENERATION SEQUENCING.

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Preimplantation genetic testing aneuploidies, blastocyst, morphology

Introduction:
In the context of IVF, it has become imperative to emphasize on embryo evaluation in order to maximise assisted conception outcome while minimising the risk of multiple pregnancy. Despite the exponential rise in knowledge and understanding of the dynamic process of embryo development in the laboratory, the classical evaluation methods based on morphology are still considered as the gold standard methods to classify and select embryos. Morphology assessment helps embryologists to detect dysmorphic or arrested embryos but chromosomal abnormalities can still be only identified by preimplantation genetic testing for aneuploidies (PGT-A). It remains controversial the strength of morphology as a tool to ensure replacement of euploid blastocysts. The present research aims to corroborate whether blastocyst morphology, using a modified Gardner and Cornell's group scoring system, correlates with ploidy status of embryos analysed with next generation sequencing (NGS). We also wanted to ascertain whether biopsy of poor quality embryo yield a euploid embryo to transfer and thus result in a favourable outcome. This is the first data set describing the correlation between blastocyst morphology and embryo ploidy status using NGS and clinical outcomes.

Materials and Methods:
296 patients underwent PGT-A. Of 1,549 blastocysts, 1,410 blastocysts had a conclusive result after PGT-A and were included for analysis. An elective single embryo transfer (eSET) policy was followed in a frozen embryo replacement cycle. A total of 179 euploid blastocysts were thawed and transferred. Clinical outcomes were categorised in four different embryo quality groups: excellent, good, average and poor.

Results:
Euploidy rates were 19/36 (52.7%, 95% CI 37-68), 199/470 (42.3%, 95% 38-47), 156/676 (23.0%, 95% CI 20-26) and 39/228 (17.1%, 95% CI 13-23) in the excellent, good, average and poor quality blastocyst groups, respectively. Fitted logistic regression analysis taking into account the following co-variables; female age, embryo chromosomal status, and day of blastocyst development/biopsy showed that good (OR 0.6; 95% CI 0.4-0.8;p=0.001), average (OR 0.5; 95% CI 0.4-0.7; p=0.001) and poor (OR 0.2;95% CI 0.1-0.5; p=0.005) morphology with excellent morphology being the reference group was predictive of the comprehensive chromosome screening result. A logistic regression analysis was also performed on clinical outcomes taking into account for the effect of blastocyst morphology and day of blastocyst development/biopsy. None of the parameters were shown to be significant suggesting morphology irrespective of the embryo quality category and day of blastocyst development/biopsy do not reduce the competence of euploid embryos (p>0.05).

Conclusions:
After euploid eSET, implantation rate was 80-86%; live birth rate per embryo transfer was 60-73% and clinical miscarriage rate was found to be less than 10% and were not significanlty affected by the embryo morphology category. These results are concordant with those reported when using array compartive genomic hybridization and highlights the competence of poor quality euploid embryos.

P-19

PGT-A: WHEN IT IS BETTER NOT TO KNOW

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aneuploidy, mosaicism, segmental chromosomal abberations, drop-out rate; Next Generation Sequencing (NGS)

Introduction:
PGT-A was implemented to select euploid embryos and thus, to improve IVF-outcome in terms of higher live birth rate (LBR), lower miscarriage rate or shorter time-to-pregnancy. With the implementation of refined techniques such as Next Generation Sequencing (NGS) the resolution to detect (small) chromosomal aberrations in embryos increased. This holds true for as chromosomal mosaicism as well as for segmental aberrations, and consequently the amount of embryos tested as euploid decreases. This observation highlights the complex nature of genetic (in)stability during early embryogenesis and reveals the substantial shortcomings of PGT-A. The early genetic plasticity counteracts with the primary goal to improve LBR. The aim of the study was to determine when PGT-A can be recommended considering the drop-out rate starting from non-biopsable embryos, amplification failure and drop-out due to testing as non-euploid.

Patients, material & methods:
This retrospective single-center study (2016-2018) includes 173 hormonal stimulation cycles (71 patients). PGT-A was performed either due to three unsuccessful embryo transfers or due to three miscarriages in the patient's history. Patients with tested structural chromosomal aberrations as well as donor cycles were excluded. Blastocyst culture was performed in single step culture medium in the Embryoscope. TE-biopsy was performed on day 5-7. WGA was performed either by PicoPlex (Rubicon Genomics) or by SurPlex (BlueGenome/ Illumina). NGS was processed using VeriSeq platform with data analysis by BlueFuse Multi (Illumina).

Results:
A total of 1,751 MII oocytes resulted in 752 blastocysts. TE-biopsy of at least one blastocyst was performed in 157 cycles (68 patients). This resulted in 609 biopsied blastocysts of which 57 showed either failure of amplification or defective sequencing. 156 (25.6%) samples showed either whole chromosome aneuploidies or uniform segmental chromosomal aberrations. Further, 117 (19.2%) biopsies revealed either chromosomal or segmental mosaicism and, 140 (23.0%) were tested with a combination of uniform and mosaic aberrations. Finally, 139 (22.8%) samples were tested as chormosomally normal. This results in mean 0.8 blastocysts/cycle tested as known euploid compared to originally 4.5 blastocysts/cycle.

Conclusions
High embryo losses due to non-biopsable blastocysts, amplification failure and non-interpretable results are well known but often neglected. Additionally, while NGS allows high resolution for small structural or numerical chromosomal aberrations as mosaic, the number of transferable blastocysts decreases enormous too. Chromosomal mosaicism (euploid/aneuploid) is a major problem of PGT-A, additional problems arises from segmental chromosomal aberrations that can be present as mosaic as well.
When PGT-A is recommended IVF-patients, physicians have to inform about high costs for patients, the increased number of IVF-cycles needed and the high cancellation rates. When estimating a 60% baby-take home rate after PGT-A the same number of babies would be born starting with 752 untested blastocysts considering an only 11% baby-take home rate (83.4 vs. 82.7 babies). This comparison shows that counselling for PGT-A due to RIF or RM has to be well prepared.

P-20

RELATIONSHIP BETWEEN EUPLOIDY RATES AND D5 KIDSCORE™ OF BLASTOCYSTS DERIVED FROM EMBRYOSCOPE

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Time-lapse microscopy, D5 KIDScore™, euploidy rate, PGT-A, Next Generation Sequencing

Introduction:
Time-lapse microscopy (TLM) offers continuous monitoring of embryo development in an undisturbed culture condition, minimalising the stress imposed by changes in temperature, oxygen and pH in the culture medium; hence optimising culture environment. Moreover, the availability of morphokinetics assessment and annotation models such as KIDScore™ in TLM can help predict the implantation potential of an embryo. Several studies have also shown a reduction in pregnancy loss in IVF with TLM. However, the association between annotation models and euploidy rates has not been reported. This is a retrospective study to assess the relationship between euploidy rate and D5 KIDScore™ of blastocysts derived from IVF in Alpha Fertility Centre, Malaysia between January to December 2018.

Material and Methods:
Eight hundred and seven (807) patients (age ranged 25 – 45) had their embryos cultured to blastocyst stage. All blastocysts were cultured in Embryoscope™ according to the manufacturer's specifications (Vitrolife, Sweden). Any utilisable blastocysts were annotated and scored using D5 KIDScore™. Blastocysts scored between 1.0 – 5.9 were categorised as Group A while those scored between 6.0 – 9.9 were categorised as Group B. Three hundred and seventy-one (371) blastocysts from Group A and 1783 blastocysts from Group B which was at least fair grade (using Gardner blastocyst grading system) were biopsied and screened using Next Generation Sequencing (Ion Torrent, USA). The mean age of patients from Group A and B were 34.4 and 34.1 respectively ($p>0.05$).

Results:
The euploidy rate of blastocysts from Group A and B were 48.8 % and 59.3% respectively whereas the aneuploidy rate of blastocysts from Group A and B were 51.2% and 40.7% respectively. Both euploidy and aneuploidy rates between Group A and Group B were statistically significant ($p=0.0002$).

Conclusions:
This retrospective study demonstrates that blastocysts with a D5 KIDScore™ of 6.0 – 9.9 have a higher euploidy rate compared to blastocysts with a D5 KIDScore™ 1.0 – 5.9. It is thus recommended for patients who had time-lapse embryo monitoring without PGT-A to transfer blastocysts with a D5 KIDScore™ 6.0 – 9.9 in lieu of the higher euploidy rate. Nevertheless, further studies with a larger sample size should be performed to confirm the above.

P-22

A NOVEL, FAST, SINGLE TUBE AMPLIFICATION AND INDEXING APPROACH FOR PGT-A FOR ILLUMINA® SEQUENCING INSTRUMENTS.

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PGT-A, WGA

Introduction:
Most methods to prepare embryo biopsy samples for Preimplantation Genetic Testing for Aneuploidy (PGT-A) for Illumina® sequencing instruments use a two-step approach of Whole Genome Amplification (WGA) followed by library preparation. A single tube, PCR indexing approach offers several advantages for laboratories performing PGT-A including protocol time efficiencies and reduced hands on time. A novel protocol has been developed based on the PerkinElmer DOPlify® WGA kit. The aim of this study was to determine the accuracy and performance characteristics of this protocol on 5 cell samples from euploid, single and double trisomy cell lines.

Material and methods:
Five-cell samples representative of trophectoderm biopsy were manually sorted from aneuploid cell lines (Coriell Institute) and euploid lymphocytes. Cell lysis and WGA were performed using a modified DOPlify® kit protocol (PerkinElmer) followed by incorporation of Illumina specific adapter sequences and unique indexes in a novel single tube approach. Amplified, indexed cell samples were purified, quantified then pooled before 50 sample multiplex and 1x75bp read length sequencing on the Illumina MiSeq® Instrument using v3 chemistry. Sequencing data was analysed for correct aneuploidy calling using the PG-Find™ software (PerkinElmer).

Results:
A total of 104 multicell samples were processed with the following karyotypes: 47,XY,+15 (n=22), 47,XX,+18 (n=20), 48,XXY,+21 (n=20), 46,XY (n=22) and 46,XX (n=20). Three samples were excluded from final analysis due to weak amplification (3%). All other samples were processed through to sequencing and displayed the expected karyotype when analysed with the PG-Find™ software, with no false positives or false negatives observed. The 50-sample multiplex generated an average of 510,000 mapped reads per sample with a 98.9% mapping rate to hg19. The novel protocol took on average 4.5 hours (2.5 hours hands on) to process 50 samples from sample receipt to MiSeq® Instrument loading.

Conclusions:
The novel one tube amplification and indexing method was shown to be a fast, accurate protocol for PGT-A utilising Illumina® sequencing instruments. Sequencing ready, 50 sample multiplex pools were prepared in 4.5 hours, readily enabling a less than 24 hour turn-around time from sample receipt to reporting. Studies processing single cells (representative of blastomere biopsy) and cell samples with smaller segmental aberrations to determine the resolution of the protocol are underway, which will be followed by clinical validation using embryo biopsy samples.

P-23

SCORING METHOD TO ESTIMATE CLINICAL PREGNANCY USING ARTIFICIAL INTELLIGENCE MODEL.

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Clinical pregnancy, PGT-a, deep learning, artificial intelligence.

Introduction:
Clinical pregnancy represents success outcome in a PGT-a in cycle. It can be defined as the presence of sacs with fetal heart beats at 7-8 weeks. Main purpose of PGT-a study is to identify euploid embryos in order to be replaced to patient endometrium. Nevertheless, PGT-a cycles differs in relation with attributes related to patients as well cycle information. The main goal of the present study is to design an predictive model capable to give a probability of clinical pregnancy before embryo transfer takes place.

Material & methods:
Dataset used included 1190 cycles that belong at New York University Fertility Center between 2012 and 2015. Patient id was encrypted with an alphanumeric code. The classifier implement was a Multilayer Perceptron. Feature engineering and standardization was carried out in the original variables. Algorithm was implemented using Python programing language.Model parameters was searched using grid search methodology. Classification performance was evaluated via 5-fold cross validation and area under the curve (AUC)

Results:
Variables used as a predictor were: maternal age, biopsy specimen method, reason for referral and quantity of euploid and aneuploid embryos generated in the cycle. In order to evaluate normality distribution among the dependent variables selected D'Agostino's K2 test was performed as a goodness-of-fit measure. Results variables showed a non gaussian distribution ($p>0.05$). Lack of normality was due to presence of outliers. To mitigate it effects we employed a technique called feature engineering applying mathematical function to the original variables. In order to include categorical variables, it were coded using numerical encoding.
Model implemented achieves an Area Under the Curve (AUC) of 80%. In other words, the sensitivity-specificity tradeoff in the classifier was of 80%. It is important to note that threshold selected was 0.5. It means that cases with an output after classification with values higher than the cut-off were classified as Pregnant. On the other hand cases with output lower than 0.5 were assigned as non pregnant. Furthermore, Sensitivity (True Positive / True Positive + False Negative) and Specificity (True Negative / True Negative + False Positive) were 82% and 69% respectively.

Conclusions:
We are aware about the presence of an intrinsic bias closely related to the Fertility Center where the PGT-a cycles was performed. In order to overcome that we are developing a new algorithm capable to solve current limitation being capable to be adapted for a specific center.
A scoring tool based on artificial intelligence can be helpful in selecting those patients with high probability to achieve clinical pregnancy during PGT-a cycle.
Artificial neural networks is a robust estimator that allowed us to overcome multicollinearity between the original variables. Overall performance of the model was high (80%) giving strong evidence that the cycles can be accurately classified.

P-24

PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDIES WITH NGS ON MINISEQ. A RELIABLE ALTERNATIVE FOR SMALL LABORATORIES

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Preimplantation genetic testing for aneuploidies, Next Generation Sequencing, mosaicism, MiniSeq

Introduction:
The demand for Preimplantation Genetic Testing for aneuploidy (PGT-A) used in IVF, is constantly increasing to improve pregnancy outcomes by transferring euploid embryos. The introduction of NGS has led to rapid transition of PGT-A from array platforms to low depth high-throughput whole genome sequencing which is now implemented in many laboratories worldwide. However, for small laboratories, the transition from array platforms to NGS should also take into consideration cost effectiveness. The aim of this study was to achieve the transition from array-CGH to NGS using the VeriSeq™ PGS (Illumina Inc.), on the MiniSeq, which is the existing platform of the Department. Consequently, the current study compares PGT-A results between array-CGH and NGS and also directly compares NGS results from MiniSeq and MiSeq, the platform for which VeriSeq™ PGS is already validated.

Materials and Methods:
In total 110 amplified biopsy samples were included and blindly analyzed in this study which was divided into three phases. The first included 30 biopsies of day-3(13) and day-5(17) embryos that were previously analyzed using array-CGH. These samples were sequenced on both MiSeqDx and MiniSeq platforms (Illumina Inc.). For the second phase, 80 amplified biopsy samples (day-5), with segmental, mosaic or full aneuploidies were run on the MiniSeq platform with paired-end (2x36) sequencing using the MiniSeq High Output Reagent Kit (75-cycles) (Illumina Inc.). The last phase was to detect the sensitivity for mosaicism by mixing serial diluted samples with different chromosomal losses/gains to give 50%, 40%, 30% and 20% aneuploidy.
Library preparations on all phases were carried out using VeriSeq™ PGS (Illumina Inc.) following manufacturer's recommendations. For the MiniSeq, the secondary analysis of alignment was performed on-instrument with BWA-MEM to produce aligned reads. An in-house pipeline was implemented to adjust the headers within bam files in order to facilitate the downstream analysis of samples on Bluefuse Multi v4.4.

Results:
Comparative results on both platforms MiniSeq and MiSeq demonstrated 100% concordance, placing our in house pipeline using MiniSeq platform as a cost effective alternative approach for PGT-A, especially for small laboratories. The two different techniques array-CGH and NGS showed concordant results in 105/107 (98%) of the samples tested and overall concordance rate of 99.8% (2410/2415) per analyzed chromosome. All differences detected were attributed to mosaicism. Three day-3 embryos were excluded from the analysis as they failed to pass the VeriSeq™ PGS quality criteria. NGS successfully detected all segmental, mosaic or full aneuploidies (smallest 15Mb) depicting superior profiles than array-CGH due to the broader dynamic range. The sensitivity of the NGS platform for mosaicism detection was defined to be 20%.

Conclusion:
Our validation study result demonstrates that VeriSeq PGS is fully compatible with the MiniSeq platform and in full concordance with results obtained from the MiSeq. The results showed that PGT-A with NGS can identify more precisely chromosomal mosaicism and segmental aneuploidy than the PGS/aCGH platform. With the cost of sequencing gradually decreasing, the cost of PGS will make the service accessible to all fertility patients in order to enhance their chances of successful pregnancy.

P-26

MAJOR FACTORS INVOLVING AN IMPLANTATION RATE OF PGT-A EUPLOID EMBRYOS IN EGG DONATION PROGRAM OVER 35 YEARS

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PGT-A, aneuploidy, egg donation, implantation rate, window of implantation

Introduction:
A potential impact of PGT-A on embryo implantation is often discussed in the reproduction of elderly women with a higher risk of oocyte aneuploidy while its impact is belied in women under 35 years at the same time.
The authors studied the influence of PGT-A in egg donation program where the advanced reproductive age and potential oocyte incompetence is mostly excluded.

Material and methods:
In total, 246 embryos were thawed and transferred in egg donation patients of the age category 35 plus in period January 2017 – December 2018. Group A consists of 188 patients with a comprehensive aneuploidy screening (PGT-A), group B consists of 58 patient with no PGT-A. There were performed elective single embryo transfers solely in both groups.

Results:
In group A an implantation rate was 46% (86/188) while in group B 20%(12/58). Natural cycles with triggering ovulation were more successful than the HRT ones (IR 53% resp. 44%).

Conclusions:
The results showed that PGT-A and transfer of euploid embryo improve an implantation rate in egg donation program in the age category 35 plus what is usually disregarded. The number of cycles to pregnancy was lower in case of a euploid embryo was transferred. The major factors involving the implantation rate besides the embryo quality and euploidy are an appropriate endometrial quality and striking the window of implantation (WOI). The implantation rate is slightly better in natural cycles compared to HRT (hormonal replacement treatment) cycles. Among other factors, we reached better results using a longer progesterone administration (minimum 5 days) and a time-lapse monitoring system used in synergy with an embryo selection for the biopsy.

P-27

CLINICAL APPLICATION OF KARYOMAPPING – EMBRYOS AS REFERENCES?

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Karyomapping, single nucleotide polymorphism, phase, reference, PCR

Introduction:
Karyomapping uses the principles of genetic linkage to detect any monogenic disorder i.e. allowing the diagnosis of the presence or absence of a disease causing allele. The process involves genome-wide single nucleotide polymorphism (SNP) analysis of the parents, a reference (e.g. an affected child) and amplified embryos DNA. 'Informative loci' are then identified and compared to the reference DNA in order to establish phase. The genotype of each embryo is established by comparing to the genotype of the reference. Despite its many advantages, Karyomapping has its limitation, for instance, no family members available as reference for karyomapping testing. Therefore, this study is to explore alternatives to counter the limitation.

Material & methods:
A total of 42 blastocysts (8 cases) were biopsied and the cells obtained were amplified using the Repli-g Single Cell Kit (Qiagen). Blood and saliva samples from the couple seeking PGD and family members used as references were obtained and DNA is extracted using QIAmp DNA Midi Kit (Qiagen). DNA samples from the couple, family members and WGA amplified embryo samples were analysed using HumanKaryomap-12 BeadChips protocol (Illumina, USA), and results were evaluated using BlueFuse Multi 4.0, Illumina. DNA samples used as references for phasing of SNP alleles included any of the following: the couple's son/daughter, the parents of the male and/or female patient and WGA amplified embryo samples. The genes involved in each single gene disorder were targeted for assessment via Karyomapping. Direct mutation analysis with PCR and Sanger Sequencing was also performed in parallel with Karyomapping. Two approaches were conducted to assess the feasibility and reliability of the method.
Firstly, to assess the feasibility of the method, 15 out of 42 blastocysts (4 cases) were assessed without prior knowledge of phase as couples had neither children nor family members available. These blastocysts were processed using a combination of Karyomapping and direct mutation detection in order to establish the phase. Embryos diagnosed by direct mutation analysis were used as references for karyomapping.
Secondly, to assess the reliability of the use of embryos as reference, the remaining 27 blastocysts in which DNA samples from family members were available as references, were processed using Karyomapping in combination with PCR testing. Embryos diagnosed by these methods were used to replace the existing references for Karyomapping and to confirm the results of the existing PGD case detecting monogenic disorder.

Results:
Results obtained were shown to be in complete agreement between karyomapping and PCR testing indicating the feasibility of the method. Diagnoses made were also 100% concordant between family members as references and embryos as references which is indicative of the reliability of the method.

Conclusion:
The results indicated that the use of embryos as references allows informative loci to be determined in cases where reference is not available in some couple. Ideally, more cases should be carried out in the future to reaffirm the reliability of this method. These approaches will be beneficial for couples that have no family members available for karyomapping testing in order to establish phase.

P-28

KARYOMAPPING USING WHOLE GENOME SEQUENCING DATA: PRECLINICAL VALIDATION OF A PERSONALISED APPROACH TO PGT-M

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Karyomapping, whole genome sequencing, AI

Introduction:
Karyomapping utilises microarray technology to track informative single nucleotide polymorphisms (SNPs) and assign haplotypes to embryos without the need for direct mutation detection (Handyside et al. 2010). Although suitable for most couples undergoing PGT-M, the use of a fixed set of SNPs may provide inadequate information in certain genetic regions or in consanguineous couples (Konstantinidis et al. 2015). Whole genome sequencing (WGS) has the potential to individualise informative SNP detection and perform concurrent direct mutation detection. However, no studies have evaluated the potential for application of the karyomapping algorithm to WGS data.

Material and Methods:
The Platinum Genomes dataset (https://sapac.illumina.com/platinumgenomes.html) was analysed as a model for the application of karyomapping to WGS data. Variant call format (VCF) files were analysed according to the principles of karyomapping to create a map of informative biallelic SNPs in each grandparental haplotype. A model of embryo biopsy was created by diluting DNA from one of the family members (NA12878) to 30pg and whole genome amplification was performed with the Repli-G single cell kit (Qiagen, Hilden, Germany) as a model of trophectoderm biopsy. Whole genome sequencing was performed in triplicate on a high output NextSeq 2500 run (Illumina, California, USA) at average 15x coverage and the number of informative SNPs detected compared to the karyomapping SNP microarray. Comparison of variant calling methods was performed using the Genome Analysis Toolkit v3.4 (Broad Institute, Massachusetts, USA) and DeepVariant (Google, California, USA).

Results:
Analysis of WGS data from the Platinum Genomes family revealed a total of 2,338,158 informative SNPs distributed evenly between grandparental haplotypes, of which 85,053 (3.6%) would have been detectable using the karyomapping SNP array. Analysis of the karyomapping window around common genes requiring PGT-M demonstrated an average 32x increase in the number of targets available for analysis in a PGT-M cycle. Analysis of 15x coverage WGS data in a model of embryo biopsy demonstrated an average 279,851 informative SNPs detected using standard variant calling and an average 285,107 using AI-based variant calling. The median distance between informative SNPs was reduced from 26,782 nucleotides to 1,212 nucleotides when comparing WGS-based karyomapping to SNP array-based karyomapping.

Conclusions:
This study provides preclinical validation for the application of the karyomapping algorithm to WGS data. The markedly increased number of informative SNPs will allow successful feasibility studies for all couples presenting for PGT-M. The decreased distance between informative SNPs detected in a model embryo biopsy will improve diagnostic accuracy, particularly in detecting small double crossover events and reducing the number of cycles in which inadequate informative markers have been detected in the embryo sample. Finally, the use of WGS for diagnostic purposes, especially for rare and undiagnosed genetic disease, will allow the creation of a high resolution digital karyomap for couples with an affected child and improve the transition to IVF and PGT-M.

P-29

CHROMOSOMAL COPY NUMBER ANALYSIS OF CHORIONIC VILLUS FROM SPONTANEOUS ABORTION BY NEXT GENERATION SEQUENCING.

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Chorionic villus, Karyotyping, Next generation sequencing, Spontaneous abortion

Introduction:
Chromosomal abnormalities are the most common reason for spontaneous abortion. Conventional cytogenetic analysis by G-banded karyotyping is generally performed for chromosomal analysis, but it has a problem with low-resolution, and is needed long term cell culture and enough experience for diagnosis. More recently, next generation sequencing has been introducing and improving for chromosomal analysis as an accurate, high resolution and throughput method. In this study we aimed to compare the consistency between conventional G-banding and NGS-based chromosomal copy number analysis for chorionic villus from spontaneous abortion. In addition, the frequency of each chromosomal aneuploidy was evaluated.

Materials and methods:
From February, 2018, to April, 2018, chromosomal analysis for 7 chorionic villus samples from spontaneous abortions (from 7 to 9 weeks) were carried out both conventional G-banding and NGS (VeriSeq-PGS, Illumia). The frequency of each chromosomal aneuploidy was investigated for 110 cases from February, 2018 to December, 2018.

Results:
NGS was able to analyze all 7 cases, but G-banding was able to detect 6 cases, and one case was cell growth failure. In 6 cases analyzed by G-banding, the results of 5 cases were consistent with the results of NGS, but one case suspected maternal cell contamination. Among 7 cases analyzed by the NGS, 2 cases were normal male karyotype (46, XY) and 5 cases were autosomal trisomy, implying that there were no cases suspected of maternal cell contamination.
Among 110 cases, chorionic villus was not observed under microscope from 18 samples, NGS result was obtained from 92 cases. Seventy-one cases were found to have abnormal chromosomes (71/92, 77.2%), and 21 cases were normal karyotype (21/92, 22.8%). Aneuploidy of chromosome 22 (21.8%), chromosome 16 (17.9), chromosome 15 (11.9%), chromosome 21 (10.3%) and Chromosome X (10.3%) were more frequently, consistent with previous report.

Conclusions:
Chromosome analysis using NGS not only obtained comparable results to conventional G-banding, but also is able to analyze more accurately and quickly.

P-30

LIVE BIRTHS FOLLOWING DAY 7 BLASTOCYST TRANSFER AFTER PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY (PGT-A)

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Live birth, Day 7 blastocyst, Preimplantation Genetic Testing for Aneuploidy (PGT-A), Frozen blastocyst transfer

Introduction:
Extended culture to blastocyst stage is being adopted in many IVF clinics, with the aim to improve embryo selection and promoting single embryo transfer. Selection of these blastocysts usually occurs up to Day 6 of embryo culture. However, a study (Su et al., 2016) has revealed that Day 7 blastocysts can be euploid and can result in healthy live births. In this study, we evaluate the euploidy rate of Day 7 blastocysts and the clinical outcome of transferring these euploid Day 7 vitrified-warmed blastocysts in Alpha Fertility Centre.

Materials & Methods:
Forty-five (45) patients had utilisable blastocysts only on Day 7 of culture between Aug-2015 and Nov-2018. A total of 58 blastocysts which were at least fair graded (Gardner, 1999) were biopsied on Day 7 and analysed using Next-Generation Sequencing (Life Technologies, USA). Vitrification was done after biopsy using Cryotec method (Cryotec, Japan). Clinical outcome was assessed following the transfer of euploid blastocysts. All successful pregnancies were monitored until delivery.

Results:
The mean maternal age was 36.0 (age range:22-44). Of the 58 blastocysts analysed, 46.6% (27/58) blastocysts were euploid, 39.6% (23/58) blastocysts were aneuploid and 13.8% (8/58) blastocysts were mosaic. Subsequently, 10 patients had frozen blastocyst transfer: 9 had single blastocyst transfer (SBT); 1 had double blastocysts transfer (DBT). All blastocysts survived intact post-warmed. The clinical pregnancy rate was 70% (7/10) with an implantation rate of 63.6% (7/11). Two (2) of these patients each delivered a healthy baby weighing 2.81 kg and 3.08 kg respectively, while 1 pregnancy is ongoing at 30+3 weeks of gestation at the time of writing. Unfortunately, the remaining 4 pregnancies resulted in miscarriages at 8+1, 8+2, 7+5 and 9+4 weeks of gestation respectively.

Conclusions:
Our study suggests that Day 7 blastocysts can indeed be euploid and yield healthy live births albeit the seemingly higher miscarriage rate. Therefore, extended culture of embryos to Day 7 should be considered as a standard practice in IVF laboratories for patients with no utilisable blastocyst on Day 5 or Day 6 of culture.

Reference:
Su, Y., Li, J. J., Wang, C., Haddad, G., & Wang, W. H. (2016). Aneuploidy analysis in day 7 human blastocysts produced by in vitro fertilization. Reproductive biology and endocrinology : RB&E, 14, 20.

P-31

CHROMOSOMAL MOSAICISM AS A RISK FACTOR FOR INCORRECT RESULTS OF PGT-A

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mosaicism, PGT-A, aCGH

Introduction:
Mosaicism may cause discordance between the result of PGT-A and the karyotype of cells outside the biopsy area. How likely is the risk of incorrect results of PGT-A due to chromosomal mosaicism? The aim of present study was comparison of molecular karyotypes of trophectoderm cells and inner cell mass. The study presents the results of re-analysis of the molecular karyotypes of 10 embryos by the comparative genomic hybridization on microarrays (aCGH).

Material & methods:
Initially, the trophectoderm biopsy was performed for all 10 embryos for PGT-A using the aCGH analysis (24sure, Illumina). All 10 trophectoderm samples were found to be aneuploid. Corresponding embryos were not recommended for transfer and were included in this study per patients' informed consent. All embryos were divided into 2 parts: trophectoderm (TE) and the inner cell mass (ICM). Each part was analyzed separately. For 2 embryos blastomeres (B) not included in the blastocyst were also available (for embryos №2 and №3). Re-analysis was performed using the aCGH analysis (24sure, Illumina).

Results:
Molecular karyotypes of TE cells and ICM 7 of 10 embryos coincided with PGT-A results. Molecular karyotypes of 3 samples demonstrated incomplete concordance with the PGT-A results. We detected mosaic form of aneuploidies of sex chromosomes in the inner cell mass of embryo №10. PGT-A result of this sample contains information about whole aneuploidy of sex chromosomes. Mosaic form of reciprocal aneuploidy was observed in the ICM of embryo №4. Additional monosomy of chromosome 16 was detected in TE of embryo №8. This aneuploidy was not detected in the ICM and in the trophectoderm biopsy sample for PGT-A. No preferential localization of abnormal cells in trophectoderm or inner cell mass was observed. The study has limitations: a) the complete separation cells one by one and TE from ICM at blastocyst stage is impossible; b) there is a risk of false determination of the localization of aneuploidy in the embryo and the risk of inability to detect aneuploidy in the case when the sample contains reciprocal aneuploidy.

№	PGT-A results	ICM	TE	B
1	arr(15)x3	arr(15)x3	arr(15)x3	-
2	arr(15)x3	arr(15)x3	arr(15)x3	arr(1-22,X,Y)cx
3	arr(12)x3	arr(12)x3	arr(12)x3	arr(20)x1,(22)x1
4	arr(8)x3	mos arr(8)x1/(1-22)x2,(X,Y)x1 (80%)	arr(1-22)x2,(X,Y)x1	-
5	arr(21)x3	arr(21)x3	arr(21)x3	-
6	arr(16)x3	arr(16)x3	arr(16)x3	-
7	arr(19)x3	arr(19)x3	arr(19)x3	-
8	arr(10)x1	arr(10)x1	arr(10)x1,(16)x1	-
9	arr(21)x1	arr(21)x1	arr(21)x1	-
10	arr(X)x1,(Y)x0	mos arr(X)x1,(Y)x0/(1-22)x2,(X,Y)x1 (80%)	arr(1-22)x2,(X,Y)x1	-

Conclusions:
Our results show the coincidence of the molecular karyotypes of different embryo's cells was detected for most samples. In cases of detection of mosaic aneuploidy in ICM, cells with the whole aneuploidy and the normal molecular karyotype were also present in TE. None of the 10 embryos was suitable for transfer per any sample results. Further research is required with increasing sample size.

P-32

THE RELATIONSHIP BETWEEN BLASTOCYST MORPHOLOGY, ANEUPLOIDY AND MOSAICISM

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embryo, aneuploidy, mosaicism, IVF, NGS

Introduction:
Embryo quality has always been considered an important predictor of successful implantation and pregnancy. Nevertheless, knowledge of the relative impact of each morphological parameter at the blastocyst stage needs to be increased. Therefore, the aim of this study was to investigate the relationship between blastocyst morphology, aneuploidy and mosaicism.

Methods:
This study collected 70 embryos from 25 women who underwent IVF-ICSI cycles in 4 IVF center in Indonesia. Embryos were cultured until blastocyst stage, biopsy was performed on day 5/6 which were the screened for aneuploidy and mosaicism by Next Generation Sequencing (NGS) method. The relationship between blastocyst, aneuploidy and mosaicism were evaluated.

Results:
We found that the frequency of euploid embryo were 40% and 60% were abnormal (28.6% aneuploid, 12.8% mosaicism, and 18.4% triploidy). From blastocyst morphology, a grade < 4 blastocyst expansion had significantly higher rate of aneuploidy and mosaicism than the other grade of expansion ($p<0.05$). Morphology grading for intercellular mass (ICM) and tropectoderm (TE) also showed a significant correlation with aneuploidy ($p<0.05$) but not for mosaicism. Another finding that maternal age and sperm morphology also affecting aneuploidy, and women who had ≥10 oocyte when oocyte retieval had a higher chance to have aneuploidy ($p<0.05$).

Conclusions:
Blastocyst morphology showed a significant correlation to aneuploidy, but not for mosaicism. However, grading morphology alone can not replace preimplantation genetic testing for aneuploidy. Morphology assesment combined with PGT-A still produce the best results.

P-33

BLASTOCYST PLOIDY STATUS DEPENDS ON SPERM PARAMETERS

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blastocyst, morphology, male factor, NGS, sperm parameters

Introduction:
Aneuploidy is a very common abnormality in human embryos. The aneuploidy rates of blastocysts in IVF is in range 45-70% depending on different factors, including the male factor of infertility. Male infertility is diagnosed, among others, on the basis of quantitative or qualitative sperm anomalies visualized by semen analysis. On the other hand, the recent data about the effect of sperm on the blastocyst ploidy in IVF are controversial. The aim of our study was to evaluate the aneuploidy rate of the obtained blastocysts for patients with the severe male factor.

Materials and methods:
In this study, the ploidy status of 229 blastocysts, obtained in 68 PGS cycles performed in 55 infertile couples with severe male factor from January 2018 to December 2018, with trophectoderm (TE) biopsy and next generation sequencing (NGS), were retrospectively analyzed. Totally 229 blastocysts with different morphology were biopsied and outsourced to commercial reference laboratory for NGS test. Correlation of blastocyst aneuploidy with sperm morphology, sperm concentration and sperm motility was evaluated with Spearman coefficient.

Results:
The rate of euploid embryos was 65.5% (150 blastocysts). In the mentioned study 29.7% of examined blastocysts were aneuploid (68 embryos) and 4.8% of blastocysts were detected as mosaic (11 embryos). We evaluated the possible relationship between the sperm parameters and the NGS results for biopsied blastocysts taking into account the morphological quality of trophectoderm (TE) and intracellular mass (ICM). There was a significant positive correlation between the euploidy rates and sperm concentration ($r_s = 0.16$, $P < 0.05$). There was no correlation between the blastocyst ploidy and sperm motility and morphology ($r_s = 0.02$, $r_s = 0.13$ respectively, $P > 0.05$). As for the correlation of NGS results with the blastocyst morphology, there was the significant positive correlation between TE quality and the number of euploid blastocysts ($r_S = 0.19$, $P < 0.01$). But there was no correlation between ICM quality and the blastocyst ploidy status ($r_S = 0.09$, $P > 0.05$). The analysis of the sperm effect on the blastocyst morphology showed a statistically significant positive link between sperm motility and morphological quality of TE and ICM ($r_s = 0.17$, $P < 0.05$ and and $r_S = 0.19$, $P < 0.01$ respectively). During the interpretation of PGS results the ploidy, aneuploidy and mosaicism were taken into account. The average age of patients' was 29.6±5.4 years old. No dependence of the blastocyst aneuploidy on the women's age was found in mentioned group.

Conclusion:
Existing data regarding the relationship between embryo morphology and its ploidy are conflicting. Results of the present study point to a significant relationship of the trophectoderm morphology with the chromosomal abnormalities in preimplantation embryos. The effect of male factor on the blastocyst ploidy status and blastocyst morphology is proved.

P-34

RETROSPECTIVE PGT-A ANALYSIS FOR MULTIPLE DISPLACEMENT AMPLIFICATION PRODUCTS OF EMBRYOS CORRESPONDING TO 104 LIVE BIRTHS BY NEXT-GENERATION SEQUENCING (NGS)

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preimplantation genetic testing for aneuploidy (PGT-A),multiple displacement amplification (MDA)next generation sequencing (NGS), chromosome mosaicism

Introduction:
Preimplantation genetic testing for aneuploidy(PGT-A) has been widely used, but its clinical application value is still inconclusive. The previous PGS 1.0 technology has been proven to have a negative impact on IVF outcomes. PGS 2.0, blastocyst biopsy combined with high-throughput detection technology, has gradually became the mainstream detection method of PGT-A.Many problems still exist in this technology: 1. Biases caused by whole genome amplification (WGA) or high-throughput detection techniques; 2.Embryo mosaicism.Mosaic embryos were used to be considered unsuitable for transfer. However recently healthy live births after transferring mosaic embryo have been widely reported. And many studies also found that some embryos diagnosed as abnormal by PGT-A were turned out to be normal after retested with their inner cell mass. It is therefore suggested that there may be false positive results in PGT-A technology, resulting in the waste of many valuable embryos with normal developmental potential. The interpretation of PGT-A results in clinical practice remains deficient.Since 2015, our center has adopted multiple displacement amplification (MDA) as the first step of all PGT cycles, so we have established a library of MDA products.

Material & methods:
We investigated PGT cycles conducted for monogenic diseases (PGT-M) between January 2015 and December 2017 in our center. Eligible cycles were in which the blastocyst biopsy and single blastocyst transfer were adopted and healthy live birth was obtained. Exclusion criteria: Cycles in which PGT-A had been performed and the embryo was selected based on PGT-A results. MDA used Repli-g MiDi kit(Qiagen, Germany) or Repli-g Single cell kit(Qiagen, Germany) .All MDA products were conducted PGT-A using next generation sequencing (NGS) by Illumina NextSeq® 550 platform. We sequenced the amplified genome of each sample at approximately 0.01x genome depth and the resolution of chromosomal abnormality detection was 4Mb.

Results:
A total of 103 MDA products were tested, of which 11 failed to amplify. Among the 92 successful amplified products, 47 used the Midi-MDA kit while 46 used the Single cell-MDA kit. The average standard deviation(SD) value of NGS data was 3.58 (2.09-9.25).The PGT-A results showed that: 35.9%(33/92) embryos were euploidy; one was trisomy 22; 30.4%(28/92) embryos were mosaic whole chromosomal aneuploidy (the proportion of mosaicism range from 20% to 41%);6.5% (6/92) embryos were segmental chromosomal aneuploidy(chromosomal fragment length of duplication or deletion range from 4.06 Mb to 191.01 Mb), and 8.7% (8/92) embryos were mosaic segmental aneuploidy; 17.4% (16/92) embryos had segmental aneuploidy combined with mosaic aneuploidy. Overall, mosaic chromosomal aneuploidy was detected in 56.5% (52/92) blastocysts which could produce healthy babies.Prenatal diagnosis or karyotype detection of the newborns were performed in 31 cases and all the results were euploidy.

Conclusions:
Our study demonstrated that PGT-A would lead to the waste of viable embryos, so it should be questioned whether all mosaic embryos and segmental aneuploidy embryos should be abandoned. How to correctly interpret the results of PGT-A is an urgent problem.

P-35

THE APPLICATION OF PGT-A FOR CARRIERS OF BALANCED STRUCTURAL CHROMOSOMAL REARRANGEMENTS

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PGT-A, structural chromosomal rearrangements, next generation sequencing, array comparative genomic hybridization

Introduction:
The frequency of chromosomal aberrations account for approximately 15% of the major congenital anomalies diagnosed before the age of 1 year in Europe, and are associated with 25% of perinatal deaths due to congenital anomalies. Balanced rearrangements represent one of the most common forms of genetic abnormality, affecting approximately 1 in every 500 (0.2%) individuals. Difficulties processing the abnormal chromosomes during meiosis lead to an elevated risk of chromosomally abnormal gametes, resulting in high rates of miscarriage and/or children with congenital abnormalities. Unbalanced chromosomal rearrangements in embryos may occur as new event, or is the product of balanced chromosomal rearrangements in one of the parents. PGT-A is one of the choices to exclude embryos with unbalanced translocation and get successful pregnancy.

Materials & methods:
Nine couples, in which one of the partners was carrier of balanced chromosomal rearrangement, were chosen for analysis. Chromosomal rearrangements were confirmed using classical G band cytogenetic approach. IVF was made by adjusting the individual characteristics for each couple. Trophectoderm biopsy was done on day 5. PGT-A was done using aCGH Illumina 24Sure arrays or using NGS – Illumina VeriSeq PGS kit. Analysis was done using BlueFuse for both approaches.

Results:
From nine couples, in three couples male was carrier of Robertsonian translocation 45,XY,der(13;14)(q10;q10). Different balanced reciprocal translocations were observed in remained six couples, from them four were female carriers and two male carriers. Overall, 90 blastocysts from 9 couples were obtained and biopsied. From those 48 blastocysts underwent PGT-A. Results are shown in table:

Population	No.of embryos	Unbalanced translocation rate, %	Sporadic aneuploidy rate, %	Total abnormality rate, %	Euploid rate, %
Total	48	39,6%	39,6%	79,2%	20,8%
Robertsonian translocation	22	13,6%	68,2%	81,8%	18,2%
Reciprocal translocation	26	61,5%	15,4%	76,9%	23,1%
Maternal	18	77,8%	11,1%	88,9%	11,1%
Paternal	30	16,67%	56,67%	73,3%	26,67%

Two successful clinical pregnancies were obtained after FET with euploid embryos.

Conclusions:
Unbalanced translocation rate is higher in reciprocal translocation carriers than Robertsonian translocations carriers. Overall unbalanced translocation rate in embryos is same as sporadic aneuploidy rate, but in case of Robertsonian translocation sporadic aneuploidy rate is much higher than in case of reciprocal translocation, which only partially could be explained by age of women in couple.

P-36

TWO HEALTHY BABIES BORN AFTER IMPLANTATION OF MULTIPLE CHROMOSOMALLY-RESCUED OOCYTES

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polar body, PGT-A, genetic rescue

Introduction:
Meiotic rescue is a well-known phenomenon but little is known about live births after oocyte rescue, because of the shortage of data on PB biopsy. PGT-A (or PGS) has been performed for many years to increase the chance of pregnancy, especially in older women. Embryo aneuploidy is a major cause of pregnancy failure and is largely of maternal meiotic origin (>95%), with the risk increasing exponentially from approximately 35 years of age. Molecular analysis of polar bodies provides indirect assessment of an oocyte's chromosomal status by determining whether the chromosomes correctly segregated during meiosis I and II. In about one third of cases, missegregation of one or two chromosomes in meiosis I can be rescued in meiosis II, leading to a euploid oocyte. We present two cases of healthy babies born after single and double chromosome-rescued oocytes

Material and methods:
A 42- (case 1) and a 44-year old woman (case 2) underwent ART treatment with PGT-A PB, after histories of 3 and 2 prior ART failures respectively. Both couples underwent ICSI with first and second PB biopsy and comprehensive chromosome analysis by Array-CGH. The mechanisms leading to missegregation in PB1 and PB2 (nondisjunction or premature separation of sister chromatids (PSSC)) were analysed using chromosome-specific STRs.

Results and conclusions:
Case 1: a single euploid fertilized oocyte was transferred, after double rescue of chromosomes 3 and 18 (PB1 -3, -18; PB2: +3, +18) at day 2, resulting in a singleton pregnancy. Case 2: a single euploid fertilized oocyte was transferred after rescue of chromosome 16 (PB1: -16; PB2:+16) at day 3. In case 1, the aneuploidy of chromosomes 3 and 18 in the first PB was caused by PSSC followed by the extrusion of 2 homologous chromosomes in the second PB. In case 2, chromosome 16 underwent PSSC and extrusion of a single chromatid in PB1, followed by nondisjunction in meiosis II and extrusion of two sister chromatids in PB2. In both cases, the rescued oocyte was the only euploid oocyte out of 12 retrieved and they were competent for generating a pregnancy. The two women gave birth at term to healthy babies (a girl of 3280 g and a boy of 3500 g). To our knowledge, this is the first report of healthy births after a multiple chromosomally-rescued oocyte.
PGT-A of PBs is a valid method with the limitation of detecting only maternal aneuploidies and so is appropriate for screening of older women. Analysis of the 2 polar bodies by aCGH is valuable not only for aneuploidy screening in ART for older women, but also to understand the mechanisms leading to missegregation and rescue in oocytes.

P-37

PREIMPLANTATION GENETIC TESTING: TO THE OPEN QUESTIONS ABOUT DATA INTERPRETATION OBTAINED BY MASSIVE PARALLEL SEQUENCING.

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PGT-A, quality assessment, CNV-charts

Introduction:
the accuracy results of preimplantation genetic testing of embryos for aneuploidy (PGT-A) by massive parallel sequencing directly depends on the effectiveness of the quality control. The aim of this study was to analyze the external and intralaboratory system of the quality assessment to optimize protocols, algorithms of troubleshooting and selection of the most informative objective criteria for the interpretation of the PGT-A results.

Materials and methods:
we analyzed the quality criteria more than 2000 human bioptates of the trophectoderm cells. PGT-A was performed using VeriSeq PGS reagent kits. Sequencing was performed with the Illumina Miseq instrument. Sequencing data analysis was done by BlueFuse Multi Software. External quality assessment of PGT-A was carried out through participation in the program of interlaboratory comparisons of the consortium UK NEQAS.

Results:
with the aim of obtaining optimal performance of the sequencing was carried out intralaboratory validation of methods of libraries preparation for sequencing. In 10% of cases, genomic profiles, which were difficult to interpret, were obtained with optimal performance criteria of whole genome amplification (WGA) and the quality of subsequent sequencing. One of the problems is the suspicion of segmental aneuploidies in a mosaic version. Recognition of false mosaicism helps, in particular, the absence of chromosome break points, the stereotypical pattern of chromosome copy number variations (CNV), which may be associated with difficulties in reading of GC-rich DNA regions. As a result of participation in the program of external quality assessment of a consortium UK NEQAS we found that partial DNA degradation in the sample makes it impossible to detect trisomies. Furthermore, studying the PGT-A statistical results of different laboratories, attention is drawn to a significant spread in the frequency of mosaicism detected by them. This may be primarily due to the complexity of interpreting so-called "noisy" CNV charts. When analyzing the sequencing results using BlueFuse Multi software, the researcher is able to estimate for each sample the total overall noise score, as well as the Region Confidence value for each chromosome. However, the low information content of these parameters to estimate the number of copies of a single chromosome often generates subjectivity in the decision of the expert. In this regard, the most important task is to minimize the technical factors that can lead to the appearance of intense artifacts in CNV-charts, which complicates their interpretation. We, together with the adjacent laboratory in Moscow, have developed a research proposal, the implementation of which will help to shed light on the technical issues of all embryo and PGT-A stages currently remain elusive.

Conclusions:
the main reason for obtaining difficult to interpret genomic profiles is the low quality of the original DNA and as a consequence – false positive or false negative results of PGT-A. To prevent false-positive and false-negative results of PGT-A, a complex analysis of objective quality criteria of different stages of PGT analysis is necessary due to the lack of the information content of each individual factor in case of moderately pronounced DNA degradation in the sample.

P-38

CASE STUDY: PREGNANCY WITH MOSAIC OF TRISOMY / MONOSOMY 18 AFTER TRANSFER OF AN APPARENTLY EUPLOID BLASTOCYST TESTED BY ACGH

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blastocyst, mosaic, NGS, trophectoderm

Introduction:
It is already known that accuracy of preimplantation genetic testing for aneuploidy (PGT-A) might be compromised by relatively high frequency of mosaics in human preimplantation embryos, even in the blastocyst stage. Giving into account low number of cells which may be removed from embryo for analysis, the fact that all the cells are biopsied in a cluster from one locus of trophectoderm (TE) and that TE represents not foetal but future placental tissue, PGT-A should not be considered as a diagnostic examination but only as a screening test that may contribute to a better assessment of embryonal viability.

Material & methods:
We present a case of a couple with multiple recurrent miscarriages in the first trimester after spontaneous conceptions. In 2016 the couple decided to undergo IVF/ICSI treatment with PGT-A by array comparative genome hybridization (aCGH) on TE samples (24sure, Bluegnome /Illumina). All (8) analyzed blastocysts were shown to be euploid. The first (double) embryotransfer resulted in pregnancy and the patient gave birth to a healthy girl (2017). After two subsequent (single) embryo transfers in 2018 patient got pregnant but the pregnancy resulted in a missed abortion in 10th week of pregnancy.

Results:
Analysis of aborted tissue by quantitative fluorescent polymerase chain reaction (QF-PCR; Devyser Complete v2/ Devyser 18 v2, Devyser AB) showed aneuploidy of chromosome 18 in a form not conflicting with trisomy of mitotic origin (all short tandem repeats (STR) markers were in 2:1 ratio). aCGH (SurePrint G3, ISCA v2, 8x60K, Agilent Technologies) confirmed trisomy 18 corresponding with 75% representation of the trisomic cell line. Re-analysis of stored amplified TE sample by next generation sequencing (NGS) method (VeriSeq, Illumina) revealed, by aCGH unrecognized, monosomy 18 at the level corresponding with 20% of monosomic cells. Both trophectoderm and aborted tissue samples showed origin from the same individual (Devyser 18 v2, Devyser AB).

Conclusions:
The presented case clearly documents limitation of PGT-A, and caution which should be taken when the test is offered to patients and particularly when doing interpretation of the results. It also supports the opinion that embryos with proven mosaic monosomy of chromosomes 13, 18, 21 should be always avoided from the transfer.

P-39

CLINICAL INTERPRETATION OF NON-STANDARD PGT-A RESULTS

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PGT-A, data interpretation, IVF efficiency

Introduction:
Preimplantation genetic testing for chromosome anomalies has been used to improve reproductive outcomes for nearly 20 years. But in some cases, the effectiveness of Preimplantation genetic testing for aneuploidies (PGT-A) may be reduced. Sources of errors during PGT-A are low sensitivity or specificity of either method or interpretation. The threshold value that separates normal result from pathological is not fully formalized for current methods of PGT-A (aCGH, NGS). The decision on the status of the sample is taken by the interpreter (the expert). Can preimplantation genetic testing for aneuploidies reduce IVF efficiency? Yes, if euploid embryo is defined as abnormal or if abnormal embryo is recommended for transfer. To estimate how much the interpretation of the same PGT-A data may differ between the readers we designed the project «Many men, many minds».

Materials & Methods:
We compared the experts’ interpretation with some arbitrary reference. The references are the clinical reports, created in Genetico Laboratory, based on the joined decision after discussion of program data between the clinical geneticist, laboratory geneticist and biologist. Participants of the study were 10 readers, 2 of which are outside Russia. We included 50 samples analyzed by aCGH (Cytochip 24sure, Illumina) and 50 samples analyzed by NGS (Veriseq, Illumina) in our project. The samples for interpretation were not representative of routine samples of Genetico Laboratory (not "typical" samples were chosen for interpretation, but rather most "hard to read" ones). The experts received data which include chromosome profiles, QC metrics, and chromosome imbalances which automatically determined by the BlueFuse Multi program (Illumina). We asked experts to provide an answer containing molecular karyotype for each sample and clinical recommendations for embryo transfer.

Results:
Interpretation of PGT-A results depends of expert’s “school of thought” – at the same lab opinions tend to be more concordant. And interpretation of chromosome profiles by another expert can lead to a change in the clinical fate of the embryo. It is advisable to discuss PGT-A results between specialists before clinical report making. Interpretation of "hard to read" data of PGT-A is difficult and the role of the human factor very big. The “low specificity” of an expert may led to samples being recognized as aneuploid, although another expert using the same data will give an estimate of “norm” (or vice versa). Reanalysis of PGT-A data may lead to significant changes in clinical decisions regarding the fate of embryos. And after several months we tested if one interpreter could have two different opinions regarding the same sample. The participants of this additional experiment became 5 experts from initial list of participants. We asked them about the interpretation of 10 samples from the initial list. We did not reveal that these samples were same the experts interpreted several months before. We saw that the same expert may have different opinions on the same sample.

Conclusions:
PGT-A allows to increase IVF efficiency, however, we have room for improvement. It is possible to work on improving the process of data interpretation.

P-40

SIMULTANEOUS PREIMPLANTATION GENETIC TESTING (PGT) FOR 5 DIFFERENT GENETIC CONDITIONS

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PGT-M, Multiplex Nested PCR, Simultaneous PGT-M and PGT-A

Introduction:
It is a common practice to perform PGT-M for one or two disorders at the same test, but it is the first PGT performed simultaneously for 5 different conditions, which is presented below.

Material and Methods:
Consanguineous couple presented for PGT-M to avoid the risk of producing another affected child homozygous for four different autosomal recessive conditions identified in their previous offspring, including : (1) early infantile epileptic encephalopathy 5 (EIEE5), caused by SPTAN1 mutation; (2) xeroderma pigmentosum-complementation group C (XPG), caused by ERCC5 mutation; (3) congenital merosin–deficient muscular dystrophy 1A (MDC1A), caused by LAMA2 mutation; and (4) phenylketonuria (PKU) caused by PAH mutation. As parents requested also aneuploiidy testing, PGT design was to combine PGT-A and PGT-M, involving mutation and linked marker testing by multiplex nested PCR, to avoid the undetected ADO of each of the genes tested.

Results:
Overall, 12 of 16 embryos reaching the blatocyst stage were tested together with NGS for PGT-A, of which 10 were affected, including 6 affected by one mutation, and four by two mutations. Only 2 embryos were unaffected carriers of all 4 gene mutations, of which one was with trisomy 13, so only a single embryos euploid and carrier of all 4 gene mutations was transferred, resulting, resulting in birth of a healthy unaffected baby. Although a cumulative risk of couple for producing an offspring affected by all the four conditions is only 0.4%, the couple still has been very unfortunate to produce such a child in their natural cycle. Although the chance for detecting unaffected embryo was also not high enough (31.6%), especially by added 50% risk for aneuploidy (15.8%), still one of 12 embryos tested was both euploid and normal carrier of all the mutations, resulting in a healthy, unaffected child.

Conclusions:
This is the world's first PGT for 5 different genetic conditions (EIEE5, XPG, MDC1A, PKU and aneuploidy) in a single test, resulting in a transfer of euploid embryo free of all the conditions tested, demonstrating feasibility and accuracy of simultaneous combined PGT for multiple genetic conditions.

P-41

EARLY EXPERIENCES WITH SCREENING FOR ANEUPLOIDY IN PREIMPLANTATION GENETIC TESTING FOR INHERITED DISORDERS

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Preimplantation genetic testing, Aneuploidy screening, clinical outcomes

Introduction:
Preimplantation genetic testing (PGT) is divided into three categories based on the indication: PGT-M (Monogenic), PGT-SR (Structural rearrangements) and PGT-A (Aneuploidy).
A large study of more than 15000 trophectoderm biopsies found the lowest rate of aneuploidy to be 25% in young women increasing with maternal age to more than 90% (Franasiak *et al.*, 2014). Thus, at least 1 in 4 embryos are expected to be aneuploid, possibly leading to implantation failure, miscarriage or an abnormal fetus. Hence, in theory, selection of euploid embryos should increase pregnancy rates and decrease miscarriage rates, but its application is still a subject of intense international discussion.
In our center we do not offer PGT-A, but as our PGT-SR setup is based on Shallow Whole Genome Sequencing, the ploidy status is revealed, and used for prioritizing embryos. Our PGT-M setup (see below) does not detect aneuploidy, why we would expect a higher rate of implantation when performing PGT-SR. We investigated this by looking at clinical outcomes following PGT-SR and PGT-M.

Materials and methods:
PGT-SR was performed by Shallow Whole Genome Sequencing while PGT-M was performed by fragment analysis of short tandem repeats and SNaPshot analysis of the specific mutation when relevant. Embryo ploidy status was obtained and used for prioritizing embryos during PGT-SR combined with morphological assessment. Embryos from the PGT-M group were only subjected to morphological assessment, as ploidy status was available. Single embryo transfer was performed for all transfers in both groups. Positive hCG and ongoing pregnancy rate (OPR) per transfer was used as clinical outcomes. Ongoing pregnancy was defined as detection of an intrauterine gestation with fetal heartbeat at gestational week 8.
Data were collected from embryos transferred from 1st January 2017 to1st December 2018.
Test of equal proportions where performed using two-proportion Z-test in R version 1.1.453.

Results:
159 and 27 embryos were transferred in the PGT-M and PGT-SR group, respectively. Mean maternal age was 30.96 in the PGT-M and 32.56 in the PGT-SR group. Positive hCG per transfer were 49.7 % and 48.1 % in the PGT-M and PGT-SR groups, respectively (P = 0.881). OPR per transfer were 34.6 % and 37.0 % in the PGT-M and PGT-SR group, respectively (P = 0.805).

Conclusion:
There are no significant differences between the two groups with respect to positive hCG or OPR. The lack of a statistical difference could not be explained by a difference in mean maternal age, as it was comparable between the two groups. Our current data indicate that aneuploidy screening does not enhance the chance of embryo implantation, but the numbers in the PGT-SR group are low. It is likely, that the slight increase in OPR when performing aneuploidy screening might be statistically significant given a larger dataset.

References:
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P-42

RETROSPECTIVE PGT-A ANALYSIS FOR MULTIPLE DISPLACEMENT AMPLIFICATION (MDA) PRODUCTS OF EMBRYOS CORRESPONDING TO 21 SPONTANEOUS ABORTIONS BY NGS

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preimplantation genetic testing for aneuploidy (PGT-A), multiple displacement amplification (MDA), spontaneous abortion, next generation sequencing (NGS), chromosome mosaicism

Introduction:
Preimplantation genetic testing for aneuploidy (PGT-A) is widely used to identify aneuploidy embryos, thereby reducing miscarriage rate and improving the clinical outcome of in vitro fertilization (IVF). But there are many reasons for miscarriage. Not all miscarriages are caused by embryonic aneuploidy. Moreover, PGT-A has its inherent defects, the results of PGT-A may not fully represent the true karyotype of embryos. Many problems still exist in this technology: 1. Biases caused by whole genome amplification (WGA) or high-throughput detection techniques; 2.Embryo mosaicism. Therefore, it remains unclear what percentage of spontaneous abortion can be prevented by PGT-A. Since 2015, our center has adopted multiple displacement amplification (MDA) as the first step in all PGT cycles. So a complete library of MDA products has been well-established.

Material & methods:
We collected PGT cycles performed for monogenic diseases (PGT-M) at our center from January 2015 to December 2017. Cycles in which the blastocyst biopsy and single blastocyst transplantation were applied, and spontaneous miscarriage appeared during early pregnancy, and MDA products of the embryos were frozen were considered eligible. Exclusion criteria: cycles in which PGT-A had been conducted and the embryo was already selected according to the PGT-A results. All MDA products were performed PGT-A using next generation sequencing (NGS) by Illumina NextSeq ® 550 platform. The software determined the ploidy status with 0.01X read coverage.

Results:
A total of 21 MDA products were detected, and 4 of them were unable to evaluate due to amplification failure or poor test data. The PGT-A results of the remaining 17 embryos showed that 47.1% (8/17) embryos were euploidy, 11.6% (2/17) embryos were segmental chromosomal aneuploidy (46,XN,-(1)(q23.3-q44)(85.03Mb) and 46,XN,+(13)(q11-q34)(95.75Mb)), 27.8% (5/18) of embryos were whole chromosomal aneuploidy mosaicism (the proportion of mosaicism range from 21 to 47%) and 2 cases were trisomy (trisomy 21 and trisomy 22). 6 patients performed karyotype analysis of abortion tissues, among which PGT-A results of 4 cases (3 cases were normal, one was trisomy 22) were consistent with the karyotype of abortion tissues. However, 2 cases showed aneuploidy mosaicism (46,XN,+(mosaic)(19)(30%) and 46,XN,-(mosaic)(6)(44%)) by PGT-A, while the karyotype of their abortion tissues were normal.

Conclusions:
Our study showed that PGT-A could prevent a proportion of spontaneous abortion. However, embryos with low ratio of mosaicism may not be the cause of miscarriage. In fact, spontaneous abortion of these cases was caused by other factors. How high the proportion of mosaicism may lead to miscarriage still requires further research to confirm.

P-43

NGS BASED PGT-A / PGT-SR : DATA FROM >7000 EMBRYOS

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Preimplantation Genetic Testing for Aneuploidy, Preimplantation Genetic Testing for Chromosomal Structural Abnormalities, Next Generation Sequencing, In Vitro Fertilization

Introduction:
Preimplantation Genetic Testing for Aneuploidy (PGT-A) has been an important and promising option for the couples experiencing repeated IVF failures in the recent years. Next Generation Sequencing (NGS) has become a popular technology in the last years for The utilization of PGT-A. In this way, pregnancy rates increased by the selection and transfer of euploid embryos with a higher chance to implant and maintain a healthy pregnancy. Additionally, use of NGS technology is advantageous in the selection of embryos that do not carry any chromosomal abnormalities due to inherited structural rearrangements with high resolution, which is extremely important especially for translocation carrier patients known as Preimplantation Genetic Testing for Chromosome Structural Rearrangements (PGT-SR). In this study, we report NGS based PGT results of 7436 embryos from 2324 patients including translocation carriers.

Materials and Methods:
SurePlex DNA Amplification System (Illumina, USA) and DOPlify (PerkinElmer Health Sciences, Australia) were used for Whole Genome Amplification (WGA) on biopsied cells. WGA samples were further processed using Veriseq PGS (Illumina, USA) and PG-Seq kits (PerkinElmer Health Sciences, Australia). Data obtained from Miseq System were analysed using BlueFuse Multi Software (Illumina, USA) and Nexus Software (BioDiscovery, USA).

Results:
7436 embryos collected from 2324 patients were processed in this study. Results of 7213 embryos were interpreted after the elimitation of amplification failure group. Among these embryos, 705 were obtained from translocation carrier patients. Our results show that 36.7% of embryos was euploid and this ratio was 25.6% for translocation carrier patients. For PGT-A and PGT-SR patients, aneuploidy rates were 16.3% and 18.2%, respectively. We observed higher complex aneuploidy in translocation patients (51.6%) compared to the rest of the samples (29.5%). In addition to these findings, we also obtained pregnancy rate data from 1245 embryos of 470 patients. Pregnancy rate per embryo transfer was 62% in the group of patients in the range of 35-39 years, which was 53% in the group of patients over 40 years of age.

Conclusions:
Our data suggest that this technology is valuable for the selection of euploid embryos and improvement of IVF success together with pregnancy rates. Additional information obtained from NGS application prevents the transfer of aneuploid embryos, which is extremely important especially for translocation carrier patients. Moreover, this technology yielded promising results in the group of patients with advanced maternal age. At the point we have arrived through this aneuploidy screening adventure in embryos started in 1993 with FISH that detects only a limited number of chromosomes, detailed information accumulation obtained from 24 chromosome screening will increase the acceptability of this technique in IVF applications every passing day.

P-44

CAN PGS USING ARRAY CGH IMPROVE IVF CLINICAL RESULTS? (THE EGYPTIAN IVF-ET CENTER EXPERIENCE)

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PGS, array CGH, aneuploidy screening, repeated IVF failure

Introduction:
Chromosomal aneuploidies are considered the main cause of failed development of embryos, implantation failures, and early reproductive losses. Preimplantation genetic screening (PGS) using array comparative genomic hybridization (array-CGH) allows selection of embryos with normal karyotype, before embryo transfer. This should increase the implantation rate and reduce the frequency of early pregnancy loss. The aim of this study is to assess if PGS using array-CGH improve IVF clinical results.

Material & methods:
During 2016 - 2018, a total of 97 cycles for 94 patients underwent blastomere biopsy by laser on day 3, and 90 fresh transfer cycles were performed. The indications were repeated IVF failure (30), repeated pregnancy loss (26), previous history of aneuploid offspring (10) and for aneuploidy screening and sex selection (28). The maternal ages ranged from 25 to 42yrs. 732 embryos from 97 cycles were subjected to day-3 biopsies, the DNA was amplified using modified whole genome amplification (WGA) protocol. The amplified DNA from 710 embryos was labeled and analyzed using array-CGH software (IBSA Genetics., Switzerland).

Results:
710 embryos was tested, 69.44% (493/710) of the embryos were aneuploid267, and 30.46% (217/710) were euploid.70 euploid embryos were cryopreserved, and 147 euploid embryos were transferred to 90 patients resulting in 50% (45/90) pregnancies, 22 deliveries with healthy babies and 20 ongoing pregnancies and 3 miscarriages.

Conclusion:
Our results show that array CGH has the potential to provide high pregnancy and delivery rates after transfer of euploid embryos.

P-45

A COMPLETE SOLUTION FOR BETA-THALASSEMIA PGT TEST

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PGT-A, PGT-M

Introduction:
Couple who are both β-thalassemia carriers usually come to PGT-M test for healthy offsprings. STR-linkage analysis is a well-known solution but Allele Drop-Out, high mutation rate and stutter peaks are still major challenges. Karyomapping is a common technique but it's costly and not be combined with PGT-A test, which may result in multiple biopsy or unaffected but aneuploidy embryos. In this study, we report two cases of β-thalassemia PGT test which uses single nucleotide polymorphisms (SNPs) for linkage analysis. By using specific primers in WGA process, the Allele Drop-out rate decreases to < 1%. This strategy is enable to combine PGT-M and PGT-A in one single test which allows to select ploidy and unaffacted embryos for transplant.

Materials and method:
Two β-thalassemia-carrier couples underwent IVF procedure, resulted in 11 embryos. Specific primers were desinged to amplify whole HBB-coding region and SNPs within 300kb flanking the HBB gene. Day 5 biopsied samples were used for whole genome amplification with designed specific primers in order to decrease ADO in HBB gene region and SNP markers, using DOPlify kit (RHS). WGA product and specific primers were used for PCR reaction to enrich HBB region and interested SNPs. Mix of WGA and enrichment PCR product was used for libraries preparation using Nextera XT DNA Library Prep Kit (Illumina) and sequenced on Miseq System. Nexus copy number was used for CNV analysis andMiseq Reporter was used for mutation detection and SNP calling.

Results:
PGT-A results showed no abnormal parameters compared to normal PGT-A test. For the total of 11 samples, three samples resulted in aneuploidy. HBB mutation detection and SNP calling results showed no ADO in all 11 samples, with 3 affected embryos and 8 unaffected embryos.

Conclusions:
- We have successfully designed a procedure which allows to combine PGT-A and PGT-M in one single test. This test is more cost-effective and decreases turn-around time compared with other techniques.
- ADO rate of HBB region and SNP makers is approximately 0%, which make it easier and more reliablefor ADO confirmation among embryos in the same cycle.

P-46

HAPLOTYPES BASED ON EIGHT VARIANTS IN SOUTHERN CHINA POPULATIONS WITH COMMON A-THALASSEMIA DELETIONS AND THEIR APPLICATION TO PREIMPLANTATION GENETIC TESTING

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Haplotype, α-thalassemia, deletion, preimplantation genetic testing

Introduction:
Around 5 to 20% of the population in the provinces of southern China are carriers of α-thalassemia mutations. In our hospital, more than half of couples treated with preimplantation genetic testing for monogenic diseases (PGT-M) are α-thalassemia carriers. The most common mutations are deletion in α-thalassemia genes (HBA1 and HBA2), including --SEA, -α 3.7, and -α 4.2. Preimplantation genetic haplotyping (PGH) is a powerful technology for PGT-M that can detect allele drop-out (ADO) accurately by detecting numerous linked or unlinked polymorphic markers in DNA segments surrounding the genes of interest. However, there is a lack of normative data references in the selection of linkage sites for disease. Therefore, a mutation-specific haplotype analysis will improve the efficiency of PGT-M for α-thalassemia.

Materials & methods:
A total of 113 couples with α-thalassemia deletion mutations were collected from the Reproductive Medicine Center in the First Affiliated Hospital of Sun Yat-sen University and the Reproductive and Genetic Hospital of CITIC-Xiangya. A pre-examination validation for PGT-M was performed on these. The SNPs with large difference between reference frequency and alternate frequency in 452 haplotypes with or without three kinds of deletions were filtered out. Candidate sites close to the α-thalassemia genes were selected, upstream and downstream of genes within 1 Mb. Then, the haplotypes model was established with several specific genotypes of SNPs inherited with the mutations. Subsequently, the model was verified to predict the genotypes in another 34 couples after PGT-M. In addition, the ADO ratio of each site in 255 embryos was analyzed.

Results:
Eight candidate SNPs (rs2562164, rs2857997, rs2857998, rs116995933, rs149153770, rs3918352, rs1203977, and rs1203979) were identified for the haplotypes model constructed for different genotypes. SEA-I (GCGTAACA), α3.7-I (AGACGACA) and α4.2-I (AGACGGAT) haplotypes were detected in 92.2%, 50.9%, and 70.8% genotypes with corresponding mutations, respectively. On average, 42.7% sites were able to be used as key SNPs in the verification of another 34 PGT-M cohort haplotypes and genotypes. The SEA-I model was detected in 91.1% of genotypes with --SEA mutation.The α3.7-I model was concordant with 37.5% of genotypes with the -α3.7 mutation. The α4.2-I models were inherited with 87.5% of genotype with the -α4.2 mutation. The average ADO ratio of the eight SNPs in the 255 embryos was 2.3%.

Conclusions:
The haplotype analysis for common α-thalassemia deletion mutations including the eight-site model improves the efficiency of PGT-M in the population of southern China. The establishment of standardized diagnostic procedures and systems can be further extended to other populations for PGT-M in the future.

P-47

PGT-M IN FAMILIES CARRYING "DE NOVO" MUTATION BY COMBINATION OF EMBRYO SANGER SEQUENCING AND KARYOMAPPING

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karyomapping, monogenic disease, de novo mutation

Introduction:
Karyomapping based on genome-wide linkage analysis and using an unique combination of "SNPs (Single Nucleotide Polymorphism) of interest" in each chromosomal region enables to compare patterns carried by parents and other family member of known genetic status (carrier of a possible pathogenic mutation) in an affected region. In case of "de novo" mutation carried by one of the parents (and detected by Sanger sequencing), there is no other family member available for a linkage analysis. The only opportunity to get reference is to perform an acquired embryonal DNA sequencig.

Material and Methods:
DNA amplification from trophectoderm samples was performed using the MDA (multiple displacement amplification) protocol recommended in the Karyomapping methodology. Direct detection of the causal mutations was performed on DNA amplification from trophectoderm by Sanger´s method of gene sequencing. Prior to the embryo analysis, the carrier mutation was verified. WGA products were sequenced and compared with reference sequence of the tested gene. A minimum of 3 embryos are required for the "de novo" mutation testing. Short - read fragments were used for Sanger seguencing. For the indirect genome - wide linkage analysis the Human Karyomap-12v1 bead chip (Illumina) and BlueFuseMulti were used. Four unrelated families were examined by Karyomapping PGT Method. All monogenic diseases were autosomal dominant with known mutation in definite gene (*SALL4* - OMIM 607343; *FBN2* - 612570; *LMNA* - 150330; *EXT2* - 608210).

Results:
In all four cases we were able to find an embryo as reference by Sanger sequencing for a conclusive result. The average number of examined embryos was 5.5 (2 - 12) per family. With exception of 1 case (*EXT2* family) we always found at least 1 embryo for an embryotransfer. In the *FBN2* - family the heart beat pregnancy is reported. The examination scheme of this family is presented in detail.

Conclusion:
Karyomapping is an applicable method to PGT-M also in families with detected "de novo" mutation. Under the assumption of a known mutation and a sufficient number of embryos as well as a coverage check in the region of interest it is possible to reveal the mutation using the sequenced embryo as a reference family member.

P-48

OBSERVING THE IMPACT OF EMBRYO CULTURING CONDITIONS ON NON-INVASIVE PREIMPLANTATION GENETIC TESTING FOR ANEUPLOIDY DETECTION (NI-PGT-A)

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Non-invasive, PGT-A, spent media, WGA

Introduction:
There are several options available to clinics culturing IVF embryos, including group and individual droplet culture, sequential or single-step medium culture and the possibility to renew the medium during culture. The absence of standardised culturing conditions or molecular testing methodologies, including whole genome amplification (WGA) used for non-invasive preimplantation genetic testing for aneuploidy (NI-PGT-A) may explain the variable rates of concordance reported between the spent embryo culture media and embryo biopsy results to-date. Culture conditions contribute to the accumulation of embryonic and contaminating DNA in spent embryo culture media. Optimisation of either the culturing conditions, molecular testing methodologies, or both, should yield the highest accuracy results for NI-PGT-A. This study examined rates of concordance between spent embryo culture media and embryo biopsies with the aim of evaluating the impact of culturing conditions on NI-PGT-A results.

Material and methods:
Spent embryo culture media was collected from single embryo culture droplets following biopsy of the embryo for PGT-A, then stored at -20°C, with ethics approval. Spent embryo culture media samples from 10ul-60ul culture droplets were whole genome amplified using DOPlify® kit reagents (PerkinElmer). WGA DNA yield was assessed following gel electrophoresis and high sensitivity Qubit instrument quantification (ThermoFisher). Next generation sequencing libraries and sequencing was performed according to the standard PG-Seq™ kit 48 sample protocol on a MiSeq® instrument sequencer (Illumina). Data was bioinformatically aligned to hg19 and analysed using PG-Seq™ kit software. WGA DNA yield, NGS metrics, and whole chromosome aneuploidy concordance with the PGT-A result for the embryo biopsy were determined.

Results:
Results were collated for each set of culturing conditions, including whether the media was renewed during culture. Whole genome amplification using DOPlify® kit reagents resulted in the amplification of 78-100% of spent embryo culture media samples (WGA failure rate 0-22%). Ploidy concordance with the embryo biopsy ranged from 33-55% for autosomal chromosomes and 47-53% for sex chromosomes using a single-step culturing system (n=3 protocols), compared with concordance rates of 60-95% and 50-97% respectively when media was changed during the 5-6 day culture (n=4 protocols).

Conclusions:
Successful NI-PGT-A using spent embryo culture media will possibly require specific culturing conditions and/or specialised molecular methodologies for accurate and representative amplification and testing of the embryonic DNA. In a step toward this, we identified that renewing culture media during IVF improves overall concordance rates between the embryo biopsy and spent embryo culture media for NI-PGT-A.

P-49

MOLECULAR PGT-M FOR VUS IN THE GENOMIC ERA: TO DO OR NOT TO DO?

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PGT-M (Preimplantation genetic testing for monogenic disorder), VUS (variants of unknown significance), CMA (chromosomal microarray), CNV (copy number variants)

Introduction:
The new chromosomal microarray CMA technology, allows the identification of genetic defects in many disorders that would previously have escaped detection. Alongside the benefits, it exposes us to copy number variants (CNV) whose pathogenic significance is unclear "Variants of unknown significance" (VUS). In our cohort we reviewed all files of couples that applied for PGT-M at our clinic with a finding of VUS. We aimed to characterize these VUS and the cases in which PGT-M was decided upon.

Materials and methods:
A retrospective cohort study in the Zohar Unit for PGT-M, Shaare Zedek medical center, 2014-2019. Files of all couples with a finding of VUS among those who applied for PGT-M with any CMA findings were reviewed. VUS were classified (likely pathogenic, VUS, likely benign) according to the American collage of medical genetics and Genomics classification at time of first consultation and to date (December 2018). Pathogenic variants were not included in the study. PGT-M was performed on blastromeres of throphoectoderm biopsy by PCR using surrounding polymorphic markers. For all microduplications, FISH was performed prior to the PGT-M in order to confirm the tandem location.

Results:
Of 45 couples requesting PGT-M for CMA findings, 24 (53%) presented with VUS. VUS was an isolated finding in 54% of cases and as an additional finding to a known pathogenic mutation in 46%. The mean age of women was 29.8. Half the couples had children at the time of PGT-M consultation. 11 couples (46%) had a termination of pregnancy (TOP) due to VUS; three solely for the VUS and eight for VUS accompanying a sonographic finding in a prenatal screening. Indications for detecting the VUS were as follows- 33% amniocentesis for maternal request not otherwise indicated, 50% amniocentesis for a prenatal ultrasound finding, 25% had a CMA testing due to an affected child and another 12.5% for a CMA finding on a previously aborted fetus.
Out of 30 VUS detected, 33% were defined VUS, 26% likely benign VUS and 40% likely pathogenic VUS. 19 VUS (63%) were microduplications (size ranging 31-2800Kbp) and 11 (37%) were microdeletions (size ranging 57-1200Kbp). Reviewing VUS classifications up to date- 33% remained unchanged, 20% were more severely defined and 46% were less severely defined.
Based on genetic counseling, PGT-M was assigned for 17/30 CNVs (56%), 13(76.4%) of which were isolated VUS. 11/13 cases of isolated VUS were performed to date with a mean number of: 1.5 IVF cycles per couple (1-3), 8.3 embryos biopsied (1-15) and a mean of 2.9 wild type embryos per couple (0-6). Of 6 couples who had an embryo transfer at our institution there were 4 clinical pregnancies.

Conclusions:
The genomic era allows the detection of varies VUS whose definition is submitted to changes as more information is gathered. Couples are already performing TOP and requesting PGT-M for VUS including likely benign VUS. It is thus of great importance to carefully use guidelines for the interpretation of these findings while counseling couples and determining the justification to practice PGT-M for their VUS finding.

P-51

PRECONCEPTION CARRIER SCREENING FOR MONOGENIC DISORDERS

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carrier screening, whole exome sequencing

Introduction:
Up to 2% of newborn children have untreatable monogenic disorder. Preconception carrier screening is performed rarely. If a sperm donor is a carrier of mutation causing of frequent monogenic disorder probability of having a child with monogenic disorders raises dramatically. The aim of the study was to investigate frequency of monogenic disorder carrying for men who meet all requirements for being sperm donors.

Material & methods:
DNA from sperm samples was extracted using New iGENatal kit. Whole exome sequencing of 25 samples was performed using Ion S5 sequencers and Ion AmpliSeq Exome RDY Kit. Read mapping and variant calling was performed using Torrent Suite software. Variant annotation was performed with Variant effect predictor tool.

Results & Conclusions:
15 men (60%) were carriers of at least 1 pathogenic mutation associated with an autosomal recessive disorder. 6 (24%) were carriers of a disorder with population carrier frequency 1/250 and higher. With no tests for second parent the probability of having a child with a monogenic disorder is higher than 1/1000. This result demonstrates the importance of preconception carrier screening, particularly in IVF programs using donor sperm.

The study is supported by the Ministry of Healthcare of Russian Federation

P-52

MULTIPLE DISPLACEMENT AMPLIFICATION CAN INCREASE THE DIAGNOSTIC EFFICIENCY IN PGT-M FOR THALASSEMIA

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diagnostic efficiency, multiple displacement amplification (MDA), preimplantation genetic testing for monogenic diseases (PGT-M), α- thalassemia, β-thalassemia

Introduction:
Preimplantation genetic testing for monogenic diseases (PGT-M) has been widely conducted to prevent the birth of children genetically affected with thalassemia, especially in southeast China. However, due to the insufficient DNA template available from an embryo-level biopsy, its detection efficiency is still far from satisfactory. A suitable whole genome amplification (WGA) method is crucial to the success of PGT-M. This study aims to evaluate whether using multiple displacement amplification (MDA) as the first step can increase the diagnostic efficiency of PGT-M for thalassemia disorders.

Material & methods:
This is a retrospective cohort study. All included patients underwent PGT-M cycles (n=939) for α-thalassemia-SEA or β- thalassemia in our center from January 2014 to February 2018. We divided the patients into two groups based on two different detection methods. For the polymerase chain reaction (PCR) group (n=428), after cell lysis we directly conducted fluorescent gap PCR for α-thalassemia-SEA diagnosis or multiplex nested PCR+ reverse dot blot (RDB) analysis for β- thalassemia diagnosis. For the MDA group (n=511), the whole genomes of single cells were directly amplified using MDA before fluorescent gap PCR or singleplex PCR+RDB procedures.

Results:
A total of 7756 embryos were tested. The overall diagnostic efficiency of the MDA group was significantly higher than that of the PCR group (96.70% vs 86.60%, $P<0.001$). The percentage of embryos available for transfer was significantly higher in the MDA group than in the PCR group (72.64% vs 62.84%, $P<0.001$). Furthermore, the carrier embryo rate of the MDA group was significantly higher than that of the PCR group (47.81% vs 32.67%, $P<0.001$).

Conclusions:
This study indicates that MDA, as the universal first step in PGT-M for α-thalassemia and β- thalassemia, can increase 10.10% diagnostic efficiency, thus may provide a better choice for the clinical PGT-M practice.

P-53

MEAN NUMBER OF ANALYZED EMBRYOS IN PGT-M CYCLES TO UNDERGO AT LEAST ONE EMBRYO TRANSFER.

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Monogenic diseases, Preimplantation genetic testing, Transferrable embryos, Genetic analysis, Blastocyst

Introduction:
Preimplantation genetic testing for monogenic diseases (PGT-M) is a clinical method developed to prevent the transmission of monogenic inherited disorders to the future offspring. The reproductive risk of carriers of single gene disorders depends on the typology of the disorder, with the probability of affected conceptions ranging from 25% in recessive or X-linked diseases to 50% in dominant diseases. Therefore, the possibility of having embryos suitable for transfer and the consequent chances of ongoing pregnancy are strictly related to the number of embryos available for the genetic analysis. The aim of this study was to evaluate how many embryos need to be analyzed for having one or more genetically transferrable embryos.

Material and Methods:
86 carriers of genetic disorders performed 99 cycles of genetic analysis between January 2010 and September 2018. They were divided into two groups according to the typology of the disorder transmission. Group 1 included recessive and X-linked diseases (58 patients, 70 cycles of analysis), while with dominant disorders were in Group 2 (28 patients, 29 cycles of analysis). Maternal age was comparable in the two groups. Following blastocyst biopsy, embryos were vitrified to complete the genetic analysis.

Results:
Clinical results were the following.

	Group 1	Group 2
No. patients	58	28
Age	35.3±3	35.3±3.2
No. cycles of analysis	70	29
No. oocyte pick-up	94	45
No. analyzed embryos Mean±SD / pick-up Mean±SD / cycle of analysis	334 3.5±1.7 4.7±1.8	151 3.4±1.8 5.4±1.6
No. transferrable embryos Mean±SD / cycle of analysis	209 3.0±1.6	79 2.7±1.7
No. transfers	95	50
No. ongoing pregnancy rate / patient (%)	26 / 57* (45.6)	13 / 27* (48.1)

*One patient still to perform embryo transfer
For both groups, the mean number of analyzed embryos to have genetically transferrable embryos are reported in the following table:

	Mean no. analyzed embryos		
		Recessive and X-linked disorders	Autosomic dominant disorders
No. transferrable embryos	1	1.6	2.1
	2	3.2	4.1
	3	4.8	6.2

Conclusions: Based on the reported data, we define for each case the minimum number of analyzable embryos needed to have one or more transferrable embryo depending on the typology of genetic disorder. Therefore, we eventually advise patients to undergo further oocyte retrievals for the best clinical outcome.

P-54

IMPLEMENTATION OF FIRST NIPT SERVICE LABORATORY IN AN INDIAN HOSPITAL

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Non invasive prenatal testing, trisomy 21

Introduction:
Non-Invasive Prenatal Testing (NIPT) for common fetal aneuploidies is rapidly increasing in India for both naturally conceived pregnancies and pregnancies conceived by assisted reproductive technologies (ART). This study aims to establish the very first next generation sequencing (NGS) laboratory for NIPT in an Indian hospital using the Ion Torrent platform to provide a complete in-house solution for pregnant women.

Materials and Methods:
In brief, all plasma DNA samples (both control and test groups) were converted to genomic DNA libraries using Fragment Library Kit (Thermo Fisher Scientific, Cat. No. 4471252) with Ion Xpress Barcode-Adapters mix (Thermo Fisher Scientific, Cat. No. 4471250) using our laboratory developed protocol. The libraries were then quantified using Qubit dsDNA HS (high sensitivity) Assay Kit, (Thermo Fisher Scientific) and diluted to equimolar amounts before pooling. The Ion Chef System (Thermo Fisher Scientific), as part of the workflow, was used for automated template preparation and loading the sequencing chip. Thereafter, low-pass whole-genome sequencing was done using the ion semiconductor sequencer Ion S5 System (Thermo Fisher Scientific,) to achieve at least 5 million sequence reads. In the dry-lab, the sequenced reads were aligned to the reference human genome (UCSC hg19) locally and their BAM files were generated. The euploid BAM files from the training set of samples were assigned to NIPT reference group and their aligned reads were partitioned into 50,000 bp bins. The number of reads in each bin becomes the unit for comparison with the test samples. GC corrections were performed based on the average coverage of genomic regions having a similar GC-content to correct the coverage differences between bins having a different GC percentage. In this validation study, the standard Z-score prediction models returned a value between -3 and +3 for all the chromosomes, indicating that there is no trisomy present. The scores for aneuploidy samples all showed a value above 4. The z-score refers to the number of standard deviations from the mean of a reference data set and in all molecular counting approaches, a z-score of more than 4 is considered a positive result.

Results:
The algorithm was blindly tested on a cohort of 100 known samples, of which the 12 were T21, eight were T18, four were T13 and 76 were from euploid pregnancies. Our algorithm correctly called all euploid and aneuploid cases. For all aneuploidies, z scores were above a value of 4. Interestingly, accurate detection of aneuploidy was also noted at foetal fraction as low as 3.2%. Therefore, based on our findings, our algorithm is expected to be sensitive and specific for up to 99.9% of pregnancies with one of the three common trisomies.

Discussion:
Based on the high performance of our NIPT test in validation studies, testing has commenced at Lifeline Hospital, including pregnancies conceived by ART. Clinical performance is currently under evaluation to derive positive predictive value (PPV) and negative predictive value (NPV) for each trisomy.

P-55

EFFICACY OF NUCLEAR TRANSFER TO PREVENT THE INHERITED MITOCHONDRIAL PATHOLOGY

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Mitochondial diseases, nuclear transfer, disease prevention, mtDNA mutation, mtDNA heteroplasmy

Introduction:
Mitochondrial pathology is characterized by maternal inheritance. Women may be hidden mutation carriers, but in some cases the risk for descendants may reach up to 100%. Due to the uneven distribution of mitochondria during cell division, the practice of preimplantation testing of embryos has significant limitations, and prenatal diagnosis may be unacceptable. The first baby after nuclear transfer procedures was born in 2016 (J. Zhang et al., 2017).

Material and methods:
The study included a married couple with a confirmed hidden woman's carriage of Leigh syndrome mutation (37 years old). The couple had a history of birth of a girl with Leigh syndrome. For ethical reasons, prenatal diagnosis and donation of oocytes were not considered for further childbirth.
The patient's mutation carrier status has been confirmed in independent laboratories (mtDNA 9176 T>C, Heteroplasmy ~ 40%). The child was diagnosed with confirmed Leigh syndrome (Homoplasmy 99.4%). The couple had 2 controlled ovarian stimulations followed by nuclear transfer procedures. Embryo biopsy was performed on Days 5-6 of development. Preimplantation genetic embryo testing PGT-A (NGS: Veriseq, Illumina) and heteroplasmy testing of pathological mutation (NGS, coverage of >1000x) were performed.

Results:
During the first stimulation, 3 blastocysts (euploid female embryo and two aneuploid embryos) were obtained. After consultation with the patients, it was decided not to use the female embryo for the embryo transfer. During the second stimulation, 6 blastocysts, incl. 3 aneuploid and 3 euploid (males) were obtained. Heteroplasmy of euploid embryos for pathological mutations: 0.51 ± 0.02%, 2.96 ± 0.05 and 3.32 ± 0.06%. The embryos obtained during ART from a patient with a mtDNA mutation carrier status were characterized by a low level of heteroplasmy of mtDNA mutation and had a low risk of mitochondrial pathology development. The patient is preparing for embryo transfer.

Conclusions:
Nuclear transfer procedures showed high efficiency in describes case. But it is still experimental and highly controversial procedure. Further investigations and long term follow up of children is required.

P-56

A NEW AGE IN PGT-M: A DECADE´S EXPERIENCE AND NEW CHALLENGES TO DEAL WITH

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PGT-M, Variant Classification, Complexity, Multidisciplinary Team

Introduction:
Genetic diagnosis has evolved dramatically mainly due to application of NGS approaches. Identification of mutations in different genes related with the disease and interpretation of pathogenicity of the new genetic variants found are some of the new challenges to face with.
Preimplantation Genetic Testing for Monogenic Disorders (PGT-M) is applied for disease-causing genetic variants (Likely Pathogenic and Pathogenic). In parallel, Assisted Reproduction Techniques (ART) without PGT is not allowed in Spain if there is a high risk of transmitting a severe genetic disease. Therefore, clinical classification of genetic variant as pathogenic/bening is crucial for patients and it directly impacts in their future reproductive options. Spanish National legislation about ART/PGT-M approaches and best practice guidelines are currently not enough to solve some of these PGT-M requests.
The aim of this work is to report our general experience since 2008 as a reference center for PGT-M in a Public Health System and the evolution of the complexity in the evaluation of the cases.

Material&Methods:
From 2008-2017 a total of 372 cycles for PGT-M (202 families and 59 different monogenic diseases) including 5 PGT-HLA cases have been studied. Embryo biopsy was performed in 268 cases: 156 cases from 2007-2015 and 112 cases from 2016-2017.
The genetic analysis was performed by cleavage aspiration and blastomere analysis was carried out by STR haplotype and Sanger sequencing.
Clinical meeting minutes available from 2017 have been revised to analyze cases that were individually evaluated by a panel of specialists due to a Variant of Uncertain Significance (VUS) or diseases with an incomplete penetrance.

Results:
During this decade the number of PGT-M requests has increased an average of 25% per year. Embryo genetic analysis was performed in 72% of the cycles with a 95% transfer rate. The Clinical Pregnancy rate (%per OR, % per Transfer) has been 23% and 29%, respectively.
First cases requiring compound PGT-M of two different genetic alterations were requested between 2016-2017: 3 cases involved two different monogenic diseases and 1 case involved a monogenic disease and a chromosomal rearrangement (3,57% of the total PGT-M cases in this period).
In 2017, four PGT-M cases with a VUS in *COL1A2*, *MYH7*, *PKD1* and *BRCA2* genes and two cases with an incomplete penetrance disease (Parkinson and FMF) needed previous evaluation by the specialist's panel. Based on the familial history, the VUS were reclassified as Probably Pathogenic in the four cases. The two cases with an incomplete penetrance were sent to the National Human Reproduction National Committee (CNRH) with a favorable resolution for the Parkinson´s case.

Conclusions:
The PGT-M scenario is getting more complex especially due to that NGS-based analysis is becoming more informative and extensive. This complexity implies that additionally to the progress in technical approaches for PGT-M, the previous genetic/clinical evaluation of PGT families is of great importance.
In this new age of PGT-M a multidisciplinary team of geneticists, gynecologists and occasionally other specialists, is becoming essential for an appropriate evaluation of PGT-M cases.

P-57

ADDITIONAL DATA FOR CONTROVERSIAL INTER-CHROMOSOMAL EFFECT SUSCEPTIBILITY IN PGT-SR PATIENTS

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Preimplantation Genetic Testing for Chromosomal Structural Abnormalities, Interchromosomal Effect, Preimplantation Genetic Testing for Aneuploidy, Next Generation Sequencing, In Vitro Fertilization

Introduction:
NGS based translocation screening has been widely used as the possibility of detecting other chromosomal aneuploidies. Some studies claimed that balanced translocation carriers may have an increased risk for aneuploidies unrelated to translocation due to inter-chromosomal effect (ICE). Translocation causes unusual segregation of chromosomes involved in translocation. Furthermore this rearrangement may have an effect on the segregation of other structurally normal chromosomes during meiosis. Here we present a report of aneuploidy and complex aneuploidy rates among the embryos derived from patients carrying balanced chromosomal rearrangements (reciprocal or robertsonian translocations).

Materials and Methods:
Whole Genome Amplification (WGA) was performed using SurePlex DNA Amplification System (Illumina, USA) and DOPlify (PerkinElmer Health Sciences - Australia). on biopsied cells. Veriseq PGS kit was used for further processing of WGA samples. BlueFuse Multi Software was used to analyse the data obtained from Miseq System.

Results:
In this study, we collected 705 embryos from 168 patients with an average age of 31. At least one of the couples was previously reported to be a translocation carrier. According to our findings, 25.6% of these embryos was euploid and 69,4% was aneuploid/complex aneuploid (mosaic embryo results were excluded). 29.33% aneuploidy was detected in translocation of interest only. The rest (39.43%) was due to aneuploidy of other chromosomes . According to our in house PGT-A data (7200 embryos collected from 2177 patients) sum of aneuploidy and complex aneuploidy rates was 45.8. Overall these results fail to provide any support for the presence of an ICE.

Conclusion:
In balanced chromosomal rearrangement carriers PGD by NGS array not only detects unbalanced chromosomal rearrangements due to chromosomes involved in translocation but also detects the possibility of other chromosomal aneuploidies.
According to our findings, aneuploidy and complex aneuploidy rates do not increase in the embryos obtained from PGT-SR group of patients. Moreover, in addition to translocation chromosomes, we observed an additional aneuploidy risk related to other chromosomes in these embryos. Our data supports previous studies suggesting that ICE in embryos derived from balanced chromosomal rearrangement carriers is elusive. On the other hand average maternal age of PGT-A patients is significantly higher than PGT-SR group (35.1 vs 31.2 respectively). Although it is thought that increased maternal age may cause aneuploidy in some of the cases, some embryos have an increased aneuploidy rate regardless of maternal age. Nevertheless euploid embryo ratio in PGT-SR group is compared to PGT-A is relatively lower (25.6 vs 36.7) thus our results emphasize the importance of 24 chromosome scan in embryos derived from balanced translocation carriers

P-58

PGD-SEQ: VALIDATION OF A NOVEL SOLUTION FOR PGT-M AND PGT-SR BASED ON TARGET ENRICHMENT

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PGD-Seq, PGT-SR, PGT-M, NGS

Introduction:
Recently, we have developed PGD-Seq, which is a solution for PGT-M and PGT-SR analysis, which can be coupled to PGT-A. PGD-Seq has several advantages over other solutions: (1) PGD-Seq is designed to work widespread in any family from any ethnicity, so no specific customization is needed; (2) the same solution can be used to detect embryos with balanced translocations; (3) it is possible to detect even small unbalanced translocations, increasing the resolution of standard PGT-SR; (4) it can be combined with PGT-A in the same run and (5) it is an easy workflow with possibilty of automation. Here we present data about the performance of PGD-Seq in challenging situations.

Material and methods:
Close to 200 cases of PGT-M and PGT-SR have been analyzed with PGD-Seq solution. Each PGD-Seq panel has been designed for a specific gene or region with a complex algorithm in order to select informative SNPs among different ethnicities. Finally, each panel involves around 200 pre-selected SNPs. Informativity study and embryo analysis have been done by PGD-Seq simultaneously in some cases. Frequently, PGD-Seq was combined with PGT-A.

Results:
Up to know, more than 150 gene analysis and 200 case studies have been developed with PGD-Seq. Among different cases, some of them were very challenging. Firstly, the same panel was used in different ethnicities. Moreover, it was used in some couples with high level of consanguinity, where we were even able to identify informative SNPs. Due to the high density of SNPs in the regions studied, we were able to identify recombinant embryos in few different cases. Finally, due to the ability of combining direct and indirect testing, PGD-Seq was used in cases without any family member available in order to complete the informativity test, or with complex family combinations.
Regarding to PGT-SR, the solution was able to differentiate normal from balanced embryos in several cases. Nowadays, FISH is commonly used in several labs for specific case studies, especially in those with a small portion of the chromosome affected. However, just with PGD-Seq, we were able to identify those small aberrations. Finally, the solution was used even in a case with an inversion.

Conclusions:
The used of PGD-Seq shows its robustness in very challenging situations, without any previous setup. Additionally, the target approach instead of whole genome one keeps the costs of the test low enough to make it affordable for most of the couples.

P-59

PREIMPLANTATION GENETIC TESTING FOR HERITABLE CONNECTIVE TISSUE DISEASES

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heritable connective tissue disease, lived experiences, PGT-M, qualitative study

Introduction:
Heritable connective tissue disorders (HCTD) are a group of mostly autosomal dominant genetic diseases, characterized by cardiovascular involvement, tissue fragility, joint hypermobility and skin involvement. Some of them are associated with life threatening events such as aortic dissection and rupture, and a high mortality rate. We recently introduced Preimplantation Genetic Testing for monogenic disease (PGT-M) with a focus on HCTD and set up an explorative qualitative study to investigate the lived experiences and feelings of women, as well as their attitudes and psychological responses to PGT-M.

Materials & Methods:
All couples are seen by a clinical geneticist and a psychologist, and blood samples of the couple are drawn for karyotyping, exclusion of carriership of cystic fibrosis, spinal muscular atrophy and fragile-X syndrome. Informative linked microsatellite markers flanking the causal (familial) mutation are identified for each couple. Once these parameters are defined, DNA, obtained from trophectoderm cells biopsied from a day 5-6 embryo, is genome-wide amplified. Subsequently, the informative linked microsatellite markers as well as the underlying disease causing mutation are analysed in each of these day 5-6 embryo's. In total, twelve HCTD couples have undergone the PGT-M procedure (*FBN1*: 4, *SMAD3*: 2, one each for *TGFBR1*, *TGFB2*, *COL3A1*, *COL1A2*, *COL2A1* and *WNT10A*). In total, 52 embryos have been tested, 19 of them carried the healthy haplotype (microsatellite analysis) and did not carry the familial mutation (Sanger sequencing) and thus were suitable for transfer to the mother. Data of seven women, aged 26-39years, were collected by semi-structured interviews and interpreted by thematic analysis. At the time of the interviews, one participant had one child through PGT-M, two participants were pregnant after embryo transfer and four women did not have a successful embryo transfer leading to pregnancy yet.

Results:
Our study shows that PGT-M is both physically and emotionally a demanding procedure. The main reason for choosing PGT-M is the severity of the disease. None of the couples wants to transmit the disease to their offspring. Since termination of pregnancy is not acceptable to them, PGT-M is preferred over prenatal diagnosis. The "drop-out race", as all interviewed women call it, going from an encouraging number of available oocytes (after pick-up), to a limited number of useful embryos, is perceived as extremely stressful. Due to the frequent hospital visits and the prescribed strict timing of hormone injections, all women stated it is impossible to keep the PGT-M procedure hidden from their friends and colleagues. Women emphasize that receiving adequate information, during every step of the PGT-M process, is essential to learn more about the entire PGT-M trajectory. They also express the need of psychological support to help them in coping with this physical and psychological demanding technique.

Conclusions:
PGT-M is a valuable option of reproductive technology for HCTD couples. Due to the significant emotional impact the PGT-M procedure causes, we advise that it should always go hand-in-hand with effective communication and psychological care in order to prevent distress in couples.

P-60

FOUR YEARS EXPERIENCE OF PREIMPLANTATION GENETIC TESTING OF FOUR MONOGENIC DISORDERS (CYSTIC FIBROSIS, BETA-THALASSAEMIA, HEMOPHILIA A AND B)

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Preimplantation genetic testing, monogenic disorder, blastocyst, linkage analysis.

Introduction:
Preimplantation genetic testing (PGT) is a significant challenge and a widely established reproductive alternative for couples with high-risk of transmitting an inherited monogenic disorder. In this study, we report single centre PGT experience at the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico of Milan, Italy, from 2015 to 2018 after the ruling of the Italian Constitutional Court (96/2015) which legalized embryo genetic analysis in couples with high genetic risk for the first time in Italy. Four diseases were firstly considered: cystic fibrosis and beta-thalassaemia, as autosomal recessive disorders, and hemophilia A and B, as X-linked disorders.

Material & methods:
Pre-clinical PGT workup was performed for 124 couples after genetic counselling. Thirty-one underwent PGD cycles for cystic fibrosis, 41 for beta-thalassemia and 3 couples for hemophilia A and 1 for hemophilia B. *In vitro* fertilization (IVF) procedures were used to generate embryos *in vitro* by intracytoplasmic sperm injection (ICSI). Blastocysts at day 5-7 were biopsied and vitrified post biopsy. Informative short tandem repeat (STR) markers were used for linkage analysis alone or in combination with family gene mutation detection by multiplex PCR. Following diagnosis, embryos with no gene mutation were transferred.

Results:
Two hundred and forty-seven embryos were obtained. Blastocyst biopsy was performed on 236 day 5-7 embryos. Successful genetic results were obtained in 195 embryos (83%), 121 of those were diagnosed unaffected (62%) and genetically transferable. No conclusive diagnosis was obtained for 41 biopsies and 15 embryos were rebiopsied. Transfer of 84 cryopreserved embryos resulted in 46 pregnancy with an implantation rate of 55% per embryo transfer (ET). Pregnancies result in 26 live births and 8 miscarriages. Thirty-six embryos still remain cryopreserved. Fourteen couples underwent prenatal diagnosis and results were consistent with PGT genetic analysis. Parameters such as allele drop-out, contamination and recombination were considered to evaluate diagnostic accuracy and reliability of genetic testing. Our data showed an allele drop-out rate of 5%, amplification failure of 10% and recombination rate of 6%.

Conclusions:
Our four years' experience on PGT demonstrates that couples with high risks of transmitting genetic mutations that cause severe medical conditions can really benefit from PGT technique, as it represents a robust reproductive option preventing abortion of an affected pregnancy with great suffering to the pair. Our PGT positive experience serves as an incentive for the use of this procedure in other genetic diseases in our centre.

P-61

PREIMPLANTATION GENETIC TESTING FOR MONOGENIC DISEASE OF SPINAL MUSCULAR ATROPHY BY MULTIPLE DISPLACEMENT AMPLIFICATION: 11 UNAFFECTED LIVEBIRTHS

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Multiple displacement amplification, haplotype analysis, Spinal muscular atrophy, preimplantation genetic testing for monogenic disease

Introduction:
PGT-M has become an effective method for providing couples with the opportunity of a pregnancy with a baby free of spinal muscular atrophy (SMA). Multiple displacement amplification (MDA) overcome the innate dilemma of very limited genetic material available for PGT-M. This study mainly evaluated the use of MDA, combined with haplotype analysis and mutation amplification, in preimplantation genetic testing for monogenic disease (PGT-M) for families with SMA.

Material and methods:
MDA was used to amplify the whole genome from single blastomeres or trophectoderm (TE) cells. Exon 7 of the survival motor neuron gene 1 (SMN1) and eleven STRs markers flanking the SMN1 gene were incorporated into singleplex polymerase chain reaction (PCR) assays on MDA products.

Results:
Sixteen cycles (19 ovum pick-up cycles) of PGT-M were initiated in 13 patients. A total of 141 embryos were diagnosed: 90 embryos were biopsied at the cleavage stage and 51 embryos were biopsied at the blastocyst stage. MDA was successful on 94.44% (85/90) of the single blastomeres and on 92.16% (47/51) of the TE cells. And the PCR efficiency were 98.4%(561/570) and 100%(182/182), respectively. In addition, the average allele drop-out (ADO) rates were 13.3%(60/392) and 9.8% (11/112), respectively. The results for SMN1 exon 7 were all matched with haplotype analysis, which allowed an accurate diagnosis of 93.62% (132/141) embryos. Twelve families had unaffected embryos available for transfer and a total of 38 embryos were transferred in 20 embryo transfer cycles. Eight transfers were successful, resulting in an clinical pregnancy rate of 40%(8/20) and an implantation rate of 28.95%(11/38). Finally,11 healthy babies were born. Among them, 5 patients were singleton live births and and 3 patients had twin births.

Conclusion:
Careful handling during the MDA procedure can improve subsequent PCR efficiency and reduce the ADO rate. We suggest that this protocol is reliable for increasing the accuracy of the PGT-M for SMA.

P-62

SUCCESSFUL STRATEGY OF COMPREHENSIVE PRE-IMPLANTATION GENETIC TESTING FOR DUCHENNE MUSCULAR DYSTROPHY AND CHROMOSOME BALANCE USING KARYOMAPPING.

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Duchenne Muscular Dystrophy (DMD), embryo selection, haplotyping, karyomapping, pre-implantaton genetic testing for monogenic disease (PGT-M)

Introduction:
Duchenne muscular dystrophy (DMD) is one of the most common muscular dystrophy in childhood. The patients suffer progressive proximal muscle weakness and die from respiratory failure and cardiomyopathy in their 30's. Pre-implantation genetic testing (PGT) is an alternative traditional prenatal diagnosis (PND). However, extremely large size of the gene and the diversity of mutations cause molecular genetic testing difficult and labor intensive. Modern haplotyping using aSNP and Karyomapping algorithm would be useful for diagnosis of monogenic disease. This study applied Karyomapping for PGT-M of DMD in 2 clinical PGT cycles in comparison to standard PCR testing techniques.

Material and methods:
Two families at risk of having DMD offspring joined the project following thoroughly counselling and inform consent was obtained. The patients 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 as well as copy number variation (CNV). Multiplex PCR with mini-sequencing was performed alongside for confirmation standard molecular mutation analysis. Multiple microsatellites within dystrophin gene were also analyzed for linkage analysis and contamination identification.

Results:
Nine embryos with good morphology from each patient were chosen for PGT. Karyomapping results of family TZ (DMD c.895G>T) revealed three normal, two carriers, two affected and two with intragenic recombination. Standard mutation analysis using multiplex fluorescent PCR incorporation with mini-sequencing and microsatellites analysis for linkage analysis confirmed haplotyping results in all embryos. In addition, karyomapping demonstrated one embryo with chromosome unbalanced, i.e. 46,XX, -6q (intragenic recombination). Therefore, three normal (two male and one female) and two carrier (both female) embryos with chromosomally balanced were fulfilled for transfer. During the first embryo transfer, one normal female embryo was chosen, no pregnancy was resulted. In the second transfer, one normal male embryo was transferred, one ongoing pregnancy was resulted. Karyomapping results of family JM (DMD exon 8-9 duplication) revealed two normal, two carriers, two affected and one with intragenic recombination. Standard microsatellites analysis for linkage analysis confirmed haplotyping results in all embryos. Additionally, karyomapping demonstrated one embryo with chromosome unbalanced, i.e. 45,XX, +2P,-22 (normal) and one embryo with uniparental disomy (UPD), i.e. 46,XX (normal). Therefore, two normal (both female) and two carrier (both female) embryos with chromosomally balanced embryos were fulfilled for transfer. All are being frozen waiting for transfer. Polymorphic marker analysis revealed the absence of extraneous DNA contamination.

Conclusions:
Two clinical PGT-M cycles using karyomapping were performed for two families at risk of having DMD offspring. This study exhibits that aSNP provides the benefit of extra information of chromosome balance and parental origins, i.e. uniparental disomy in one of the embryo. Therefore, kryomapping can omit the risk of transfer chromosomally unbalanced embryos, termination of abnormal chromosome pregnancy later and the birth of abnormal chromosome babies. Therefore, karyomapping provides an accurate, quick, time saving for protocol development, universal PGT-M method for every monogenic disease of various types of mutations and also the advantage of CNV and parental origin information which is common abnormalities in pre-implantation embryos.

P-63

THE DEVELOPMENT AND PILOT CLINICAL STUDIES OF NON-INVASIVE CAPACITY SCREENING (NICS) ASSAY

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NICS,PGS,non-invasive

Introduction:
For investigating the detection success rate and accuracy of non-invasive capacity screening(NICS) and determining its effectiveness on improvement of embryo transfer, we reviewed the research results and clinical advances of MALBAC-based NICS from multiple groups worldwide between 2016 to 2018.

Material and Methods:
1. Detection success rate :
qPCR was used to investigate the starting DNA amount limit and the detection success rate of NICS in 1483 culture medium samples.
2. Detection accuracy
246 embryos with morphological scoring over CC were collected. Culture medium samples were subjected to NICS and the results were compared with whole embryo's aneuploidy results to evaluate the accuracy of NICS as an option of pre-implantation chromosome screening. Also, NICS were applied to 41 families with chromosome translocations to determine its accuracy on pre-implantation chromosome diagnosis.
3. Clinical validation
MCT: 120 patients were enrolled and RCT were implemented for non-inferiority comparison of clinical outcomes between NICS and PGS. And 72 patients were recruited for superioritystudy by comparing clinical outcomes between traditional morphology assessment and NICS.

Results:
1. The overall success rate reached to 94.6% among all 1483 culture medium samples with NICS and it mounted to 99.2% when DNA amount ≥ 3pg.
2. The NPV of NICS were over 90% during pre-implantation chromosome screening and this value reached to 97% when applying NICS to pre-implantation chromosome diagnosis. The other performances of NICS including sensitivity, specificity, PPV were comparable to PGS.
3. The clinical results of NICS and PGS from 120 patients did not exhibit significant differences. The clinical outcomes of 72 cases showed that the miscarriage rate of NICS is 0%, and the results are still updating.

Conclusions:
NICS is a novel genetic testing that allows an accurate selection of blastocysts with better developmental potential for transfer and might improve the clinical outcomes.

P-64

CAN FISH PROBE STRATEGIES INFLUENCE MOSAIC EMBRYO RATES IN ROBERTSONIAN TRANSLOCATION PGD?

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PGD, embryo development, implantation, viability, robertsoniantranslocation

Introduction:
Robertsonian translocation (RT) results from centric fusion of two acrocentric chromosomes. RT carriers are phenotypically normal, but they are known to be at increased risk of repeated miscarriages or of pregnancies resulting in the birth of a child with congenital anomalies, mental retardation. Preimplantation Genetic Diagnosis (PGD) is therefore a solution for RT carriers. A suitable probe strategy on cleavage stage allows us to differentiate balanced embryos, unbalanced embryos, mosaic and chaotic embryos. According to ESHRE recommendations, only balanced embryos are considered for transfer. However, transfer of some mosaic embryos has resulted in live births. We performed the first comparative analysis between a two or a three probes FISH strategies, in order to enhance the safety and security of embryos transferred and to establish the best reliable and low-cost strategy for RT PGD management.

Material and methods:
Retrospective analysis of 253 preimplantation genetic diagnosis (PGD) cycles were performed on 91 men and 42 women carriers of different Robertsonian translocations t(13;14), t(14;21), t(14;15), t(15;21), t(13;15), t(13;21), t(14;22), t(15;22), t(21;22), t(13;13). The first strategy involved two relevant probes use. Each probe is located within the terminal long arm. The second strategy required a third probe addition on one of the two translocated chromosomes. The additional probe is located close to centromeric regions.

Result(s):
Regardless of probe strategies, 253 PGD cycles were performed in the aim to exclude imbalances in both carriers. On a total of 1,549 biopsied cells, 1,458 were successfully analyzed. Among these, 232 were transferred. Clinical pregnancy was achieved in 108 patients, and 85 healthy babies were delivered. No significant difference was observed in the balanced embryo rates (40.5% and 40.4% in a two probes and a three probe strategies respectively). Among these balanced embryos, diagnostic established on single or two mononucleate cells, wasn't affected by the probe strategies. However, a significant difference ($p <= 0.0001$) was observed in mosaic embryo rates. Mosaic embryo rate decreased with three probes strategy in comparison with two probes strategy. On the other hand, chaotic embryo rate increased with three probes strategy in comparison with two probes strategy.

Conclusion(s):
According to the present findings, concerning balanced embryo rates, the two probes strategy seems more suitable for Robertsonian translocation PGD analysis, firstly because it led to similar results as the three probes strategy. Secondly, this study also highlighted an increase mosaic embryo rate observed with the two probes strategy. Recent data indicated that mosaic embryos may represent a second category to transfer after balanced embryos. Obviously, balanced embryos must be transferred in priority. However in absence of balanced embryos, transfer of a mosaic embryo should be considered. In this case, genetic counseling about risks and potential benefits is of utmost importance to ensure informed decision-making by patients.

P-65

SUCCESSFUL STRATEGY OF COMPREHENSIVE PRE-IMPLANTATION GENETIC TESTING FOR BETA-THALASSEMIA-HEMOGLOBIN E DISEASE AND CHROMOSOME BALANCE USING KARYOMAPPING.

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beta-thalassaemia-hemoglobin E disease, embryo selection, haplotyping, karyomapping, pre-implantaton genetic testing for monogentic disease (PGT-M)

Introduction:
Thalassemia syndrome and hemoglobinopathy are the world commonest monogenic disease and cause health and economic burden worldwide. Pre-implantation genetic testing (PGT) is an alternative to traditional prenatal diagnosis (PND). However, the nature of a vast variety of mutations makes molecular genetic testing sophisticated and labor intensive. Modern haplotyping using SNP array (aSNP) and Karyomapping algorithm would omit molecular analysis development process and provide chromosome balance information at the same time. This study applied Karyomapping for PGT-M of beta-thalassemia-hemoglobin E (Hb E) disease in 2 clinical PGT cycles in comparison to PCR testing techniques.

Material and methods:
Two families at risk of having beta-thalassemia-Hb E disesase offspring decided to join the project following thoroughly counselling and inform consent was obtained. The patients 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 as well as copy number variation (CNV). Multiplex PCR with mini-sequencing was performed alongside for confirmation standard molecular mutation analysis. Informative polymorphic marker was also included for contamination identification

Results:
Thirteen and nine embryos with good morphology from families YW and CS were chosen for PGT, respectively. Karyomapping results of family YW (beta–thalassemia (c.17A>T)-Hb E (c.26G>A) disease) revealed four normal, two beta-thalassemia trait, one Hb E trait and six affected with beta-thalassemia-Hb E disease. Standard mutation analysis using multiplex fluorescent PCR and mini-sequencing confirmed haplotyping results in all embryos. In addition, Karyomapping demonstrated three embryos with chromosome unbalanced, i.e. 45,XY, -19 (affected), 47,XY, +16 (beta-thalassemia trait) and 47,XX, +21 (beta-thalassemia trait). Therefore, four normal (three male and one female) and one Hb E trait embryos were fulfilled for transfer. One normal male embryo was chosen for transfer and one normal male baby was delivered. Prenatal and postnatal DNA testing confirmed PGT results. Karyomapping results of family CS (beta–thalassemia (c.17A>T)-Hb E (c.26G>A) disease) revealed six Hb E trait and three affected with beta-thalassemia-Hb E disease. Standard mutation analysis using multiplex fluorescent PCR and mini-sequencing confirmed haplotyping results in all embryos. Additionally, Karyomapping demonstrated two embryos with chromosome unbalanced, i.e. 45,XY, -21 (affected) and 45,XY, -22 (Hb E trait). Therefore, five Hb E trait embryos were fulfilled for transfer. One Hb E trait embryo was chosen for transfer and one ongoing pregnancy was resulted. Polymorphic marker analysis revealed the absence of extraneous DNA contamination.

Conclusions:
Two clinical PGT-M cycles using Karyomapping were performed for two families at risk of having beta–thalassemia (c.17A>T)-Hb E (c.26G>A) disease offspring. Additional standard multiplex PCR with mini-sequencing analysis confirmed haplotyping results of Karyomapping. However, aSNP provides the benefit of extra information of chromosome balance. This study demonstrated that kryomapping can omit the risk of transfer chromosomally unbalanced embryos, termination of abnormal chromosome pregnancy later and the birth of abnormal chromosome babies. Therefore, Karyomapping provides an accurate, quick, time saving for protocol development, universal PGT-M method for every monogenic disease, and also the advantage of CNV information which is common in pre-implantation embryos.

P-66

THE DETECTION OF MITOCHONDRIAL DNA IN THE BASTOCOELIC FLUID OF EXPANDED BLASTOCYSTS

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blastocoelic fluid, mitochondrial DNA, PGT

Introduction:
The pivotal role of mitochondria in ATP production, as well as the high mutation rate of their genome (mtDNA) are well known. Although variants fixation within the population depends to the phenotype functionality, some recurrent variants with deleterious effect survive to natural selection.We already described a relation between mitochondrial haplogroups and aneuploidy susceptibility. Since the segregation process is strictly dependent on adequate ATP provision, the mutation load analysis of mtDNA in blastocysts may be informative of embryo viability. The aim of this study was to verify whether mtDNA analysis could be conducted on the fluid extracted from the blastocoelic cavity through a procedure that is moderately invasive compared with the conventional forms of biopsy.

Material and Methods:
In the first part of the study, the blastocoelic fluid (BF) retrieved from 10 expanded blastocysts previously inferred as aneuploid by a-CGH was amplified for the mitochondrial D-loop region and sequenced by Sanger. Maternal age of the 10 patients ranged between 37 and 41 years. In the second part of the study, D-loop sequencing was carried out in five additional sets of sequential biopsies including both polar bodies (PB I; PB II), BF, trophectoderm (TE) and the corresponding whole embryo (EM), accounting for a total of 25 samples. Also in this case, the 5 sets had been diagnosed as aneuploid by chromosome analysis on PBs. All sequences were aligned to mtDNA reference sequence prior to haplogroup inference.

Results:
In the first part of the study, the complete D-loop amplification and sequencing was successful for all the BF samples. Haplogroups were inferred with 60% of lineages belonging to Macro-haplogroup H, while the remaining 40% was represented by the lineages I, W and K. These haplogroups are characteristic of the European population.In the second part of the study, D-loop amplification and sequencing was possible in all samples except for the PBs from one sample set. The intra-group sequence comparison highlighted full correspondence in 4 sets, while the remaining set showed a heteroplasmic position in one PB I.

Conclusions:
The feasibility to amplify and sequence mtDNA from the BF may offer a reliable alternative to other forms of conventional biopsy. In this context, it would be interesting to validate the method also to the complete mitochondrial genome (16Kb), testing in this way also its integrity.

P-67

BIOPSIED CELLS FROM FROZEN EMBRYOS IN PREIMPLANTATION GENETIC TESTING FOR MONOGENIC ARE INFERIOR TO FRESH EMBRYOS

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preimplantation genetic testing for monogenic, biopsy, frozen embryo, whole genome amplification

Introduction:
Preimplantation genetic testing for monogenic (PGT-M) is carried out by using both direct and indirect methods. However, due to the limited number of facilities where PGT-M can be implemented, its execution sometimes involves an embryo biopsy using frozen embryos transported from other infertility facilities as well as fresh embryos obtained by in vitro fertilization and embryo culture at the facility. In the present study, the effect of freezing on the embryos was examined by comparing the frozen and fresh embryos on the basis of number of biopsied cells, amount of amplified DNA, and undiagnosed embryo rates in biopsied cells.

Material & methods:
The subjects used in the present study comprised 38 blastocysts obtained from 13 cycles of 7 couples who underwent PGT-M at our clinic between 2017 and 2018. Of the 38 embryos, 28 were fresh, obtained from 9 cycles of 5 couples, and 10 were frozen, obtained from 3 cycles of 3 couples. The diseases to be diagnosed were: Duchenne muscular dystrophy (1 case), myotonic dystrophy (3 cases), adrenoleukodystrophy (2 cases), and peroxisomal disease (1 case). Trophectoderm biopsy was performed using a laser, and a light microscope was used to count the number of biopsied cells. REPLI-g Single Cell Kit (QIAGEN) was used for whole genome amplification of the biopsied cells. Further, in order to enhance the accuracy of the analysis, haplotyping by short tandem repeat markers along with direct mutation detection were performed. This also ensured minimal potential errors caused by the undetected allele dropout and/or contamination.

Results:
The age of the females from whom fresh and frozen embryos were obtained was 34.7 and 32.7 years, respectively. Furthermore, the average number of biopsied cells between the fresh and frozen embryos did not differ much (7.8 and 8.6 respectively). However, the amount of amplified DNA from the frozen embryos (485.3 ng/µl) was noted to be significantly smaller than that from the fresh embryos (696.5 ng/µl). In addition to this, the incidence of undiagnosed embryos in case of frozen embryos was much higher than that in the case of fresh embryos (40.0% vs. 3.6%, $p < 0.05$).

Conclusions:
The results revealed that the biopsied cells from the same number of frozen embryos as the fresh embryos generated a relatively smaller amount of amplified DNA and higher rate of undiagnosed embryos. This difference can be attributed to the possibility of cell damage due to embryo freezing. The result of the current study suggests that biopsies should be performed on fresh embryos rather than on frozen ones.

P-68

PREIMPLANTATION GENETIC TESTING OF MONOGENIC DISEASE: EXPERIENCE IN RUSSIA

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PGT-M, single-gene disorder, retrospective analysis, PGT-A

Introduction:
Preimplantation genetic testing of monogenic disease (PGT-M) is technically challenging, because extremely small amount of biomaterial increases allele dropout (ADO) rates, contamination significance, failed amplification (FA) events. We report here our experience of PGT-M in “Genetico” center in Russia.

Material & methods:
A retrospective analysis of all requests and cycles of PGT-M referred to our center was performed. Embryo biopsies from 29 medical centers were sent for PGT-M. Embryos were mostly biopsied on day 5-6. PGT-M assays combine direct diagnosis of the pathogenic variants and linkage analysis of highly heterozygous STRs. The nested PCR was used for DNA amplification from different sources: single cells or whole genome amplification (WGA) products of embryo biopsy, total DNA. PGT-A by NGS or aCGH was performed for unaffected embryos upon patient’s request.

Results:
Of 109 couples referred to our center for PGT-M, 92 completed preliminary test for PGT-M for 42 genetic condition and HLA gaplotyping: 24 autosomal-dominant (AD) requests, 54 autosomal recessive (AR), 13 X-linked requests. The most frequent indication was spinal muscular atrophy (17 couples) and cystic fibrosis (8 couples). All these couples referred for PGT-M not after preconception screening, but because of affected child birth. We performed 85 PGT-M cycles with 413 embryos. Almost all of these samples undergo WGA for possibility to combine PGT-M with PGT-A, also each test-system was validated for single-cell either. The WGA failed in 16 cases (3,9%). The reliability was decreased for 12 embryo results (3%), because of increased number of ADO or marker FA events. Median number of markers included in test-systems was 12 and for embryo analysis it was 10. These highly informative test-systems contributed to low number of inconclusive results – only for 7 samples (1,8%) (Girardet et al. 2018). The recombination events are not the reason to decrease the reliability if sufficient number of informative markers employed. The recombination event was revealed in 39 samples (9,4%). Median distance between markers of test-system (2,97 Mb) suggests lower recombination rate 3,75%. Of all analyzed embryos 131 (31,7%) did not inherited any of pathogenic alleles (AD – 61; AR – 48; X-linked – 11), 135 (49%) were carriers (AR – 122; X-linked – 11), 136 (32,9%) were affected (AD – 49; AR – 67; X-linked – 10). For 156 (58,6%) unaffected embryos PGT-A was performed and 91 (34,1%) were suitable for transfer. Chromosomal abnormalities for three embryos were revealed at PGT-M stage and confirmed by PGT-A. At the moment we have information about 43 transfers, 19 pregnancies and 7 healthy births and no affected pregnancy or birth.

Conclusions:
Highly informative test system and accurate analysis of results can lead to both - high accuracy of obtained results and decreased number of embryos, that were rejected because of inconclusive results. PGT-M appears to be more robust analysis than PGT-A.

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P-69

IMPROVED IVF SUCCESS OF COMBINED PGT-M AND PGT-A APPLICATIONS

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Preimplantation Genetic Testing for Aneuploidy, Preimplantation Genetic Testing for Monogenic/Single Gene Disorders, Next Generation Sequencing, In Vitro Fertilization

Introduction:
Preimplantation Genetic Testing (PGT) has been performed since 1989 and utilized in the diagnosis of all genetic conditions at preimplantation stage (1). In the previous years, only a disorder could be diagnosed with the use of multiplex nested PCR in PGT applications. Increased use of trophectoderm biopsy and vitrification together with Whole Genome Amplification (WGA) technology enabled the detection of single gene disorders together with 24 chromosome screening. This approach allowed us to select healthy and chromosomally euploid embryos.
Even though single gene disorder carrier patients are mostly young and fertile, they still have high risk of spontaneous miscarriage. In the recent years, it has been reported that when the embryos are screened for aneuploidy (PGT-A) in addition to single gene disorder testing (PGT-M), pregnancy rates can be increased from 45% to 68% and spontaneous miscarriage rates decreased from 15% to 5%. 34 single gene disorder patients, whose embryos were found to be healthy in terms of single gene disorder, were included in this study and these embryos were further screened for aneuploidy using Next Generation Sequencing (NGS) technology (2).

Materials & Method:
On the 5th and 6th days of embryonic development, WGA was performed using SurePlex DNA Amplification System (Illumina, USA) and DOPlify (PerkinElmer Health Sciences - Australia). Following multiplex nested PCR on WGA samples, fragment analysis for haplotyping and sequence analysis for mutation detection were performed. Embryos found to be suitable for transfer regarding the single gene disorder have further been screened for aneuploidy using NGS technology with VeriSeq PGS Kit (Illumina, USA).

Results:
This study was conducted for single gene disoder and 24 chromosome screening in 44 patients with an average age of 34. 280 embryos derived from these patients were included in PGT-M. Among 153 embryos that were found to be suitable for transfer after PGT-M, PGT-A was performed on 121 embryos upon patients' requests and 49% of these embryos were euploid. Embryo transfer could not be performed in 12 patients due to the absence of euploid embryos. According to the data we acquired, 64% pregnancy was achieved in the rest of the patients.

Conclusion:
While pregnancy ratio can be improved with this approach, Allele drop out (ADO) and contamination errors are still major limitation which can lead to misdiagnosis of embryos. ADO ratio was demonstrated to be 25% in PGT for blastomere cells, whereas this ratio drops down to 5% when 5-7 cells obtained by trophectoderm biopsy are tested. Thus trophectoderm cells are more appropriate for PGT-M applications combined with PGT-A.
Since stable and homogenous amplification of the entire genome is a crucial step, optimised WGA technologies will be a key factor in these applications.

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P-70

PREIMPLANTATION GENETIC TESTING FOR YQH-AUTOSOME TRANSLOCATION CARRIERS: ANALYSIS OF GAMETE SEGREGATION

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PGT-SR, translocation, satellite DNA, meiosis

Introduction:
Y-autosome translocations occur in approximately 1:2000 in population and represent special group of reciprocal exchanges. Due to a balanced nature they do not influence on phenotype in general. However, Y-autosome translocation can dislocate sex bivalent formation and chromosome synapsis in the first meiotic division prophase that led to gamete aneuploidy or pachytene arrest. More often Y-derived DNA, predominantly Yq12 band, is translocated onto the short arm of acrocentric chromosomes, mainly chromosomes 15 and 22 due to a strong correlation in their satellite DNA sequences. In women inheritance of such derivative acrocentric chromosomes could be associated with the risk of ovarian malignancy, but the particular mechanism of this pathological effect is not entirely clear since the Yq12 band consists of tandemly organized satellite DNA sequences. It can be associated with the transcription of satellite DNAwhich occurs during carcinogenesis.

Material & methods:
Two families with Yq12/15p and one – with Yq12/22p translocations were analyzed during IVF cycle with PGT in International Centre for Reproductive Medicine. The mean age of men and women was 36±2.8 and 34.25±1.4 years respectively. Karyotyping analysis was performed using GTG banding technique on metaphase chromosomes from peripheral blood lymphocytes. Fluorescence *in situ* hybridization (FISH) was used to clarify the breakpoint on chromosomes and confirm the chromosomes-specificity of the probes. Additionally, seven chromosomes (13, 14, 15 or 22, 16, 18, 21, X), which are not involved in rearrangement, were analyzed.

Results:
FISH with chromosome specific probes was performed on blastomere from cleavage-stage embryo. A total of 42 blastomeres were analyzed. In 43% of the blastomeres «adjacent-1» segregationwith derivate autosome and X or Y chromosomes was revealed. Segregation 3:1 was observed in 10% of the cells, with no predominant inheritance of the derivative autosome. In 21% of cells the type of chromosome segregation could not be determined due to mosaicismor polyploidy. 45% of the cells were aneuploid for autosomes, which are not involved in the translocation, which may be connected with interchromosomal effect.

Conclusions:
Carriers of Yq12/15p or Yq12/22p translocations have a predominant inheritance of the derivative autosome, which can be associated with the disruption ofsex bivalent segregation.
This research was supported by Russian Foundation for Basic Research (grant #18-34-00279).

P-71

PREIMPLANTATION GENETIC DIAGNOSIS OF NEURODEGENERATIVE DISEASES

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PGD, ARMS-qPCR, FAP, spinocerebellar ataxia, Huntington's disease

Introduction:
Preimplantation genetic diagnosis (PGD) has become a crucial approach to help carriers of inherited disorders give birth to healthy offspring. Here, we review the PGD methodologies and explore the use of polymerase chain reaction (PCR)-based technologies for PGD in neurodegenerative diseases that are clinically relevant and have typical features such as late-onset and severely debilitating.

Material and Methods:
Trophectoderm biopsies were performed at Day5/6 blastocyst stage. PGD using amplification refractory mutation system quantitative PCR (ARMS-qPCR) and/or linkage analysis was performed for 10 cases of various neurodegenerative diseases, underwent 13 oocyte retrieval cycles for *in vitro* fertilization (IVF) with PGD at the core laboratory of Dr. Ming Chen, a major PGD laboratory in Taiwan, during 2013-2016.

Results:
A total of 13 oocyte retrieval cycles were conducted. Among the 59 embryos analyzed, 49.2% (29/59) were unaffected and 50.8% (30/59) were affected. There were 12 embryo transfer cycles, of which 3 became pregnant and all pregnancies were delivered. The implantation rate and livebirth rate were 23.1% (3/13) per oocyte retrieval cycle and 25.0% (3/12) per embryo transfer cycle, respectively. Allele dropout (ADO) was noted in two embryos that were classified as unaffected by ARMS-qPCR but were evidenced as affected after prenatal diagnosis, rendering the false negative rate as 6.3% (2/32). Four among the 13 cycles underwent PGD by ARMS-qPCR coupled with linkage analysis and all of them were correctly diagnosed.

Conclusions:
PGD by PCR-based methods (*e.g.*, ARMS-qPCR and linkage analysis) is a feasible strategy whereas ADO is always a concern if ARMS-qPCR is used as the sole technology in PGD, especially in autosomal dominant diseases. Robust methodologies, proper genetic counseling that covers technical and ethical aspects of genetic testing, and confirmatory invasive prenatal diagnosis are important in PGD of neurodegenerative diseases.

P-72

THE COUPLES' CHOICES ON PREIMPLANTATION GENETIC TESTING FOR MONOGENIC AFTER GENETIC COUNSELING IN JAPAN

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PGT-M, genetic counseling,

Introduction:
In Japan, preimplantation genetic testing for monogenic (PGT-M) can only be done under Japan Society of Obstetrics and Gynecology (JSOG) approval facility. Inside the approval facility, case has to get an approval from JSOG. Due to such strict condition, there are only 5 facilities that have experienced the PGT-M. Even to the genetic diseases that are subjects of PGT-M among other countries, it is not so in Japan and only limited to severe genetic diseases with onset at childhood. In this study, we will consider the issues of PGT-M in Japan from our genetic counseling and their subsequent decision by couples who wanted PGT-M at our clinic.

Material & methods:
The subject is 31 couples who made reservation of consultation with our clinic for the purpose of PGT-M from August 2014. In order to obtain approval of PGT-M by JSOG, the couple need to receive genetic counseling not only at the PGT-M implementation facility but also at two different facilities.

Results:
21 couples out of 30 couples who received genetic counseling at our clinic wished to apply PGT-M to JSOG.
A couple with a child with an Alpha-thalassemia X-linked mental retardation syndrome failed to undergo consultation for genetic counseling at our clinic because the condition of the child worsened.
After genetic counseling at our clinic, seven couples did not want PGT-M.
One couple with a child with congenital glycosylation disorder of glycosylation (type Ik) gave up PGT-M because it was difficult to attend our clinic with treatment of the affected child since it took more than 3 hours to our clinic.
Four couples abandoned PGT-M because it takes a long time to approve.
Also, in a couple with Myotonic dystrophy 1 male patient, the fact that they are not suited for PGT-M was not easily accepted and they had to be re-consulted by other facilities before they could accept the fact. The couple of congenital myopathy central core disease was selected for PGT-M because of adult onset disease.
In other two couples, one couple applied for PGT-M at different facility, and the other has not yet been able to make the decision after genetic counseling.

Conclusions:
Although there may be couples who want to take PGT-M, there are not enough facilities that have experienced the PGT-M in Japan. In addition, indication of PGT-M in Japan is severely limited to diseases in which symptoms that strongly disdain daily life are developed and the survival is dangerous before reaching adults. In the future, based on overseas adaptation criteria, Japan's adaptation criteria should be relaxed

P-73

SIMULTANEOUS PGT-M APPLICATIONS FOR MULTIPLE GENETIC CONDITIONS.

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Preimplantation Genetic Testing for Monogenic/Single Gene Disorders, In Vitro Fertilization, Whole Exom Sequencing

Introduciton:
Pre-implantation genetic diagnosis for monogenic disorders (PGT-M) have been extensively applied for genetic conditions resulting from mutations at single genetic locus . Vast amount of data have arised from Whole Exom Sequencing (WES) and Whole Genome Sequencing (WGS) technologies it is not always straight forward to filter out the mutation responsible for the disorder. Furthermore consanguinity increases the risk of cooccurence of two or more etiologically different genetic diseases in a single family. For this reason increased number of patients referred to PGT-M applications started to demand simultaneous testing of multiple genetic disorders. Drastically some families referred for one condition later had further offsprings with additional single gene defects. We are reporting PGT-M application for two or more genetic conditions performed in our center.

Materials and Methods:
PGT-M was performed by testing blastocyst/blastomere samples depending on the familial pathogenic variants. For this purpose biopsied cells were lysed and fragment analysis for haplotyping and sequence analysis for mutation detection have been performed following multiplex nested PCR. PGT-M testing was performed simultaneously for multiple genetic conditions in 17 couples. 6 infromative STR markers were used for each loci in addition to mutation site.

Results:
was performed for 17 couples and 36 disorders including (Beta Thallasemia, DMD, Glycogen storage disease, Mitochondrial complex I deficiency, Maple syrup urine disease etc.) For 2 couples 3 different conditions were screened simultaneously. While 5 out 7 embryos was appropriate for transfer in one of these patients only 1 out of 8 embyos was transferable for the second case. For 15 couples where 2 conditions were screened 32 embryos out of 104 were appropriate for transfer. Embryo transfer was performed in all patients except for one patient who had only 1 embryo for .

Conclusion:
Insufficient information in databeses regarding the pathogenicity of variants for rare diseases leads to a difficults in counselling patients for PGT-M. Frequent use of WES/WGS for families has become an effective tool for detecting the mutations to enable PGD application for families without previous diagnosis of their affected child. Unfortunately in many cases unusal phenotypes in consangenous couples forces us to exclude all variants including variants of unknown significance (VUS) that may be associated with phenotype. This a challanging approach since PGT-M should be designed for each condition together with linked STR markers for each of them to achieve high confidence results. Additionally the more we try to exclude the less likely we will find an appropriate embryo for transfer and families should be informed of this possibility before consenting to PGD applications. Because of this delineation of VUS will enable us to interprete patients history and give more efficient counselling to PGT-M patients thus limiting the number of embryos excluded due to these variants.

P-74

NORMAL LIVE BIRTH FROM TWO CARRIERS OF RARE INSERTIONAL TRANSLOCATIONS UNDERGOING PGD

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FISH, Interchromosomal insertion, PGD, Sperm

Introduction:
Despite the use of more precise techniques that indicates greater incidence, meiotic segregation of interchromosomal insertion has rarely been studied. Theoretically, the risk to have a child at term with malformation or mental retardation is high but there are no tools to determine this risk. We have investigated the Preimplantation genetic diagnosis (PGD) management of patients with interchromosomal insertion (IT) by fluorescent in situ hybridization (FISH) to obtain a better understanding and improve the genetic counseling.

Material & methods:
Two couples were enrolled in our PGD unit. The male of the first couple carries the IT 46,XY,ins(4;11)(q22;p12p13) and the female karyotype of the second couple is 46,XX,ins(2;10)(q21;q25.2q22). We have investigated, by FISH technique, the meiotic segregation analysis of the sperm of the IT male carrier and embryos providing from the two carriers. One FISH analysis round was performed for the male IT carrier. Five specific probes of chromosomes 4 and 11 were applied to nuclei from spermatozoa and blastomeres from embryo biopsy. An independent X2 test was used to compare abnormal spermatozoa from the patient and the control. Two FISH analysis rounds were performed for the female IT carrier. Six specific probes of chromosomes 2 and 10 were applied to blastomeres of PGD embryos.

Result(s):
The sperm-FISH segregation analysis showed a significantly increased percentage of unbalanced gametes (52.7%) compared with the control sperm (3.4%, $p<0.05$). Among the unbalanced spermatozoa, a preferential 2:2 segregation mode was observed with a rate of 48.2% which was significantly increased compared with the control sperm (0.45%). The PGD performed by FISH analysis on eleven embryos at Day 3 revealed three balanced embryos (27.3%) and eight embryos with chromosomal abnormalities (72.7%). For the female carrier, on the seven embryos analyzed by FISH, three were balanced (42,9%) and four displayed chromosomal abnormalities (57,1%). Each PGD cycle give birth to a healthy child for each couple.

Conclusion(s):
These results permit to reassure the physicians in the management of IT carriers and better guide the genetic counseling. PGD is a suitable diagnostic tool for IT carriers. Sperm FISH analysis contributes to a better IT behavior and segregation understanding. The reanalysis of not transferred whole embryos is essential to avoid misdiagnosis and therefore to contribute to a better IT behavior and segregation understanding. Several studies are needed to specify the risk to ITs carriers and to better assess the risk incurred by the couples.

P-75

A FAMILY OF MATCHED PARENT-CHILD HLA HAPLOTYPES: A CASE STUDY FROM BAHRAIN

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Leukemia, AML, BMT, HLA Typing

Introduction:
Bone marrow transplant (BMT) is a medical procedure performed to replace the soft, fatty bone marrow that has been damaged or destroyed by disease, infection, or chemotherapy. Most bone marrow transplants are usually performed on patients with certain blood cancers (leukemias) and other blood disorders. Cases of fully matched offspring(s) with parents are very rare and would occur when both parents share at least one haplotype which is commonly seen in several generations of consanguineous marriages (Hajeer et al. 2012). The probability of finding a full parent-child HLA match is higher in consanguineous marriages common in the Middle East, West Asia and North Africa (Hamamy, 2011). We present a case of a unique family in Bahrain with four children (two sons and two daughters). The four siblings and their parents were tested for HLA to check whether they were a match with a family member who needed a bone marrow transplant after being diagnosed with Acute Myeloid Leukemia (AML).

Materials and Methods:
Six DNA samples were extracted from peripheral blood using the Qiagen QIAamp DNA Mini Kit according to manufacturer's instructions (Qiagen, Hilden, Germany). The extracted DNA samples were quantified using the Qubit® 3.0 Fluorometer. A separate PCR protocol was set up for each locus to be amplified and for each individual sample to be tested. Successful amplicons were confirmed by a 1% agarose gel electrophoresis. Sequencing reactions were run with group specific and locus specific primers using the Conexio Genomics SBT Resolver kits. The sequenced products were purified using the ethanol precipitation method and capillary electrophoresis using the ABI 3130XL Genetic Analyzer (Applied Biosystems).The data was analysed using the CareDx Pty Ltd's ASSIGN™ SBT software.

Results:
The results showed that of the four children both sons were a 100% match with the mother, while one of the two daughters was a 100% match with the father.

Conclusion:
This case presents a unique situation where parents and children have fully matched HLA alleles due to a strong family history of consanguinity. This is the first case reported in Bahrain where children were found to be a 100% HLA-matched with their parents. Further studies are suggested to arrive to a broader conclusion to understand whether this is an isolated case or more of such cases would be found to be a trend in the Bahraini population.

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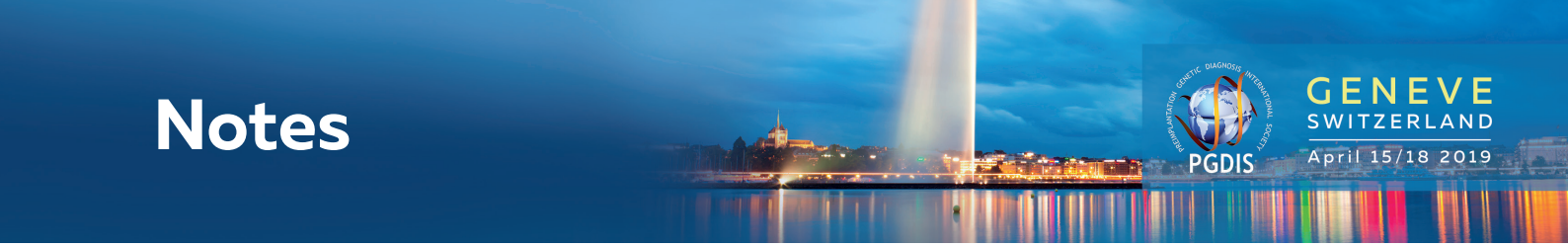

Notes

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