16th INTERNATIONAL CONFERENCE ON Preimplantation Genetics

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY
PGDIS

PROGRAM AND ABSTRACTS

Valencia 2017
26 / 29 March

Welcome

*We are very pleased to welcome you to the **16th International Conference on Preimplantation Genetic Diagnosis**, organized by the Preimplantation Genetic Diagnosis International Society in Valencia.*

We aimed to put together an exciting and cutting-edge scientific program and to place together Scientifics, Geneticists, Clinicians and Embryologist interested in the challenging field of Reproductive Genetics.

We do hope that you will enjoy the scientific discussions and the social program, as well as the city of Valencia with its sightseeing, monuments and lifestyle.

Carlos Simón
Local Organizing Committee

Carmen Rubio
Local Organizing Committee

Svetlana Rechitsky
President of PGDIS

International Scientific Committee

- Carlos Simón, Spain (Chairman)
- Joe Leigh Simpson, USA
- Alan Handyside, UK
- Svetlana Rechitsky, USA
- Luca Gianaroli, Italy
- Semra Kahraman, Turkey
- Anver Kuliev, USA
- Santiago Munne, USA
- Jacques Cohen, USA
- Dagan Wells, UK
- David Cram, China
- Don Leigh, Australia
- Jamie Grifo, USA
- Carmen Rubio, Spain
- Alan Thornhill, UK
- Antony Gordon, UK
- Darren Griffin, UK
- Kangpu Xu, USA
- Cristina Magli, Italy

Local Organizing Committee

- Carmen Rubio, Spain (Co-Chairman)
- Carlos Simón, Spain (Co-Chairman)

10.00-14.00 INNOVATING THE FUTURE OF REPRODUCTIVE GENETICS - organized by Illumina

Sala de Comisiones 1+2 (1st Floor)

Chairman: Alan Handyside

10:00–10:15	Introduction and the Illumina Difference: Sally Cartwright, Illumina
10:15–10:45	Developing the Next Generation of Preimplantation Genetic Screening (PGS) Technology: Illumina Product Development: Parul Choudhary, Illumina
10:45–11:15	Software Designed with the Application in Mind: Paul Hateley, Illumina
11:15–11:30	Coffee break
11:30–12:00	PGS: How can Scoring and Reporting be Improved: Alan Handyside & Robert Blanshard, Illumina
12:00–12:15	NextSeq® 550 and the World of Reproductive Genetics: Tyl Taylor, Illumina
12:15–12:45	Karyomapping for Single-gene PGD: Miroslav Hornak, Repromeda
12:45–13:15	Design and Validation of Sequencing Panels: Julio Martin, iGenomix
13:15–13:45	Beyond PGS: Expanding the Reproductive Continuum to Noninvasive Prenatal Testing (NIPT): Tony Gordon, Cooper Genomics

10.00-14.00 EMBRYO MANIPULATION COURSE - organized by Igenomix

Sala de Comisiones 3+4 (1st Floor)

Chairmen: Mª Eugenia Póo and Xavi Vendrell

10:00-10:05	Introduction: Xavi Vendrell
10:05-10:45	Reconstitution of Oocyte by spindle nuclear transfer for mitochondrial DNA Mutation: John Zhang (USA)
10:45-11:00	Q&A
11:00-11:40	Strategies for blastocyst biopsy: Are all blastocyts biopsiable?: Mª Eugenia Póo (Spain)
11:40-11.50	Q&A
11:50-12:20	Coffee break
12:20-13:00	How to achieve reproducible outcome of blastocyst biopsy across different biopsy practitioners: Antonio Capalbo (Italy)
13:00-13:10	Q&A
13:10-13:50	Blastocentesis protocol: Is it a reliable method for Comprensive Chromosome Screening?: Maurizio Poli (Italy)
13:50-14:00	Q&A

15.00-19.30 **ENDOMETRIAL RECEPTIVITY COURSE** (organized by Igenomix)

Introduction: Carlos Simón (Spain)

Sala de Comisiones 3+4 (1st Floor)

15.05-15.45	Diagnosis of Endometrial Receptivity from the Bench to the Patient using an evidence based medicine approach: Carlos Simón (Spain)
15.45-16.00	Q&A
16.00-16.45	How, When and to Whom: Maria Ruiz (Spain)
16.45-17.00	Q&A
17.00-17.30	Coffee break
17.30-18.15	The uterine microbiome: Inmaculada Moreno (Spain)
18.15-18.30	Q&A
18.30-19.15	Collaborative Clinical Research In progress: Diana Valbuena (Spain)
19.15-19.30	Q&A and conclusions

15.00-19.00 **ADVANCING REPRODUCTIVE GENOMICS WITH MICROARRAY AND NEXT GENERATION SEQUENCING APPROACHES -** organized by Termofisher

Sala de Comisiones 1+2 (1st Floor)

Detection of carriers with microarray technology
The technologies behind preimplantation genetic analysis
Adam Harris, Thermo Fisher Scientific
All-in-one workflow for whole chromosome aneuploidy and point mutation detection
Luis A. Alcaraz, PhD, Bioarray, S.L.
RNA analysis powering testing of endometrial receptivity
David Blesa Jarque, IGENOMIX
Simplified analysis of preimplantation genetic data
Alain Rico, Thermo Fisher Scientific

For Research Use Only. Not for use in diagnostic procedure

Day 1 - Monday 27th March - Auditorio 2 (Ground Floor)

08:45-09:00 OPENING AND WELCOME OF PGDIS 2017
Anver Kuliev (USA) and Carlos Simón (Spain)

09:00-09:30 PLENARY LECTURE
Genetic contribution to human disease, the impact of personalized medicine: Ángel Carracedo (Spain)

09:30-10:30 SESSION 1: UNDERSTANDING THE GENETIC RISK OF THE COUPLE
Chairmen: Anver Kuliev (USA) and Carlos Simón (Spain)

09:30-10:00 Personalized vs. Extended panels: Santiago Munné (USA)

10:00-10:30 Should we be testing all sperm/egg donors: Julio Martin (Spain)

10:30-11:00 COFFEE BREAK

11:00-13:00 SESSION 2: INTRINSIC FACTORS CONTRIBUTING TO EMBRYO ANEUPLOIDY
Chairmen: Tony Gordon (UK) and Mireia Sandalinas (Spain)

11:00-11:30 Common variants associated with mitotic-origin of aneuploidy in human embryos: Rajiv Mc Coy (USA)

11:30-12:00 Relationships between cancer and aneuploidy: Melvin DePamphilis (USA)

12:00-12:30 Implications of MTHFR gene polymorphisms in embryo viability and aneuploidy: Dagan Wells (UK)

12:30-13:00 Embryo aneuploidy induced by the sperm: Moncef Benkhalifa (France)

13:00-14:00 LUNCH BREAK - Exhibition Area
FREE POSTER SESSION - Multiusos 1 room (1st Floor)

14:00-15:30 **SESSION 3: FREE COMMUNICATIONS (RELATED TO CARRIER SCREENING AND PGD)**

Chairmen: Filipa Carvalho (Portugal) and Xavi Vendrell (Spain)

14:00-14:15 OC-01. THERAPEUTIC SUCCESS OF HSCT BY PREIMPLANTATION HAPLOTYPING, FOLLOW-UP OF 520 CYCLES. Cetinkaya, Murat

14:15-14:30 OC-02. COMBINED PGS AND PGD FOR THALASSEMIA. Brockman, Matthew

14:30-14:45 OC-03. HAPLOTYPING AND COPY-NUMBER PROFILING OF SINGLE CELLS BY MASSIVE PARALLEL SEQUENCING. Masset, Heleen

14:45-15:00 OC-04. FROM PRENATAL DIAGNOSIS OF FETAL ABNORMALITY TO PREIMPLANTATION GENETIC DIAGNOSIS FOR SKELETAL DYSPLASIA USING NEXT-GENERATION SEQUENCING TECHNOLOGIES. Penacho, Vanessa

15:00-15:15 OC-05. SEGMENTAL ANEUPLOIDIES IN PGD CYCLES FOR SINGLE GENE CONDITIONS BY KARYOMAPPING. Vesela, Katerina

15:15-15:30 OC-06. PREDICTION OF MENDELIAN DISORDERS RISK IN ASSISTED REPRODUCTIVE TECHNOLOGY PATIENTS OF MIDDLE EASTERN ETHNICITY BASED ON DETECTION OF GERMLINE VARIANTS USING AN EXPANDED PRECONCEPTION CARRIER GENETIC SCREENING TEST. Martin, Julio

15:30-16:00 COFFEE BREAK

16:00-17:00 **SESSION 4: EXTRINSIC FACTORS CONTRIBUTING TO EMBRYO ANEUPLOIDY**

Chairmen: Darren Griffin (UK) and Amparo Mercader (Spain)

16:00-16:30 Controlled Ovarian Stimulation: Jason Franasiak, (USA)

16:30-17:00 Embryo culture conditions and more: María José de los Santos Molina (Spain)

17:00-17:30 Impact of Lifestyle & Environment on Embryo aneuploidy: Ursula Eichenlaub-Ritter (Germany)

Day 2 - Tuesday 28th March - Auditorio 2 (Ground Floor)

08:00-08:15 **Summary of highlights previous day and Introduction to second day**
Svetlana Rechistky (USA)

08:15-08:45 PLENARY LECTURE
Mouse model of chromosome mosaicism reveals lineage-specific depletion of aneuploid cells and normal developmental potential: Magdalena Zernicka-Goetz (UK)

08:45-10:30 SESSION 5: CHALLENGING PGD-A DIAGNOSIS. MOSAICISM
Chairmen: David Cram (China) and Gary Harton (USA)

08:45-09:00 How to report and counsel mosaicism and segmentals in PGD-A: Don Leigh (Australia)

09:00-09:15 Mosaicism and segmentals in POCs and prenatal diagnosis: David Cram (China)

09:15-10:15 Specific Views (from submitted abstracts)
Chairmen: Gary Harton (USA), David Cram (China)

09:15-09:30 OC-07. CHALLENGES IN INTERPRETING THE RELEVANCE OF SEGMENTAL MOSAICISM DETECTED BY NGS. Grkovic, Steve

09:30-09:45 OC-08. THE EXTENT OF CHROMOSOMAL MOSAICISM INFLUENCES THE CLINICAL OUTCOME OF IN VITRO FERTILIZATION TREATMENTS. Spinella, Francesca

09:45-10:00 OC-09. EMBRYO AND PATERNAL FACTORS ASSOCIATED WITH BLASTOCYST CHROMOSOMAL MOSAICISM. Lledo Bosch, Belén

10:00-10:15 OC-10. CUSTOM NGS ALGORITHM FOR CONSISTENT AND ACCURATE DIAGNOSIS OF MOSAICISM IN TROPHECTODERM BIOPSIES. Vera-Rodriguez, Maria

10:15-10:30 Conclusion: Joe Leigh Simpson (USA)

10:30-11:00 COFFEE BREAK

11:00-13:00 **SESSION 6: HOW TO IMPROVE CLINICAL OUTCOME IN PGD-A**
Chairmen: Katerina Vesela (Czech Republic) and Mónica Parriego (Spain)

11:00-11:30 Laboratory conditions/biopsy strategies: Laura Rienzi (Italy)

11:30-12:00 When to transfer? Fresh vs. deferred transfer: Semra Kahraman (Turkey)

12:00-12:30 Review of the clinical evidence of MtDNA for embryo viability: Luca Gianaroli (Italy)

12:30-13:00 What about the maternal endometrium. The search for the personalized WOI and Microbiota: Carlos Simón (Spain)

13:00-14:00 LUNCH BREAK - Exhibition Area
FREE POSTER SESSION - Multiusos 1 room (1st Floor)

14:00-15:30 **SESSION 7: FREE COMMUNICATIONS (RELATED TO PGD-A, MOSAICISM, BIOPSY)**
Chairmen: Edith Coonen (Netherlands) and Carles Giménez (Spain)

14:00-14:15 OC-11. ANEUPLOIDY RATES ARE ASSOCIATED WITH THE BLASTOCYST BIOPSY TECHNIQUE. Whitney, John B

14:15-14:30 OC-12. MITOCHONDRIAL DNA COPY NUMBER MEASURED BY MITOSCORE IS ASSOCIATED TO TROPHECTODERM QUALITY. De Los Santos, Maria José

14:30-14:45 OC-13. THE INCIDENCE AND ORIGIN OF SEGMENTAL CHROMOSOME ABNORMALITIES IN HUMAN IVF EMBRYOS DETECTED DURING PGD AND PGS. Hornak, Miroslav

14:45-15:00 OC-14. CLINICAL SIGNIFICANCE OF UNDIAGNOSED MOSAICISM IN IVF EMBRYOS. Perry, Emma

15:00-15:15 OC-15. MITOSCORE VALUES ARE NOT AFFECTED BY ATMOSPHERIC OXYGEN CONCENTRATION DURING EMBRYO CULTURE. Mifsud, Amparo

15:15-15:30 OC-16. HOW TO CHOOSE PRENATAL TESTING OPTIONS FOR PREGNANT WOMEN AFTER PREIMPLANTATION GENETIC SCREENING: GENETIC COUNSELING CHALLENGES. Tamura, Chieko

15:30-16:00 COFFEE BREAK

16:00-17:30 SESSION 8: BORDER-LINE INDICATIONS FOR PGD

Chairmen: Martine De Rycke (Belgium) and Alan Handyside (UK)

16:00-16:30 PGD for HLA (ESHRE data collection): Georgia Kakourou (Greece)

16:30-17:00 Experience in PGD for Cancer: Svetlana Rechistky (USA)

17.00-17:30 Current limitations of Karyomapping: Alan Handyside (UK)

Day 3 - Wednesday 29th March - Auditorio 2 (Ground Floor)

08:00-08:15 **Summary of highlights previous day and Introduction to second day:** Alan Handyside (UK)

08:15-09.00 **PLENARY SESSION**
Gene editing for nuclear and mithocondrial DNA mutations:
Juan Carlos Izpisua (USA)

09:00-10:30 **SESSION 9: FREE COMMUNICATIONS (RELATED TO NEW TECHNOLOGIES, NON INVASIVE, ETC)**
Chairmen: Santiago Munné (USA) and David Blesa (Spain)

09:00-09:15 OC-17. DETECTION OF SEGMENTAL ANEUPLOIDY AND MOSAICISM IN PREIMPLANTATION EMBRYO MODEL BY NEXT GENERATION SEQUENCING METHODOLOGIES. Biricik, Anil

09:15-09:30 OC-18. EVIDENCE TO SUGGEST A UNIQUE 3D ORGANIZATION OF CHROMOSOMES WITHIN THE SPERM NUCLEUS: IMPLICATIONS FOR FERTILIZATION AND EARLY EMBRYONIC DEVELOPMENT. Tempest, Helen

09:30-09:45 OC-19. A NOVEL ALGORITHM FOR DETERMINING THE LEVEL OF MOSAICISM IN PREIMPLANTATION GENETIC SCREENING (PGS) WITH NEXT-GENERATION SEQUENCING (NGS). Castejon Fernandez, Natalia

09:45-10:00 OC-20. COMBINED PGD AND PGS BY NGS ON THE SAME BIOPSY USING A SINGLE INDEX. Jasper, Melinda

10:00-10:15 OC-21. MATERNAL AGE HAS NO INFLUENCE ON MITOCHONDRIAL DNA (MTDNA) CONTENT IN CHROMOSOMALLY NORMAL EMBRYOS. Ogur, Cagri

10:15-10:30 OC-22. DETECTION LIMIT OF PARTIAL INSERTIONS AND DELETIONS FOR PGS IN TERMS OF NGS BY ANALYZING 242 EMBRYOS OF COUPLES WITH BALANCED TRANSLOCATIONS. Blanca, Helena

10:30-11:00 COFFEE BREAK

11:00-13:00 **SESSION 10: THE NEAR FUTURE**

Chairmen: Samir Hammamah (France) and Antonio Díez-Juan (Spain)

11:00-11:30 Cumulus analysis to predict pregnancy potential: Johan Smitz (Belgium)

11:30-12:00 Ovarian rejuvenation. Is that for real?: Antonio Pellicer (Italy) and Sonia Herráiz (Spain)

12:00-12:30 Epigenetics in the male factor: Michael Skinner (USA)

12:30-13:00 Concurrent preimplantation genetic diagnosis for single gene disorders and comprehensive chromosomal aneuploidy testing without whole genome amplification: Rebekah Zimmerman (USA)

13:00-14:00 LUNCH BREAK - Exhibition Area
FREE POSTER SESSION - Multiusos 1 room (1st Floor)

14:00-15:30 **SESSION 11: REMOVING BARRIERS TO ADOPT GENETIC TEST IN REPRODUCTIVE MEDICINE**

Chairmen: Joe Leigh Simpson (USA) and Alan Thornhill (UK)

14:00-14:30 Carrier Screening. Disease prevention vs cost effectiveness: Jason Franasiak (USA)

14:30-15:00 Obstacles to implement PGD-A in ART: David Jiménez (Spain)

15:00-15:30 How are we perceived by our clinical fellows? Critical review of SART and ESHRE data: Kangpu Xu (USA)

15:30-16:30 **SESSION 12: LATE-BREAKING SESSION. RCTS/ NON- INVASIVE PGD**

Chairmen: Carmen Rubio (Spain) and Antonio Capalbo (Italy)

Non invasive analysis of the embryonic genome: a critical review of non invasive approaches for PGD and PGS
Antonio Capalbo (Italy)
PGD-A in advanced maternal age: final results of a day-3 biopsy RCT
Carmen Rubio (Spain)

Jason Franasiak (USA)

16:30-17:00 **CLOSING REMARKS AND PRESENTATION OF NEXT MEETING**

Congress Venue

Palacio de Congresos de Valencia
Avda. Cortes Valencianas, 60
46015 Valencia

Technical Secretariat

GRUPOPACIFICO
The power of meeting

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Other useful information

CONGRESS SECRETARIAT TIMETABLE

Sunday March 26th	9.00-19.00
Monday March 27th	7.30-17.00
Tuesday March 28th	8.00-17.00
Wednesday March 29th	8.00-17.00

EXHIBITION AREA TIMETABLE

Monday March 27th	7.30-17.00
Tuesday March 28th	8.00-17.00
Wednesday March 29th	8.00-17.00

Language

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

EACCME Accreditation

UNION EUROPÉENNE DES MÉDECINS SPÉCIALISTES

The 16th International Conference on Preimplantation Genetic Diagnosis, organized by the Preimplantation Genetic Diagnosis International Society (PGDIS).

The European Accreditation Council for Continuing Medical Education (EACCME).

Event code: 15309

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 Monday March 27th 2016 between 9.00 to 10.30 (fixing support will be supplied by the Techinical Secretariat) and they will remain in the Posters' area until Wednesday 29th 2016, when they will have to be removed by authors no later than 14.30.
- 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

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.

List of accepted Posters – Displayed on Multiusos 1 Room (1st Floor)

P-01. **NEXT GENERATION SEQUENCING (NGS) METHODOLOGY RELIABLE DETECTS SEGMENTAL ANEUPLOIDIES WITH MOSAIC PATTERNS.**
Fiorentino, Francesco; Anil, Biricik; Cotroneo, Ettore; Bono, Sara; Surdo, Matteo; Spinella, Francesca.

P-02. **NEXT GENERATION SEQUENCING TO DETECT LOW GRADE MOSAICISM AND ITS EFFECT ON THE LIVE BIRTH RATE.**
Morales, Ruth ; Lledo, Belen; Ortiz, Jose A. ; Eva, García-Hernandez; Ten, Jorge; Llacer, Joaquin; Bernabeu, Rafael.

P-03. **DEVELOPMENT OF A 5 HOUR PGS PROTOCOL FOR A DAY 5 FRESH TRANSFER.**
Proptopsaltis, Sandra; Jasper, Melinda.

P-04. **VALIDATION OF EMBRYOCELLECT™ WITH SUREPLEX AMPLIFIED EMBRYO BIOPSIES.**
Robinson, Christine; Modamio, Silvia; Fernández, Silvia; Daina, Gemma; Garcia, Lydia; Jasper, Melinda.

P-05. **DOES THE CHANGE OF TECHNOLOGY FROM ACGH TO NGS IMPACT ANEUPLOIDY RATES?**
Coprerski, Bruno; Souza , Mariane; Rubio, Carmen; Simón, Carlos; Riboldi, Marcia.

P-06. **MATERNAL AGE TRIGGERS THE FORMATION OF CHROMOSOMAL LOSSES MORE THAN GAINS AND/OR SEGMENTAL ANEUPLOIDIES IN PREIMPLANTATION EMBRYOS.**
Ogur, Cagri; Gultomruk, Meral; Caferler, Julide; Capar, Betul; Findikli, Necati; Bahceci, Mustafa .

P-08. **CATTLE KARYOMAPPING TO OPTIMISE FOOD PRODUCTION AND DELIVERY OF SUPERIOR GENETICS: THE FIRST LIVEBORN CALVES.**
Turner, Kara; Silvestri, Giuseppe; Smith , Charlotte; Dobson, Gemma; Black, David; Handyside, Alan; Sinclair, Kevin; Griffin, Darren.

P-09. **PREIMPLANTATION GENETIC DIAGNOSIS FOR TRANSLOCATIONS AND INTERCHROMOSOMAL EFFECT ASSESSED BY ARRAY CGH.**
Mateu, Emilia; Rodrigo, Lorena; Peinado, Vanesa; Milán, Miguel; Campos, Inmaculada; García-Herrero, Sandra; Mercader, Ampar; Bronet, Fernando; Larreategui, Zaloa; Florensa, Mireia; Remohí, José; Pellicer, Antonio; Simón, Carlos; Rubio, Carmen.

P-10. **INTRODUCTION OF A NOVEL, UNIVERSAL NGS-BASED RESEARCH METHOD FOR PREIMPLANTATION GENETIC DIAGNOSIS AND SCREENING.**
Devogelaere, Benoit.

P-11. **VALIDATION OF TWO WHOLE GENOME AMPLIFICATION METHODS FOR PGD ON MONOGENETIC DISEASES AND ANEUPLOIDY SCREENING.**
Chow, Judy F C; Yeung, William S B ; Lee, Vivian C Y ; Lau, Estella Y L; Ng, Ernest H Y.

P-12. **INCIDENCE OF CHROMOSOMAL ANEUPLOIDIES AT EMBRYONIC LEVEL WITH COMPARISON BASED ON TYPE OF BIOPSY AND MATERNAL AGE: FIRST INDIAN EXPERIENCE.**
Khajuria, Rajni; Rodrigo, Lorena; Valbuena, Diana; Rubio, Carmen; Simón, Carlos.

P-13. **NGS-ANALYSIS OF CHORIONIC VILLI OF MISCARRIAGES AND CONFORMING TROPHECTODERM CELLS OF TRANSFERRED BLASTOCYSTS.**
Mykytenko, Dmytro; Pylyp, Larysa; Lavrova, Kate; Spinenko, Lyudmyla; Lamtyeva, Vera; Zukin, Valery.

P-14. **RE-ANALYSIS OF ANEUPLOID EMBRYOS AFTER TE-BIOPSY AND ACGH BY NGS ON INNER CELL MASS BIOPSY.**
Hruba, Martina; Vlckova, Romana; Pittrova, Monika; Hradecky, Libor; Zemanova, Jitka; Novakova, Pavla; Wirleitner, Barbara; Schuff, Maximilian; Stecher, Astrid; Kralickova, Milena; Murtinger, Maximilian.

P-16. **TRANSFER OF ANEUPLOID EMBRYOS FOLLOWING PREIMPLANTATION GENETIC DIAGNOSIS: THE ADDED VALUE OF A HAPLOTYPING-BASED GENOME-WIDE APPROACH.**
Melotte, Cindy; Dimitriadou, Eftychia; Debrock, Sophie; Devriendt, Koen; De Ravel, Thomy; Legius, Eric; Peeraer, Karen; Vermeesch, Joris Robert.

P-17. **NGS ANEUPLOIDY SCREENING IN EMBRYO CELLS AND MISCARRIAGE MATERIAL.**
Glinkina, Zhanna; Kurtser, Mark; Visotsky, Aleksandr; Trotsenko, Ivan.

P-18. **WHAT NEXT GENERATION SEQUENCING BRINGS TO PREIMPLANTATION GENETIC TESTING.**
Unsal, Evrim; Aktuna, Suleyman; Ozer, Leyla; Aydin, Merve; Duman, Turker; Pelin, Celikkol; Baltaci, Aysun; Baltaci, Volkan.

P-19. **IMPLEMENTATION OF THE NEXT GENERATION SEQUENCING BASED PREIMPLANTATION GENETIC SCREENING IN CLINICAL PRACTICE: CHALLENGES AND BENEFITS.**
Marinova, Elena; Rizov, Momchil; Popova, Maya; Tsaneva, Dimitrinka; Antonova, Iskra; Nikolov, Georgui; Penchev, Valentin.

P-20. **A SINGLE BLASTOCYST BIOPSY RESULTING IN A MOSAIC AND EUPLOID PROFILE: A CASE REPORT.**
Jones, Amy; *Whitney, John; Zou, Ping; Weitzman, Glenn; Freeman, Melanie; Schiewe, Mitchel.*

P-21. **MITOCHONDRIAL GENOME COVERAGE FOR COPY NUMBER DETERMINATION AND DETECTION OF DISEASE; THE IMPACT OF WGA.**
Fraser, Michelle; Warren, Kimberly; Robinson, Christine; Jasper, Melinda.

P-22. **INVESTIGATION OF THE RELATIONSHIP BETWEEN EMBRYO PLOIDY, NUCLEAR MITOCHONDRIAL MISMATCH AND EMBRYO MORPHOLOGY.**
Jawdat, Razan Saeed; Lane, Nick; Sengupta, Sioban.

P-23. **THE STRUCTURE AND LOCATION GRADATION OF OOCYTE MEIOTIC SPINDLE AND ITS RELATIONSHIP TO EMBRYOS' QUALITY AND EUPLOIDY.**
Gontar, Julia; Ilyin, Igor; Buderatskaya, Natalia; Fedota, Olena; Parnitskaya, Olga; Ilyina, Kateryna; Kazachkova, Nadiya; Kapustin, Eduard; Lavrynenko, Sergey; Lakhno, Yana.

P-24. **ANEUPLOIDY STATUS IS ASSOCIATED WITH THE LENGTH OF TIME THAT THE PRONUCLEI ARE VISIBLE.**
Gould, Rebecca L; Griffin, Darren K

P-25. **EVALUATION OF REPRODUCTIVE LABORATORIAL PARAMETERS OF PATIENTS WITH X CHROMOSOME MOSAIC KARYOTYPE.**
Christofolini, Denise; Bianco, Bianca; Gastaldo, Guilherme; Santos, Monise; Dornas, Mayla; Parente Barbosa, Caio.

P-26. **MITOCHONDRIAL ASSESSMENT IN DAY 3 BIOPSY, A PROGNOSTIC FACTOR FOR POSITIVE PREGNANCY.**
Eibes Peteiro, Paula; Fakih, Amanda; Thulaseedas, Deepthydas; Nawaz, Mohammed; Chaturvedi, Ritika ; Shyju, Soumi; Hellani, Ali.

P-27. **THE LIKELIHOOD OF TRANSFERRING A EUPLOID EMBRYO AFTER PGD-ANEUPLOIDY CYCLES DEPENDS NOT ONLY ON FEMALE AGE BUT ALSO ON THE NUMBER OF OOCYTES COLLECTED.**
Cetinkaya, Murat; Tufekci, Mehmet Ali; Cinar Yapan, Cigdem; Pirkevi Cetinkaya, Caroline Selma; Kahraman, Semra.

P-28. **VALIDATING PGS BY PROBING THE KARYOTYPIC CONCORDANCE BETWEEN ICM AND TE.**
Viotti, Manuel; Victor, Andrea; Brake, Alan; Tyndall, Jack; Murphy, Alex; Lepkowsky, Laura; Lal, Archana; Griffin, Darren; Zouves, Christo; Barnes, Frank.

P-29. **PATIENTS UNDERGOING PREIMPLANTATION GENETIC SCREENING MAY BENEFIT FROM ARTIFICIAL OOCYTE ACTIVATION.**
Nagorny, Viktor; Veselovsky, Viktor; Mykytenko, Dmytro; Zukin, Valery.

P-30. **EXCELLENT ONGOING PREGNANCY RATE WITH COMBINED USE OF PCR FOR MONOGENIC DISEASE AND NGS FOR ANEUPLOIDY SCREENING.**
Lee, Vivian Chi Yan; Chow, Judy Fung Cheung; Yeung, William Shu Biu; Ng, Ernest Hung Yu.

P-31. **THE EFFECTS OF LASER MANIPULATION ON BIOPSY KARYOTYPE IN PGS.**
Victor, Andrea; Viotti, Manuel; Barnes, Frank; Brake, Alan; Zouves, Christo; Griffin, Darren.

P-32. **SINGLE EMBRYO TRANSFER AS AN IMPERATIVE CHOICE FOR PATIENTS OVER 38 YEARS OLD IN AUTOLOGOUS IVF PGS CYCLES.**
Barash, Oleksii; Willman, Susan; Ivani, Kristen; Wachs, Deborah; Rabara, Fleurdeliza; Weckstein, Louis.

P-33. **EUPLOIDY RATE OF DAY 7 BLASTOCYSTS DERIVED FROM IN VITRO FERTILISATION (IVF).**
Yap, Wei Yuan; Lee, Colin Soon Soo; Lim, Yun Xin; Siew, Shuk Chen.

P-34. **THE USE OF LOW OXYGEN CONCENTRATION DURING EMBRYO CULTURE SEEMS NOT TO AFFECT BLASTOCYST ANEUPLOIDY RATE.**
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ABSTRACTS

Session 2: Intrinsic factors contributing to embryo aneuploidy – 11:00 – 13:00h

COMMON VARIANTS ASSOCIATED WITH MITOTIC-ORIGIN OF ANEUPLOIDY IN HUMAN EMBRYOS

Mccoy, Rajiv [(1)]; Demko, Zachary [(2)]; Ryan, Allison [(2)]; Banjevic, Milena [(2)]; Hill, Matthew [(2)]; Sigurjonsson, Styrmir [(2)]; Rabinowitz, Matthew [(2)]; Fraser, Hunter [(3)]; Petrov, Dmitri [(3)].
[(1)] University of Washington, Seattle, United States; [(2)] Natera, Inc., San Carlos, United States; [(3)] Stanford University, Stanford, United States.

Introduction: While meiotic-origin aneuploidies are known to increase in frequency with maternal age, substantial unexplained variation in aneuploidy rates exists among IVF embryos obtained from women of the same age. We sought to investigate potential maternal or paternal genetic factors influencing rates of embryonic aneuploidy.

Material & methods: We collaborated with Natera to aggregate and analyze preimplantation genetic screening (PGS) data from 20,798 single blastomeres biopsied from day-3 cleavage-stage embryos from a total of 2362 unrelated mothers. Embryo biopsies, along with DNA from both parents, were previously genotyped using SNP microarray and aneuploidies inferred using the Parental Support algorithm. This approach facilitated the identification of meiotic-origin trisomies, in turn allowing us to distinguish putative meiotic and mitotic errors to narrowly define aneuploid phenotypes for subsequent association testing. We then conducted a genome-wide association study of meiotic- and mitotic-origin aneuploidy, testing for associations between individual parental genetic variants and proportion of aneuploid embryos. Results of this study were published in McCoy et al. (2015).

Results: No genome-wide significant ($p < 5 \times 10^{-8}$) associations were identified between rates of meiotic-origin aneuploidy and maternal or paternal genotypes. A strong association was identified between common variants on chromosome 4, regions q28.1 to q28.2, and aneuploidy of putative mitotic origin (logistic GLM, $\beta = 0.218$, SE = 0.0270, $p = 8.68 \times 10^{-16}$). The associated haplotype (defined by genotype imputation) spans approximately 400 Kb and is tagged by genotyped SNP rs2305957. The haplotype encompasses eight genes, including the gene Polo-like kinase 4 (*PLK4*), the master regulator of centriole duplication. *PLK4* represents a strong causal candidate, as mutation and dysregulation of *PLK4* has previously been associated with centrosome amplification, mitotic spindle abnormalities, and chromosome segregation defects in adult somatic cells. Initial follow-up analyses (unpublished results) further support a causal role of *PLK4* and implicate mitotic spindle aberrations as the mechanism driving the association. Additional data from 15,388 trophectoderm biopsies indicate that the associated mitotic aberrations impair survival to the blastocyst stage, as patients with high-risk genotypes had significantly fewer blastocysts available for testing at day 5 (Poisson GLM, $p = 0.00247$). This result was recently replicated and extended in an independent study (Zhang et al., 2017). Despite this apparent negative impact on embryonic survival, the associated variant segregates at intermediate frequencies (~30% MAF) across diverse human populations, suggesting that its deleterious effect may be balanced by a yet-uncharacterized positive effect on fitness.

Conclusions: We identified a common genetic variant that strongly influences aneuploidy rates through a maternal effect on the fidelity of early embryonic mitosis. Data indicate that this effect may be caused by abnormalities in centrosome duplication, mediated *PLK4*, in turn contributing to variation in preimplantation embryonic survival.

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ANEUPLOIDY AS BOTH A CAUSE AND A CURE FOR CANCER

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Cancer is the second leading cause of death worldwide. The frequency of most cancers increases with age, because they result from the accumulation of genetic mutations, most of which result from errors in DNA replication as stem cells produce and maintain a particular tissue [1]. Cancer driver mutations are essential for carcinogenesis, but the rise of aneuploidy (chromosome instability) contributes to tumor heterogeneity, drug resistance, and treatment failure. Aneuploidy is promoted by polyploidy and results from missegregation of chromatids during mitosis. Cells with a single polyploid nucleus are rare in mammals, but aberrant genome duplication in the form of unscheduled endoreplication can give rise to aneuploidy and cancer. We screened ~95% of the human genome for genes that are essential to restrict genome duplication to once per cell division [2]. Since at least 14 of the 42 genes identified in this screen prevent aneuploidy and cancer in mice, there exists a clear link between aberrant genome duplication and the development of aneuploidy and cancer.

Remarkably, aberrant genome duplication might also provide a cure for germ cell cancers. We discovered that induction of aneuploidy could selectively kill pluripotent cancer stem cells. Geminin is a protein unique to the metazoa that prevents unscheduled origin licensing and affects cell fate acquisition. Geminin is a metazoan protein that is essential to prevent DNA re-replication dependent apoptosis in cells derived from various human cancers, but not in cells derived from their parent tissue [3] - with one remarkable exception. Totipotent and pluripotent mouse cells are exquisitely sensitive to Geminin depletion, an event that triggers DNA re-replication, double strand DNA breaks, a DNA damage response, and apoptosis [4]. However, after these cells differentiate, they can proliferate in the absence of geminin [5]. Since pluripotent cells are responsible for germ cell tumors in embryos or adults, there exists a link between Geminin activity and the genesis of benign as well as malignant tumors. Geminin might well be the Achilles' heel of germ cell cancers, as well as any cancer that depends on pluripotent cancer progenitor cells.

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Session 4: Extrinsic factors contributing to embryo aneuploidy – 16:00 – 17:00h

EMBRYO CULTURE CONDITIONS AND MORE

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It is very well accepted that embryo culture conditions may have undesirable effects upon the embryo's molecular and cellular physiology, adding stress factors to the culture and hence decreasing embryo viability
One of the possible effect of suboptimal culture conditions can be translated into the induction of a higher incidence of chromosomal imbalance in human embryos that are generated in vitro.

Whereas embryo meiotic errors are mainly associated to patient related factors such as women age and in less extend male age, mitotic errors or postzygotic errors will occur mainly in the laboratory, during oocyte and embryo handling.
Therefore stress factors such as changes on temperature, pH, osmotic pressure, culture media, the use of unappropriated oxygen concentration during embryo culture, the cryopreservation policy or even IVF laboratory contaminants might facilitate the suggested root causes of mitotic errors such as are anaphase lag, endoreplication and mitotic non disjunction.

Some recent publications point out the possible implication of different laboratory settings into the euploidy rates of the analyzed embryos. However taking into consideration the high number of possible parameters that can influence on embryo quality is very difficult identify and weight at what extend each of the possible variables affect on the aneuploidy rate of the embryos.
Literature search place in evidence the lack of studies demonstrating the effect of such in vitro culture conditions on the chromosomal constitution of human embryos so basic studies are need to address this topic in the future.

Session 5: Challenging PGD-A diagnosis. Mosaicism - March 28th 08:45 – 10:30h.

IT´S A MOSAIC RESULT- WHAT DO I SAY NOW?

Leigh, Don.
Sidney; Australia

With the advent of preimplantation 24 chromosome aneuploidy screening and the switch to blastocyst stage biopsy, implantation rates have reached an all time high and miscarriage rates have dropped to a low- even for the older women in the IVF process. However, not all embryos implant even after screening and there are still miscarriages amongst this group (albeit at a relatively low rate). With the switch to sequencing based aneuploidy detection and the typical linear profile plotting available with this methodology, a new category of aneuploid status has appeared- the mosaic. With a typical trophectoderm biopsy consisting of 5-10 cells, differences in cell chromosome status amongst the cell cohort can be seen as a deviation somewhere between the zero base line (representing 2 copies for the autosomes) and -1 (monosomy) and +1 (trisomy) This has been reported to be up to 30% of analysed embryos but typically is around the 10% level for most groups. So what is "normal" and appropriate for transfer and what is considered unsuitable for transfer? What will the laboratory say to the clinician? What will the clinician discuss with the patient? In some clinics, what will the laboratory say to the patient during any pretest briefing? The International PGD Society has formulated guidelines but these are general in nature and in many ways a prelude to a more informed approach as clinics report more and more on outcomes associated with the transfer of these "somewhere in between" embryos.

Discussion will revolve around current findings and how these relate to information that may be appropriate to patient consent and ultimately their understanding.

MOSAICISM AND SEGMENTALS IN POCS AND PRENATAL DIAGNOSIS

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Berry Genomics; Beijing, China

Introduction

A wide spectrum of chromosome abnormalities are commonly observed in pre-implantation embryos, including trisomies, monosomies, segmental imbalances, mosaics and complex aneuploidies. The vast majority of these chromosome abnormalities, if undetected, will cause implantation failure or miscarriage in the early first trimester. Only a very small proportion will persist during pregnancy and manifest as chromosome disease syndromes in the newborn. Validated methods for reliable and accurate detection of segmental aneuploidies and mosaics represents the next stage in comprehensive 24-chromosome embryo testing. The purpose of the study was to validate a higher resolution NGS platform for comprehensive 24-chromosome analysis of embryos and compare the nature and frequency of chromosome abnormalities in blastocysts and products of conception (POCs) obtained from miscarriage samples.

Materials and Methods

High resolution copy number variation sequencing (CNV-Seq) performed on the Illumina NextSeq500 platform was used to detect chromosome abnormalities. In brief, 3M sequencing reads were precisely and uniquely mapped and then allocated to 20 kb bins along the length of each chromosome. Copy number changes were detected by comparison of sequencing read numbers in sequential bins of the test samples. For validation studies, we used a number of different cell line models and embryos with known segmental imbalances and levels of mosaicism. Clinical samples comprising 1454 blastocysts from PGD-A cycles and 1810 miscarriage POCs samples were retrospectively analyzed and compared for chromosomal aneuploidies.

Results

In validation studies of cell lines carrying known pathogenic microdeletions, CNV-Seq reproducibly detected DiGeorge, Sotos, Jacobsen and Wolf-Hirshhorn syndromes, and in cell models of mosaicism, detected Chr21 and ChrX mosaicism at levels of 50% and 20%. In embryos, CNV-Seq detected segmental imbalances between 1-4 Mb in size at the expected termini positions of

translocation chromosomes. CNV-Seq analysis of 1454 blastocysts in PGD-A cycles identified 711 euploid (49%) and 743 aneuploid (51%) embryos. The embryonic aneuploidies detected included trisomies/monosomies (26%), segmental imbalances (44%), mosaics (9%) and complex aneuploidies (21%) comprising trisomies/monosomies with additional segmentals or mosaics. By comparison, CNV-Seq analysis of 1810 miscarriage samples identified 881 (47.5%) euploid and 929 (42.5%) aneuploid fetuses. The fetal aneuploidies detected included trisomies (47.5%), 45,XO (9.9%), segmental imbalances (8.5%), mosaics (13%), polyploidy (8.9%) and complex aneuploidies (12.2%). Comparison of the two data sets showed that the vast majority of embryo segmental imbalances were non-pathogenic whereas the vast majority of segmental imbalances in fetal POCs were associated with genes important for normal fetal development.

Conclusions

Segmental imbalances and mosaicism are significant embryonic chromosomal abnormalities, representing around 50% of all aneuploidies. By extrapolating from the type and frequency of the different chromosomal aneuploidies detected in embryos and in miscarriage POCs, our findings suggest that a significant proportion of embryos with segmental aneuploidies or high level mosaicism succumb in the early first trimester of fetal development. On this basis, PGD-A testing using a higher resolution methodology such as CNV-Seq may provide an incremental improvement in reproductive outcomes by reducing the incidence of unexplained miscarriages following the transfer of embryos identified as “euploid” by lower resolution methods.

Session 6: How to improve clinical outcome in PGD-A – 11:00-13:00

LABORATORY CONDITIONS/ BIOPSY STRATEGIES:

Rienzi, Laura.
Clinica Valle Gulia. Roma. Italv

The implementation of preimplantation genetic testing (PGT) requires a global improvement of the clinical and laboratory practices. Currently, blastocyst stage biopsy is the safest, accurate and reliable strategy to conduct PGT for aneuploidies and, if required, for monogenic diseases. Indeed, Scott and colleagues (Scott et al., 2013) showed in a randomized non-selection trial on sibling embryos that blastomere biopsy negatively impacts the reproductive potential, while blastocyst biopsy does not. Polar body biopsy instead is still nowadays to be considered the least cost-effective strategy.

To conduct blastocyst stage biopsy though, extended embryo culture and cryopreservation need to be effectively performed. Extensive investigations have shown that different culture conditions, which may impact embryo developmental potential, such as temperature, pH and low O2 concentrations, have to be managed properly. Moreover, vitrifications instead of slow freezing need to be adopted to ensure a efficient treatment (Rienzi et al., 2017). Embryo culture in time-lapse systems may improve culture conditions, since it is an undisturbed environment, but it does not provide predictive criteria upon embryo chromosomal status.

Three different blastocyst stage strategies have been published up to date: with zona opening at the cleavage stage, with zona opening in day 5/6 whenever the embryo reaches the fully-expanded blastocyst stage, or with simultaneous zona opening and trophectoderm cells retrieval. No paper has been published up to date, which compares them, but the latter two methods may involve a lower stress for the embryo and prevent the risk of Inner Cell Mass hatching. Moreover, the method involving simultaneous zona opening and trophectoderm cells retrieval has been reported as reproducible and standardizable among different biopsy practitioners in a multicenter study (Capalbo et al., 2016).

The future perspectives of non- (culture media analysis) or minimally-invasive (blastocentesis) approaches to perform comprehensive chromosomal testing still require scrupulous and rigorous pre-clinical and clinical validations to show their real value. At present, they cannot be considered reliable, since discordant and controversial reports have been published up to date. However, other -omics investigations of blastocoele fluid and/or spent culture media may provide novel criteria in the future for embryo evaluation.

WHEN TO TRANSFER? FRESH VS. DEFERRED TRANSFER:

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Istanbul Memorial Hospital, Istanbul, Turkey

Despite dramatic improvements in PGD, there has been only one randomized controlled study regarding the optimal embryo transfer strategy for euploid embryos; fresh day-5 biopsy and fresh transfer on day-6 or cryopreservation of all embryos including day-5 and day-6 in preparation for a frozen ET ?
The fresh blastocyst transfer approach necessitates not only the availability of expanded blastocysts on the morning of day 5, but also of at least one these being euploid, thereby reducing the chance of transfer.
In the freeze all approach, both day-5 and day-6 embryos can be biopsied and cryopreservation of the whole cohort enables a higher proportion of patients to reach their ET goal.

In order to investigate the optimal ET strategy in PGD-A cases, a total of 1486 cycles performed between 2011-2016, fresh (n=690) versus feeze all (n=790) were evaluated at Istanbul Memorial Hospital, ART&Genetics Center. For both fresh and frozen embryos advanced maternal age was the main indication followed by translocations and repeated pregnancy losses. All positive outcomes, implantation, ongoing pregnancy and live birth rates were found to be significantly higher in the freeze all group. Similarly, in cases with advanced maternal age (38-43 years), ongoing pregnancy and live birth rates were significantly higher in the freeze all group.

When fresh and freeze all cycles in different age groups were compared, in the youngest age group (<35 years), there was no significant difference in the ongoing pregnancy rate, but in older age groups (35-37y, 38-40y, 41-42y, 43-44y), as maternal age increased so did the relative ongoing pregnancy rate in the freeze all group. This is probably related to endometrial receptivity, as it is possible that in older patients the rested endometrium is more crucial.

Within the freeze all groups, the cumulative implantation and live birth rates increased for patients with more than one euploid embryo (n=406) compared to those with only one euploid embryo .

The viability rate of vitrified-warmed blastocysts based on a total of 564 thawed embryos, (247 top quality and 317 good quality), was as high as 96.7%. It confirms that vitrification as a cryopreservation technique, is a reliable , efficient and a safe one.

In conclusion the freeze all protocol provides more time for comprehensive diagnostic tests which often need more than one day to provide results.

Furthermore, the freeze all strategy decreases stress in the embryology laboratory because it allows more flexibility regarding the timing of embryo biopsy. Having the biopsy results of the whole cohort available within the same period allows for the inclusion of all blastocysts.
Importantly, it allows euploid embryos to be transferred in a non-stimulated cycle.

Finally, this strategy reduces the risk of multiple pregnancy and life-threatening OHSS to almost zero.

To come back to the original question which is better, fresh or deferred embryo transfer? Our results at Istanbul Memorial Hospital showed that, in conjunction with new sophisticated genetic tools, deferred transfer produces better clinical outcomes.

REVIEW OF THE CLINICAL EVIDENCE OF MTDNA FOR EMBRYO VIABILITY

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Since the beginning of IVF, embryologists have directed major efforts to identify key pathways of normal embryo development and factors that contribute to embryo viability. Morphological evaluation remains the primary approach of embryo assessment despite its limitations, due not only to the subjectivity of the embryologist, but also because of the evaluation system itself, which does not provide any information whatsoever about embryo ploidy and metabolic activity.

Recent advances in genetic screening technologies greatly improved our capacity to investigate the embryonic DNA, thus allowing the identification and transfer of euploid embryos. This approach represented a great achievement, since aneuploidy is the major cause of failed implantation and spontaneous abortion. For this reason, the testing of preimplantation embryos focused primarily on nuclear DNA to detect numerical abnormalities and deselect the corresponding embryos. More recently, attention was diverted to the investigation of mitochondrial DNA (mtDNA) as a potential marker of embryo viability and implantation potential. This approach stemmed from the consideration that mitochondria are the highly specialized organelles principally charged with the production of cellular energy.

It is well known that the number of mitochondria differs from cell to cell. In gametes and embryos, this figure is extremely variable during the different stages of development. It has been demonstrated that defects in their distribution and function can negatively affect the embryo ploidy condition and viability (Carrol, 2012; Jessberger, 2012).

In the attempt to establish a possible correlation between mtDNA copy number and clinical outcome, two studies have tried to relate the embryonic mtDNA content to maternal age, chromosomal complement, implantation potential and blastocyst development. In both cases, the mtDNA content was calculated as a ratio to nuclear DNA. Despite some differences mainly due to the diverse size and cell type of the analyzed samples, both groups demonstrated that the quantity of mtDNA represents a marker for embryo viability. This led to define a threshold, above which the probability of embryo implantation is extremely low (Diez-Juan et al., 2015; Fragouli et al., 2015).

A criticism to these studies was made by another group based on the argument that when calculating the quantity of mtDNA in relation to nuclear DNA, the denominator of this ratio needs to be adjusted for each embryo according to the effective content of nuclear DNA. Variations in the nuclear genome are actually brought by sex (the Y chromosomes has a lower DNA content compared to the X chromosome) and by numerical aneuploidy (a monosomic embryo has a lower DNA content compared to either a disomic or a trisomic embryo). Therefore, after adding a corresponding correction factor to avoid extra/over estimation of mtDNA, no correlation was found between the mtDNA content and maternal age, chromosomal status, and implantation rate (Victor et al., 2015). More research is needed in this direction to obtain robust data that can corroborate the presented results. Meanwhile, an approach other than the quantitative methodology can be investigated. During oogenesis, a great amount of energy is requested to accomplish all the involved steps, including meiosis. It is well known that female oogenesis is a very peculiar process, during which chromosome segregation is particularly prone to errors in a modality that is strictly related to age. A correlation has been established between aneuploidy and defective energy supply, leading to conclude that mitochondria in the mature oocyte (they are maternally inherited) are crucial for the following steps of fertilization and early embryonic development. To better understand the process of oogenesis, we studied the segregation of mitochondria at the first and second meiotic division. Therefore, we sequenced mtDNA in a series of triads composed by oocyte and corresponding polar bodies, and compared the results with the blood.

After sequencing the mitochondrial non-coding region (D-Loop) of 17 oocytes, full correspondence was found with the respective blood samples. However, the concordance with polar bodies dropped to 89.6%, suggesting that oocytes could have an active mechanism to preserve a condition of normality, similar to what has been observed for chromosome segregation during meiosis (compensated aneuploidy). Accordingly, some haplogroups were shown to be more prone than others to generate aneuploidy, with a significantly higher incidence of chromosomal errors in some of them (Gianaroli et al., 2015). The same trend was observed when sequencing the coding region (Gianaroli et al., 2014). When comparing the two polar bodies, there were no differences in the quantity of mutations between them, but a higher pathogenic score was detected in the first polar body. Also in this case, the presence of a selection process operating during meiosis was postulated. As the mitochondrial condition of the second polar body is closer to that of the oocyte, the by-product of the second meiotic division could be a good predictor of the genetic status of the oocyte (De Fanti et al., 2017). In conclusion, the study of mitochondria in oocytes and embryos could represent an important tool in the definition of the most efficient approach towards the selection of viable concepti. More information is needed, but the preliminary data obtained so far point to the relevance of energy supply in determining the fate of the embryo.

WHAT ABOUT THE MATERNAL ENDOMETRIUM. THE SEARCH FOR THE PERSONALIZED WOI AND MICROBIOTA

Simon, Carlos.
Igenomix. Valencia University-INCLIVA-IVI. Stanford University. Baylor College of Medicine. Paterna - Valencia, Spain

The endometrium is considered receptive during an individually defined period, the window of implantation (WOI), when the mother permits a blastocyst to attach and implant. For a successful pregnancy, the synchronic coordination between the embryonic development and the endometrial status is crucial. Based on greater than 10 years of extensive research, our group has developed a molecular diagnostic tool, the endometrial receptivity analysis (ERA) based on the specific transcriptomic signature that identifies the receptive endometrium in natural and hormonal replacement therapy cycles by analysing 238 endometrial genes. This ERA test has shown that up to 30% of patients are non-receptive at the expected time of the cycle and has allowed the prediction of their specific WOI and the personalization of the embryo transfer. The efficiency of ERA has been assessed in different conditions as implantation failure, obesity, endometriosis and diminished endometrial thickness. Also, an ongoing international RCT has shown that personalization of the endometrial factor in the diagnostic work-up of the infertile couple must be considered.

Because implantation is a multifactorial process, the endometrial microbiota and its impact on embryo implantation in IVF patients has been evaluated based on the structure and abundance of the bacterial communities in the uterine cavity. In asymptomatic women, the endometrium has been shown to be mainly dominated by bacteria from the *Lactobacillus* genus (LD microbiota). However, shifts in this microbial profile towards a non-*Lactobacilli* dominated microbiota (NLD microbiota) is associated to poor reproductive outcomes, even in those patients with receptive endometrium, and should be considered as an emerging cause of implantation failure and pregnancy loss. These findings expand the evaluation of endometrial receptivity at different levels (molecular, microbiological etc.) to improve clinical reproductive outcomes with personalized medicine.

Session 8: Border-line indications for PGD 16:00 – 17:00

PGD FOR HLA (ESHRE Study)

Kakourou, Georgia.

ESHRE SIG Reproductive Genetics University of Athens, Athens, Greece

Since the first cases of PGD-HLA in 2001, >1000 cases have been performed, making it a well-established procedure. However, PGD-HLA is still offered by relatively few PGD centers, the currently available data is fragmented and limited publications preclude evaluation of parameters (of assisted-reproduction (ART), embryology, genetic diagnosis) that potentially influence the positive outcome, the birth of genetically suitable donor-baby(ies) and cure of the affected sibling. An ESHRE PGD Consortium retrospective multicentre cohort study, initiated in 2014, aimed to address this issue and evaluate the true clinical utility of PGD-HLA. Thirty-two PGD centres with published/known PGD-HLA activity were invited to participate in the study. Fourteen centres submitted data between February-September 2015, using a custom-designed secure database (Redcap) with unique code/centre. Data parameters covered family history, assisted-reproduction procedures, embryology, genetic diagnosis, donor-babies born and HSCT. Following quality evaluation of submitted cycles, data analysis included 704 PGD-HLA cycles from 364 couples performed between August 2001 and September 2015. HLA-matching with concurrent exclusion of monogenic disease accounted for 83% of cycles, of which in 58.2%, the indication was beta-thalassaemia. Mean maternal age was 33.5 years, 7.5% of couples were infertile. With 1.93 cycles/couple, 9751 oocytes were retrieved (13.89/cycle), 5552 embryos were analyzed (7.88/cycle, 85.3% on day-3), using PCR-based protocols (97.5% cycles). Of 4392 embryos diagnosed (6.26/cycle, 79% of embryos analyzed), 644 were genetically suitable (16.2% of those analysed for HLA alone; ~10% of those analysed for HLA with monogenic disease exclusion). 56.6% of couples achieved an embryo-transfer (598 embryos in 382 cycles), producing 163 HCG-positive pregnancies (pregnancy-rate/embryo-transfer: 42.67%, pregnancy-rate/initiated cycle: 24.3%). One hundred and twenty seven babies were born, with 30 pregnancies ongoing. HSCT was performed in 55 cases (7.8% cycles initiated), 76.2% of reported procedures were without complications. Genetic probability remains a major limitation to overall success. Other factors highlighted through data analysis include maternal age, number of oocytes and number of STRs used for haplotyping analysis, which were associated with number of embryos analyzed and transferred, chance of live birth and number of embryos diagnosed respectively. The findings of the study may be limited, as not all PGD centres with PGD-HLA experience participated. Furthermore the study is based on retrospective data collection, from centres with variable practices and strategies for ART, embryology and genetic diagnosis. Overall PGD-HLA is a valuable procedure and the number of children cured, though small, is significant. The study highlights the need to adequately follow-up and evaluate PGD-HLA cycles. Acknowledgement: ESHRE funded database customization and data analysis.

PREIMPLANTATION GENETIC TESTING (PGT) FOR BORDERLINE INDICATIONS – PGT FOR CANCER

Rechistky, Svetlana; Kuliev, Anver.
Reproductive Genetics Innovations, Illinois, United States

Aim: Inherited cancers have not traditionally been an indication for prenatal diagnosis, as this would lead to pregnancy termination, which may not be justified on the basis of genetic predisposition alone. On the other hand, the possibility of choosing the embryos free of cancer predisposing genes for transfer would obviate the need for pregnancy termination, as only unaffected pregnancies are established. PGT for such conditions is acceptable on ethical grounds because only a limited number of the embryos are selected for transfer.

Method: PGT strategies differed depending on the type of inheritance, mutation origin and available parental haplotypes. Polar body and/or embryo biopsy were performed followed by the mutation testing and haplotyping, combined with 24-chromosome aneuploidy testing in patients of advanced reproductive age. Relevant linked markers in both parents were analyzed even if only one is a carrier. Blastocyst biopsy was the method of choice in a concomitant testing for genetic predisposition with 24-chromosome aneuploidy testing, performed by array-CGH or NGS.

Results: Cancers were the largest group of conditions with genetic predisposition in our experience of 5037 PGT cycles for monogenic disorders (PGT-M), which resulted in replacement of 6038 embryos in 3669 transfers, yielding 2004 pregnancies and 2056 healthy children, representing the world's largest series of PGT-M. It includes PGT for 702 PGT cycles performed for 383 couples at risk for producing 24 different inherited cancers, including BRCA 1 and 2, Li-Fraumeni syndrome (LFD1), familial adenomatosis polyposis 1 (FAP1), colorectal cancer hereditary nonpolyposis, type 2 (HNPCC2), colorectal cancer hereditary nonpolyposis, type 4 (HNPCC4), Von Hippel-Lindau syndrome (VHL), familial posterior fossa brain tumor (hSNF5), retinoblastoma (RB), neurofibromatosis 1 and 2 (NF1 and NF2), Nevoid basal cell carcinoma (NBCCS) or Gorlin syndrome, tuberous sclerosis (TSC type 1 and type 2), multiple endocrine neoplasia, type 1, type 2a and type 4 (MEN 1, 2A and 4), Rhabdoid tumor (RTPS1), Lynch syndrome, hereditary diffuse gastric cancer (HDGS), Peutz-Jeghers syndrome (PJS), multiple exostoses, ataxia teleangiectasia (AT) and Fanconi anemia (FA). Overall, this resulted in replacement of 684 (1.4 embryos per transfer on the average) predisposition free embryos in 484 transfer cycles, yielding 282 (58.2%) unaffected pregnancies and birth of 316 healthy children free from mutations predisposing to cancer. Increasing number of PGT-M cycles is performed together with 24 chromosome aneuploidy testing, with 1194 cycles performed at the present time, resulting in improvement of the pregnancy rate (from 50 to 69.6%) and almost two-fold reduction of spontaneous abortions rate (from 13.6% to 9%), so optimising the clinical outcome in patients of advanced reproductive age.

Conclusion: The data show that the borderline indications have become an important addition to the PGT-M practice, making possible to offer PGT to a great variety of common diseases of early and later life, such as cancers determined by genetic predisposition, currently not preventable in majority of cases with the available traditional approaches.

Session 10: Near the future - March 29th 11:00 – 13:00h

OVARIAN REJUVENATION. IS THAT FOR REAL?

Sonia Herraiz [(1)], Mónica Romeu, Susana Martinez[(1)], Carlos Simón[(2)]., Antonio Pellicer[(1)].
[(1)] IVI, Valencia, Spain; [(2)] IGENOMIX, Valencia, Spain.

Nowadays low ovarian reserve due to advanced **maternal age** is a major cause of human infertility. Attempts to overcome poor response (PR)have been so far focused on stimulating the ovaries. Previous studies suggest a positive effect of BoneMarrow derived stem cells transplant(BMT)on the ovarian niche of damaged ovaries and raise the possibility that resting follicles may benefit from a positive influence of BM cells or soluble factors by their activation to growth.This concept is reinforced by the report of women who unexpectedly restore fertility after allogeneic BMT and similar approaches have been developed to regenerate tissues such as the endometrium and the myocardium.Thus, our purpose was to evaluate ovarian rejuvenation in PR women by BMT and to elucidate the underlying mechanisms to optimize the recruitment of resting follicles.The first experiments were done in animals. We asked whether BMT would reach the ovaries and whether these cells would stimulate follicular growth.Human ovarian cortex from PR was xenogratfed into SCID mice (n=18)and one week after, animals were injected via tail vein with: $1x10^6$ BMT cells, CD133+ cells or PBS (Control).Prior to injection, cells were labeled with MIRB to allow cell tracking. We observed that injected cells were able to migrate near blood vessels and close to preantral follicles of ovarian xenografts from both BMT and CD133 groups since day 1. A significant increase in the proportion of growing follicles(Control=12.5±10.5%; BMT=43.9±0.7%; CD133=43.8±6.2%)was observed in BMT and CD133 ovarian grafts, including also increased vascularization and E2 secretion when compared to control.Then, we designed a prospective pilot study with 12 PR women [NCT02240342].BM derived stem cells were mobilized to peripheral blood with a G-CSF and isolated by aphaeresis.Then, a volume of non-selected aphaeresis containing $50x10^6$ CD133+ cells was delivered into one ovarian artery by catheterism.Patients were considered as their own control as cells were injected in just one ovary while the contralateral was considered the control one.Serum AMH and AFC were monitored for up to 5 months and compared to basal levels.Then, BMT success criteria were established to determine the correlation between the treatment effects in PR patients and the analyzed components of the grafts.An increase in the **AFC** in the treated ovary (≥3foll.) was considered the primary outcome while two consecutive increases in AMH levels was the secondary success criteria. When the preliminary results were assessed in the first ten patients recruited of our study, an improvement of the ovarian function was detected in 67% of the PR women underwent BMT.In fact, both criteria were accomplished by 30% of the recruited patients while 10% just reached the AFC increase and 20% the AMH improvement.The AMH rose was >200% of their baseline level in 80% of the PR.To elucidate the undelaying mechanisms of this improvement, we establish the association between AFC/AMH and the presence of plasma soluble factors released by BM stem cells.We found that Fibroblast Growth Factor-2(FGF-2)and Thrombospondin(THSP-1)in plasma were positively associated with improvement in ovarian reserve biomarkers.

ENVIRONMENTALLY INDUCED EPIGENETIC INHERITANCE OF SPERM EPIMUTATIONS AND DISEASE: EXPOSURE AND DISEASE DIAGNOSTICS IN SPERM

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Epigenetic alterations of sperm due to effects of environmental toxicants, nutrition or stress significantly amplify the impact and health hazards of these exposures. One of the most sensitive periods to exposure is during fetal gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation occurs, however, adult exposures have also been shown to impact sperm epimutations. Previous studies have shown that endocrine disruptors can cause an increase in adult onset disease such as infertility, prostate, ovary and kidney disease, cancers and obesity. Interestingly, this effect is transgenerational (F1, F2, F3 and F4 generations) and hypothesized to be due to a permanent (imprinted) altered DNA methylation of the germ-line. The transgenerational epigenetic mechanism appears to involve the actions of an environmental compound to permanently alter the epigenetic (e.g. DNA methylation) programming of the germ line that then alters the transcriptomes of developing organs to induce disease susceptibility and development transgenerationally. In addition to DNA methylation, alterations in sperm ncRNAs have also been observed. A variety of different environmental compounds have been shown to induce this epigenetic transgenerational inheritance of disease including: fungicide vinclozolin, plastics BPA and phthalates, pesticides, DDT, dioxin, hydrocarbons, and chemotherapies. Interestingly, exposure specific epigenetic alterations were observed between the specific exposures. The suggestion that environmental factors can reprogram the germ line to induce epigenetic inheritance of disease is a new paradigm in disease etiology that is particularly relevant to preconception diagnostics of sperm.

CONCURRENT PREIMPLANTATION GENETIC DIAGNOSIS FOR SINGLE GENE DISORDERS AND COMPREHENSIVE CHROMOSOMAL ANEUPLOIDY TESTING WITHOUT WHOLE GENOME AMPLIFICATION

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Foundation for Embryonic Competence, Basking Ridge, United States

For over two decades preimplantation genetic diagnosis (PGD) has helped prevent the transmission of single gene disorders (SGDs) to the offspring of high-risk couples. The most widely used methods involve establishing linkage using either short tandem repeats (STRs) or single nucleotide polymorphisms (SNPs) (i.e. Karyomapping). More recently, strategies have been developed that allow simultaneous diagnosis of multiple genetic factors such as a SGD in combination with comprehensive chromosome screening (CCS) for aneuploidy. Some studies have involved performing two separate biopsies on the same embryo to perform the SGD and CCS testing independently, while others have shown whole genome amplification (WGA) on a single biopsy with the WGA product being distributed across the SGD and CCS testing. WGA on embryo biopsies has been shown to have an allele drop out (ADO) risk of 3-12%, which can lead to a misdiagnosis. Additionally, the most common platform for CCS has historically been array-based which can be costly and time and labor intensive. Therefore, we sought to develop a new, simple, low-cost, and universally applicable strategy, which allows simultaneous PGD of SGDs or small duplications or deletions and CCS from the same biopsy without using WGA to minimize the ADO risk.

First the ADO rate was examined by looking at how many times a known heterozygous genotype was falsely called homozygous. The ADO frequency on a single cell from a fibroblast cell line was 1.64% (18/1,096). When two or more cells were tested, the ADO frequency dropped to 0.02% (1/4,426). Amplification failure was also examined and determined to be 1.38% (55/4,000) overall, with 2.5% (20/800) for single cells and 1.09% (35/3,200) for samples that had two or more cells. The technical validation of the qPCR-based SGD PGD and CCS was performed using 152 embryos from 17 families. 100% were successfully given a diagnosis, with 0% ADO or amplification failure detected by our method. The genotyping consistency with reference laboratory results was >99%.

This method has been used clinically for over 2 years and testing has been completed for over 500 families and 2800 embryos. With the most recent universal uptake of expanded carrier screening our method, that allows for direct mutation detection, has been used to prepare PGD testing plans for numerous couples at risk who cannot include family members for linkage. Successful testing has also been completed for many complex cases involving *de novo* mutations, germline mosaicism and embryos previously labeled as inconclusive due to recombination between STRs. Lastly, while initial studies and applications included concurrent testing with qPCR-based CCS, these methods are also applicable with concurrent testing with our recently developed targeted next generation sequencing-based CCS test.

Session 11: Removing barriers to adopt genetic test in reproductive medicine- 14:00 – 15:30h

CARRIER SCREENING. DISEASE PREVENTION VS COST EFFECTIVENESS

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Patients come to fertility specialists to increase the probability of having a healthy child. According to the Online Mendelian Inheritance of Man there are nearly 24,000 heritable disease and phenotypes with which physicians and patients must contend. The American College of Obstetricians and Gynecologists have recently published committee opinions which add expanded carrier screening as an acceptable and recommended method of preconception screening. This allows traditional methods of ethnic based screening to be supplanted by more universal methods. In the face of data which shows that self-reported ethnicity can change with time and is impacted by the individual's culture, religion, language, behavior and psychology, this takes an uncertain factor off the table for patients and providers. Further, in the era of increased cost efficiency in genomic medicine, expanded carrier screening presents an increasingly cost effective method of preconception counseling. The curation of these panels needs to be an area of great focus and continued maintenance. It is recommended that these conditions have a carrier frequency of >1:100, have a well-defined phenotype, have a detrimental effect on quality of life, cause cognitive or physical impairment, require surgical or medical intervention, and have early onset in life. As we continue to discover new genetic causes of disease these panels will inevitably evolve. The identification of carrier status in couples allows for enhanced clinical decisions to be made. This includes options such as preimplantation genetic diagnosis, use of donor gametes, adoptions, or even a discussion about discontinuing effort to build a family. Traditionally utilization of PGD in at risk couples has been low given the limits of IVF including time, low success rates, high cost, and multiple gestation risk. However, with modern IVF, many of these concerns are greatly diminished allowing for more access to couples to this technology. For the clinician, this paradigm which avoids the need for ethnic based assessment allows for improved liability coverage and presents a cost effective approach to care when the cost of traditional screening methods in addition to the healthcare savings when heretofore undiagnosed carrier states result in an affected child are calculated. Reducing the burden to both patients and providers will likely lead to continued increased utilization in preconception care.

HOW ARE WE PERCEIVED BY OUR CLINICAL FELLOWS? CRITICAL REVIEW OF SART AND ESHRE DATA

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Since the beginning of preimplantation genetic diagnosis (PGD) a quarter century ago, our clinical colleagues have been very much interested in the development of this new technology. Indeed, they are oat the forefront of PGD providers to our patients. In the age of internet, information (news, stories, and descriptions, etc., accurate, unbiased or biased can be instantly obtained at the bedside, dinner table, subway trains, or high in the sky. Medical information can be obtained in the same manner. We are facing patients filled with all kinds of information when they seek infertility treatment. Moreover, genetic testing has been expanded to greater depths as more rare disorders have been identified. Our clinical colleagues are also bombarded with all kinds of information as for PGD. Rich information are available from professional websites, such as the European Society of Human Reproduction and Embryology (ESHRE), or American Society of Reproductive Medicine (ASRM).

ESHRE has a long history of PGD involving, starting from 1999 when the first ESHRE data collection was reported in Human Reproduction (1999). Since then, another 12 data collections have been published, the last one appeared two years ago (Hum Reprod. 2015). Technology development has been closely monitored. Many technical details are dissected. For example, the frequencies and causes of misdiagnosis was carefully analyzed, providing guidance to the laboratories for their practice. Also pros and cons of every new technologies have been debated.

American Society of Reproductive Medicine and Society of Assisted Reproductive Technology (SART), and Center for Disease Control (CDC), has long been monitoring IVF outcome and requiring its members to report their IVF success rate in order to better inform patients. Recently, CDC data has included more items from PGD due to increasing patient demand. From 458 IVF centers reported to CDC in 2014, 36 % of them are not provide PGD/PGS at all, 56.8% of the centers are proving <1% to 10% patients for PGD/PGS, and 6.1% of the centers are performing 11-30% of their patients and the highest percentage of patient underwent PGD was 86%. There is a clear trend in increasing PGD/PGS in 2015, more centers are providing PGD/PGS and higher percentages of patients are PGD/PGS patients. Fertility and Sterility, the society's journal has also actively involved in publishing PGD/PGS results, opinion papers, guidelines, as well as the debate.

In summary, with mounting accumulated scientific evidence, our clinical colleagues, patients as well as insurance industry are fully convinced that PGD for monogenetic diseases, chromosomal rearrangements and some sex-linked disorders, including male with sex chromosome abnormalities is a very effective way to provide real benefits to the potential parents. However, whether or not PGS or PGT-A will improve overall outcome of in vitro fertilization is still under debate. Better understanding of biology for preimplantation mosaicism and the clinical consequences of transferring mosaic embryo(s) is urgently needed. A detailed analysis, delineating all the confounding factors, including age, number of embryos available for biopsy (for selection), reproductive history and patient's psychological tolerance to miscarriages and/or abortions etc., is warranted.

Session 12: Late-breaking session. RCTS/ Non-invasive PGD

NON INVASIVE ANALYSIS OF THE EMBRYONIC GENOME: A CRITICAL REVIEW OF NON INVASIVE APPROACHES FOR PGD AND PGS

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Trophectoderm (TE) biopsy is increasingly adopted worldwide, as reported by ESHRE PGD consortium (Moutou 2014). Indeed, TE biopsy advantages over other biopsy stages include the analysis on multiple cells leading to more accurate results, and the ability to avoid the removal of inner cell mass cells (ICM), thus minimizing negative impacts on embryo's viability [Scott RT, 2013]. However, TE biopsy requires high technical skills and micromanipulation expertise and, if these are lacking, embryos can be critically damaged.

For this reason, less invasive procedures are now being investigated to allow genetic testing without embryonic cells, hence reducing costs and technical skills required for sampling. With this aim, blastocoel fluid (BF) and spent blastocyst media (SBM) are being evaluated as minimally invasive and completely non-invasive sources of embryonic DNA.

Fundamental points to investigate include the reproducibility of sample retrieval, the origin of DNA in BF and SBM and whether it is representative of the embryo's genetic constitution. It has been reported that DNA may be released by cleavage-stage embryos through processes of cellular fragmentation, apoptosis and necrosis (Stigliani et al., 2014). This results in fragmented DNA diffusing both inwards into the blastocoel and outwards into the media through the zona pellucida, both in cell-free and membrane-contained forms.

Preliminary studies from multiple laboratories on use of blastocoel DNA on embryo ploidy prediction led to conflicting results [Tobler 2015; Magli 2015]. Our validation study on 23 BF samples for PGS application shows significantly high amplification failure and diagnostic non-concordance rates.

Despite the first clinical application of DNA in SBM has been reported for aneuploidy testing (Xu 2016), there are increasing doubts that culture media can provide a reliable representation of embryo's genetic constitution. Indeed, it has been shown that cumulus cells, polar bodies, manufacturing process and exposure to the environment can introduce exogenous DNA into the culture media [Hammond 2017].

In our preliminary study, we used TaqMan RT PCR to assess the use of DNA from BF and SBM in PGD for monogenic disorders. We analyzed 62 BF and 72 SBM for 11 monogenic disorders using probes for both mutation sites and informative flanking SNPs. SBM showed higher amplification and diagnostic rates over BF samples. Using parental haplotypes, we could define the parental origin of the DNA detected. Due to significantly different allele drop-out (ADO) rates between maternal and paternal loci, we showed that maternal DNA exceeded the paternal one, suggesting DNA contamination from cumulus cells or PBs.

Moreover, we report evidence of maternal DNA contamination demonstrated by the detection of a mutation of maternal origin in multiple SBM samples retrieved from homozygous *wt* embryos. Finally, it is possible that further optimization of DNA isolation methods and analytical platforms may improve the detection rate of embryonic DNA. However, there is a lack of evidence that the DNA within BF and SBM is exclusively of embryonic origin.
Current data suggest that spent culture media is highly unreliable for embryo's genetic assessment, and future methodologies will need rigorous validation prior to clinical applications.

PGD-A IN ADVANCED MATERNAL AGE: FINAL RESULTS OF A DAY-3 BIOPSY RCT

Rubio, Carmen; Bellver, José; Rodrigo, Lorena; Castillón, Gema; Guillén, Alfredo; Vidal, Carmina; Giles, Juan; Ferrando, Marcos; Cabanillas, Sergio; Remohí, José; Pellicer, Antonio; Simón, Carlos.
IGENOMIX, Valencia, Spain

Fertility declines as women age, due to both a diminished ovarian reserve and an impaired oocyte quality that leads to an increase in embryo aneuploidy. Classical studies in miscarriages and livebirths showed the increased trisomic rates in established pregnancies according to maternal age and a similar trend has been observed in day-3 and trophectoderm biopsies in Preimplantation Genetic Diagnosis for Aneuploidy (PGD-A).

Conventional embryo morphology evaluation as well as morphokinetics can not properly discriminate euploid from aneuploid embryos. Particularly trisomic embryos that behave very similarly to euploid embryos. For this reasons, PGD-A started to be applied in advanced maternal age, with controversial results, mainly when day-3 biopsy and fluorescence In situ hybridization were employed.

We presented our prospective randomized study in women between 38 and 41 years of age, comparing livebirths rates with blastocyst transfer as a control group with a study group in which day-3 biopsy was performed, and array CGH was applied for 24-chromosome analysis. In the study group, euploid embryos at blastocyst stage were transferred. The PGD-A group exhibited significantly fewer embryo transfers (68.0%, vs. 90.5% for control; p=0.0001), and lower miscarriage rates (2.7%, vs. 39.0% for control; p=0.0007).

Delivery rate after the first transfer attempt was significantly higher in the PGD-A group per transfer (52.9% vs. 24.2%; p=0.0002), and per patient (36.0% vs. 21.9%; p=0.0309). No significant differences were observed in the cumulative delivery rates per patient six months after closing the study. However, the mean number of embryo transfers needed per live birth was lower in the PGD-A group compared to control group (1.8 vs. 3.7), as was the time to pregnancy (7.7 vs. 14.9 weeks).

After this study we concluded that PGD-A could improve reproductive outcome in these patientes. Also, since now throphectoderm biopsy and Next Generation Sequencing (NGS) are commonly applied, lower cost per treatment can be offered and success rates could be even higher.

Reference: Rubio C, Bellver J, Rodrigo L, Castillón G, Guillén A, Vidal C, Giles J, Ferrando M, Cabanillas S, Remohí J, Pellicer A, Simón C. In vitro Fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril. 2017 May;107(5):1122-1129.

FREE COMMUNICATIONS AND POSTER ABSTRACTS

Session 3: Free communications (related to carrier screening and PGD) – March 27th 14:00-15:30h.

OC-01

THERAPEUTIC SUCCESS OF HSCT BY PREIMPLANTATION HAPLOTYPING, FOLLOW-UP OF 520 CYCLES.

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Introduction: Many inherited and acquired pediatric hematological disorders can be cured only by hematopoietic stem cell transplantation (HSCT). However, the success of HSCT depends on how well human leukocyte antigen (HLA) haplotypes of recipient and the donor match to each other. Unfortunately, an HLA identical related donor is only available for 15-30% of the cases and the probability of finding an HLA matched unrelated donor is extremely small. Preimplantation genetic diagnosis combined with HLA typing is an alternative technique, which offers the possibility of selecting unaffected embryos that are HLA-identical with the sick child with the aim of possible use of stem cells from the resulting baby in future. This alternative source of HSCT known as “preimplantation HLA matching” has been a well-established and realistic treatment option especially for pediatric hematological disorders since 2001.

Material & methods: Here we aim to present the updated therapeutic success of this technique which resulted in full recovery of 51 (51/55) sick children who received HSCT from healthy infants conceived after preimplantation HLA typing for the following 12 different indications; beta-thalassaemia, Wiskott–Aldrich syndrome, Fanconi anemia, sickle cell anemia, acute myeloid leukemia, acute lymphoblastic leukemia, Glanzmann’s thrombasthenia, Diamond–Blackfan anemia, X-linked adrenoleukodystrophy, mucopolysaccharidosis type I, hemophagocytic lymphohistiocytosis and severe congenital neutropenia (Kostmann Disease).

Results & Conclusion: A total of 520 cycles were carried out for 272 couples. 217 couples underwent 422 treatment cycles for both monogenic disorders and preimplantation HLA typing, and 55 couples underwent 98 cycles for preimplantation HLA typing-only. A total of 4368 embryos were biopsied, of which 89.6% were diagnosed successfully. For the HLA-only testing group, 15.9% of analysed embryos were found as HLA-compatible and transferrable, whereas in the other group where mutation analysis were also carried out, only 12.4% of analysed embryos were found as both disease-free and HLA compatible resulting in an overall 12.9 % of embryos as transferable. In 59.3% of cycles, at least one suitable embryo could be found and transferred, resulting in a live birth rate of 31.7% per transfer. 108 healthy babies were born from 94 births, and some more pregnancies were ongoing at the time of writing this abstract.

HSCT has been carried out in 55 families where both recipients and donors met the appropriate criteria for transplantation and maintained a good state of health. The engraftment parameters, chimerism values, transplant-related mortality, relapse and disease-free survival time were used as assessment criteria on the outcome of each HSCT. No serious complication was observed among recipients and donors. Graft failure occurred in four children with beta-thalassemia where a second HSCT was planned (4/55). 51 children survived free of disease at a median follow up period of 42 months (2-144).

Preimplantation HLA matching is a reliable technique and provides a realistic option for couples seeking treatment for an affected child when no HLA-matched donor is available.

OC-02
COMBINED PGS AND PGD FOR THALASSEMIA.
Brockman, Matthew; Hodgson, Bree; Warren, Kimberly; Jasper, Melinda.
RHS Ltd, Thebarton, Australia.

Introduction: Thalassemia has become one of the most prevalent blood disorders around the world and is frequently screened for in PGD. The disease is caused by mutations within the HBA and HBB genes involved in haemoglobin production. In the IVF setting, PGD for thalassemia detection in embryos requires optimisation to ensure amplification of all regions of clinical interest. The ability to combine whole genome amplification and thalassemia specific PCR primers multiplexed in a single PCR reaction to allow aneuploidy detection (PGS) and to limit allele drop-out (ADO) for preimplantation genetic diagnosis (PGD) maximises the screening opportunity for a single embryo biopsy. The aim of this study was to demonstrate the application of a novel sequence enrichment protocol using DOPlify for combined PGD and PGS by NGS for thalassemia.

Material and methods: Single and 5-cells sorted from an aneuploid cell line (Coriell Institute for Medical Research) were subjected to WGA using the DOPlifyTM TSE (Targeted Sequence Enrichment) protocol (RHS Ltd) without (control samples) or with the inclusion of globin sequence specific PCR primers in the same reaction. Enrichment of the beta locus during the WGA was assessed using semi-quantitative PCR and NGS. Kapa HyperPlus libraries (Kapa Biosystems) were prepared from the beta globin enriched WGA DNA or the globin specific PCR amplicon amplified from the enriched WGA and a total of 40 samples were subsequently multiplexed and sequenced on a MiSeq sequencer according to standard 2x75bp protocol (Illumina). The sequencing data was bioinformatically aligned to hg19 to determine aneuploidy status. In addition, ADO and depth of beta globin specific sequencing reads was evaluated.

Results: Correct aneuploidy diagnoses were achieved for all single and multi-cells with trisomy 21, and with abnormal sex chromosome numbers XXY (approximately 900,000 reads per sample). Enrichment of the beta globin gene sequences was confirmed by semi-quantitative gene specific PCR and NGS when compared to the control WGA DNA sample without enrichment. The NGS protocol used was typical for PGS but was not expected to yield the depth of reads required for PGD. The purpose of the analysis was to demonstrate a reduction in ADO rate through enrichment. No sequencing reads mapping to the beta globin gene were reported for control samples, compared to 11 reads mapping to beta globin (exon 3) and 76 reads mapping to beta globin (exon 1 and 2) in the enriched WGA samples. When the beta globin gene was sequenced independently, as would be done for PGD, read depths in excess of 300x for exons 1, 2 and 3 were achieved with 100% of the exons covered.

Conclusions: The targeted and concurrent enrichment of globin sequence during WGA reduced ADO and when separately indexed, provided a suitable depth of coverage for PGD without compromising PGS results. The enriched WGA products can be analysed using NGS or pre-established PGD workflows such as Sanger sequencing or size determination.

OC-03

HAPLOTYPING AND COPY-NUMBER PROFILING OF SINGLE CELLS BY MASSIVE PARALLEL SEQUENCING.

Masset, Heleen; Dimitriadou, Eftychia; Zamani Esteki, Masoud; Voet, Thierry; Vermeesch, Joris R..
KU Leuven, Leuven, Belgium.

Introduction: Genome-wide haplotyping (such as karyomapping or haplarithmisis) enables the reconstruction of genome-wide haplotypes and copy-number profiles and is able to determine the parental origin of haplotypes at the single cell level. This methodology is being implemented as a generic method for preimplantation genetic diagnosis (PGD) in many IVF laboratories. The method can be applied on blastomeres biopsied from cleavage embryos or trophectoderm sampled from blastocysts. This allows the diagnosis of disease alleles, numerical and structural chromosomal abnormalities genome-wide. Current methods use SNP arrays to deduce the haplotype. The resolution of SNP arrays is limited and the platform is costly. The ultimate genetic screen of a single cell would be whole genome sequencing. However, this is currently too expensive. To overcome these limitations, we developed a single cell based genotyping-by-sequencing (GBS) method.

Materials and methods: Proof-of-principle experiments were performed on cells from the HapMap cell lines. DNA is amplified by RepliG. Three different restriction enzymes were used to digest the DNA and reduced representation libraries were generated. The fragments were sequenced on Illumina HiSeq2500. Subsequent haplotyping was performed by haplarithmisis. To allow for sequencing based genotyping, the siCHILD computational workflow was amended.

Results: We first demonstrate that a median coverage of 20 allows for a genotyping accuracy of 99.9 % for bulk DNA. Across informative SNPs covered in both single cell and genomic DNA from the same individual we obtain a 70 % concordance for the single cell genotype compared to the bulk DNA genotype. Preliminary results show a confidence of 99% within a distance of 18 informative SNPs flanking a homologous recombination site for correct single cell haplotype inference compared to the haplotype derived from bulk DNA. Subsequently a validation study is performed by comparing the GBS based haplotypes with the SNP array based haplotypes which were derived on embryos from clinical PGD cycles. Results of this ongoing study will be presented.

Conclusions: Here, we present a novel methodology for genotyping single cells through next-generation sequencing. By applying the GBS protocol as a method for genome reduction and the advancing sequencing technologies, we foresee this methodology as a valuable alternative for genotyping using SNP arrays.

OC-04
FROM PRENATAL DIAGNOSIS OF FETAL ABNORMALITY TO PREIMPLANTATION GENETIC DIAGNOSIS FOR SKELETAL DYSPLASIA USING NEXT-GENERATION-SEQUENCING TECHNOLOGIES.
Penacho, Vanessa; Amoros, Diego; González-Reig, Santiago; Galán, Francisco; Blanca, Helena; Castejón, Natalia; Alcaraz, Luis A.
Bioarray S.L., Elche, Alicante, Spain.

Introduction:

Skeletal malformations and dysplasias involve more than 300 syndromic and non-syndromic disorders, characterized by considerable phenotypic and genetic heterogeneity. Regarding to Jeune syndrome, common signs and symptoms include a small chest and short ribs which restrict the growth and expansion of the lungs, often causing life-threatening breathing difficulties. In cases where chromosomal abnormalities are not detected along a pregnancy, Trio-WES (whole genome amplification) is revealed as a powerful technique for genetic testing of gene mutations associated with those malformations observed by ultrasound. Concurrently, if causative point mutations are detected, preimplantation genetic diagnosis (PGD) coupled with informativity testing is mandatory before *in vitro* fertilization (IVF) in order to transfer disease-free embryos successfully.

Material and Methods:

We present the case study of a healthy couple with a previous history of two consecutive selected abortions due to skeletal dysplasia, evaluated by second-trimester ultrasound. A protocol for preconception assessment based on genetic diagnosis of the second affected abortion was developed, in order to prevent inheritance diseases in subsequent pregnancies. In the set-up phase, we performed genetic diagnosis on DNA isolated from both the malformed abortion material and parental blood samples by targeted Trio-WES, using Ion-AmpliSeq™ Exome Kit on Ion-Proton™ platform (Thermo Fisher Scientific). Afterwards, two IVF cycles were performed combining PGS (preimplantation genetic screening) and PGD between August and October 2016. After oocyte insemination and day-5 biopsy, DNA whole genome amplification was followed by PGS/PGD/informativity testing in all embryos. SNPs (single nucleotide polymorphism) detected through exome sequencing were used for the informativity test.

Results:

After Trio-WES study, it was found that both members of the couple were genetic carriers of Jeune syndrome. Likewise, both mutations were detected in compound heterozygosity state in *DYNC2H1* gene (pathogenic p.(Thr2106fs*7), and likely pathogenic p.(Asp3015Gly)), after the analysis of fetal tissue. After 5-day embryo biopsy, a combined PGD/PGS cycle was carried out. Along informativity testing, SNP-genotyping linked to gene regions involved by mutations was performed according to original Trio-WES data, giving us an accurate genetic profiling of embryos before implantation. A total of 65 informative SNP markers were selected in 2000 Kb flanking region on both sides of *DYNC2H1* gene and Ion-AmpliSeq™ primer pools were designed. Targeted next-generation sequencing on Ion-PGM™ System along with Ion-AmpliSeq™ and Ion-ReproSeq™ workflow was performed. After both IVF cycles and among the seven embryos analyzed, 2 were euploid and 5 aneuploid. Both euploid ones were also healthy carriers for only one mutation, therefore they were suitable for transfer. This has not been performed yet due to gynecological issues in the patient. Presented framework will improve whole PGD/PGS in terms of turnaround-time, reliability and cost-effectiveness.

Conclusions:

We applied Trio-WES results, combined with PGS/PGD and informativity testing in order to obtain healthy embryos for couples at risk for transmitting skeletal dysplasia. Beyond this, couples with undiagnosed pathologies could be benefited from this combined Trio-WES-PGD assay. We decided to couple the most powerful genetics tools available, with the aim of offering a very reliable and cost-effective method for those patients without previous clinical history reported.

OC-05

SEGMENTAL ANEUPLOIDIES IN PGD CYCLES FOR SINGLE GENE CONDITIONS BY KARYOMAPPING.

Vesela, Katerina; Hornak, Miroslav; Horak, Jakub; Kubicek, David; Tauwinklova, Gabriela; Oracova, Eva; Hromadova, Lenka; Travnik, Pavel; Vesely, Jan; Vesela, Tereza Agata.
Repromeda Biology Park, Brno, Czech Republic.

Introduction: The present era of PGD for single gene defects is associated with karyomapping - a genome-wide and robust array technology. It enables to evaluate both single gene conditions, and structural imbalanced anomalies as well as to perform an integrated aneuploidy screening enabling detection of maternal or paternal origin of aneuploidy in one test along with the single gene conditions. Over and above all the advantages Karyomapping helps us to understand the origin and character of early embryo aneuploidies included the segmental (sub-chromosomal) ones. The authors focused their attention to the segmental aneuploidies frequency in embryos in PGD cycles in couples with single gene conditions and a potential role of paternal age.

Material & methods: In period May 2014 – January 2017 in total 182 PGD cycles for various single genes conditions along with the aneuploidy screening were carried out using Karyomapping (Illumina - HumanKaryomap 12 SNP bead chips, BlueFuse Multi software). Totally 794 embryos were analyzed (50 blastomere samples and 744 trophectoderm samples). Group A consists of 30 cycles with segmental aneuploidies while group B consists of 152 cycles without segmental aneuploidies.

Results: 249 out of 794 embryos (31%) were aneuploid. Totally 90% of all whole chromosome aneuploidies were of maternal origin (289 out of 319), while only 10% were of paternal origin. By contrast the segmental aneuploidies (totally 50) consisted of 36 paternal aneuploidies (72%) in 30 embryos and 14 maternal segmental aneuploidies (28%) in 12 embryos. Group A consists of 30 cycles with segmental aneuploidies, the average paternal age was 36,63 (+-6,16) while group B consist of 152 cycles without segmental aneuploidies and the average paternal age was 36,13 (+-6,56). Segmental aneuploidies represent about 5% of all embryos and there was found at least one embryo with segmental aneuploidy in about 16,5% of cycles.

Conclusions: Segmental aneuploidies were found preferentially in paternally derived chromosomes. The frequency in PGS cycles is higher then in PGD cycles for single gene conditions (11% versus 5%). The cause and mechanism of segmental aneuploidies is still unclear however we could assume that they arising de novo and could be potentially involved by DNA fragmentation in sperm. There was no correlation observed with paternal age. Further studies are necessary to disclose more information.

OC-06

PREDICTION OF MENDELIAN DISORDERS RISK IN ASSISTED REPRODUCTIVE TECHNOLOGY PATIENTS OF MIDDLE EASTERN ETHNICITY BASED ON DETECTION OF GERMLINE VARIANTS USING AN EXPANDED PRECONCEPTION CARRIER GENETIC SCREENING TEST.

Martin, Julio [(1)]; Fatemi, Human [(2)]; Rodriguez-Iglesias, Beatriz [(1)]; Alonso, Roberto [(1)]; Jimenez, Jorge [(1)]; Simon, Carlos [(1)].
[(1)] IGENOMIX SL, Paterna, Spain; [(2)] IVI Fertility Clinics LLC, Abu Dhabi, United Arab Emirates.

Objective: To predict the Mendelian genetic risk of individuals of Middle Eastern (ME) ethnicity by analysis of protein-coding region genetic variation using a NGS-based preconception carrier genetic screening test.

Design: Retrospective analysis of genetic variation results from carrier testing of patients seeking ART due to subfertility; data were obtained from 312 individuals (156 couples) of ME ethnicity. Consanguinity was present in 56% of couples (87).

Methods: In total, NGS-based sequencing was conducted for testing 543 genes (501 autosomal recessive and 42 X-linked) primarily involved in severe childhood phenotypes. This approach was accompanied with non-NGS tests covering clinically significant mutations in frequently-mutated genes not detected by NGS, i.e., SMA common exon 7 deletion, Fragile X CGG repeat, alpha thalassemia, Duchenne MD del/dup, etc. Permission to perform this study was obtained by the ethics comittee (institutional review board) (code 1411-VLC-075-CS).

Results: Excluding HBA gene silent carriers (-α/αα) we found that 72% of ME ethnicity individuals were carriers of pathogenic variants. Average number of mutations for positive ME individuals was 1,24. In this population, the most frequently detected pathogenic variants with DM category (disease-causing mutation) in the HGMD database were: NM_000243.2(MEFV):c.2230G>T; NM_000350.2(ABCA4):c.5882G>A; NM_019098.4(CNGB3):c.1208G>A; NM_000048.3(ASL):c.392C>T; NM_000518.4(HBB):c.20A>T; NM_004004.5(GJB2):c.35delG. Regarding high-risk for the offspring, 16% of the consanguineous couples were found to have high risk for the offspring due to a common pathogenic variant in the same recessive gene. On the other hand, the non-consanguineous couples of ME ethnicity had a 5.8% high risk, comparable to the 5% risk of Caucasian couples (Martin et al. 2015). All high-risk couples received appropriate genetic counselling including a recommendation for preimplantation genetic diagnosis (PGD) during ART treatment. As indicated by others, before recommending PGD curation of clinical interpretation for variants classified as pathogenic in some mutation databases, i.e. HGMD, was necessary.

Conclusions: NGS-based expanded carrier genetic screening test constitutes a powerful tool to predict risk for Mendelian diseases. In patients undergoing ART the use of carrier screening and PGD for at risk couples allows to prevent inherited conditions. In the Arab world, were there is some of the highest rates of consanguineous marriages worldwide this strategy is still more pertinent as shown here.

Session 5: Challenging PGD-A diagnosis. Mosaicism / Specific Views (from submitted abstracts) – March 28th 08:45-10:30h.

OC-07

CHALLENGES IN INTERPRETING THE RELEVANCE OF SEGMENTAL MOSAICISM DETECTED BY NGS.

Grkovic, Steve; Traversa, Maria; Bonifacio, Michael; Bowman, Mark; Marren, Anthony; Mcarthur, Steve.
Genea, Sydney, Australia.

Introduction: Following the switch to NGS for comprehensive chromosome screening (Illumina VeriSeq kit, MiSeq instrument), Genea has implemented nine categories for the reporting of segmental mosaicism. In addition to those recommended in the 2016 PGDIS guidelines, three extra classifications were defined as: (a) probable sample-driven irregularities, (b) embryos exhibiting mosaicism for more than 2 chromosomes and (c) segmental mosaicism of 20 MB or greater. As the last of these new categories was the most contentious, the primary objective was to validate the technical limitations of NGS for the detection of segmental mosaicism, using samples that were representative of clinical specimens. A secondary aim was to assess how representative a mosaic segmental result is for the entire embryo.

Materials & methods: Three different approaches were employed. (1) To evaluate the technical reproducibility of the VeriSeq protocol, new sequencing libraries were prepared and reanalyzed for greater than 50 segmental mosaic WGA samples. (2) Detection limits, both in terms of percentage mosaicism and the size of the chromosome segment, were investigated by titrating WGA products from embryos identified as unbalanced for defined reciprocal translocations with WGA products from NAD embryos. (3) To assess the biological reproducibility of mosaicism, abnormal embryos (defined as at least one full aneuploidy) that also exhibited mosaic segmental shifts were subjected to multiple rebiopsy and reanalysis by NGS.

Results: (1) 100% concordance was observed for resequencing of mosaic segmental WGA samples.

(2) Whole autosome and X and Y titrations were detectable down to 10%, with the overall noise of the profile being the limiting factor. Segmental titrations greater than 20 MB could be detected down to 20%, although at 20% and lower it became difficult to distinguish shifts from background noise. For smaller (9-16 MB) segments, in general titrations containing 30% or greater ABN samples could be detected. However, a higher level of variation from the predicted percentage shift was observed for these smaller regions, with some becoming problematic to distinguish from background noise at 30% titration.

(3) Multiple rebiopsy of abnormal embryos confirmed full trisomies and monosomies, whereas whole chromosome mosaicism was observed to varying degrees in the rebiopsy samples. In contrast, limited evidence of segmental mosaicism was detected in the rebiopsy samples.

Conclusions: The resequencing and titration results indicate that the VeriSeq protocol exhibits a high level of technical reproducibly for the detection of mosaicism in WGA samples. Titration of translocation samples suggested that shifts of 20% or more should be considered as representative of potentially real segmental mosaicism for samples without excessive background noise. However, the technical capabilities of NGS need to be interpreted in the context of the rebiopsy results, which provided a higher level of support for full chromosome mosaics being of biological relevance than it did for the mosaic segmentals. Further studies are required to confirm these findings, which may lead to embryos in which segmental mosaicism is detected being classified at a lower risk category for transfer and/or alterations in the cutoff limits for the reporting of mosaicism.

OC-08

THE EXTENT OF CHROMOSOMAL MOSAICISM INFLUENCES THE CLINICAL OUTCOME OF IN VITRO FERTILIZATION TREATMENTS.

Spinella, Francesca [(1)]; Biricik, Anil [(1)]; Bono, Sara [(1)]; Minasi, Maria Giulia [(2)]; Cotroneo, Ettore [(1)]; Baldi, Marina [(1)]; Cursio, Elisabetta [(2)]; Diano, Laura [(1)]; Greco, Ermanno [(2)]; Fiorentino, Francesco [(1)].
[(1)] Genoma Group, Rome, Italy; [(2)] European Hospital, Rome, Italy.

Introduction: Embryonic mosaicism is a phenomenon characterized by the presence of two or more genetically distinct cell lineages, typically one with a chromosome abnormality and the other possessing a normal chromosome constitution. In a recent published study, we have demonstrated that 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 in vitro fertilization (IVF) resulting in mosaic embryos but no euploid embryos. Here we investigated whether the extent of chromosomal mosaicism might influence the development potential of mosaic embryos

Material & methods: The transfer of mosaic embryos at different aneuploidy percentage was offered to 73 women for whom IVF had resulted in no euploid embryos between May 2013-March 2016. The comparison of the clinical outcome obtained after transfer of mosaic embryos with low (<50%) and high (≥50%) aneuploidy percentage, was performed in order to assess a statistically significant difference in the development potential between the two groups. To obtain reference curves for determination of mosaicism percentage we assessed 114 diploid/aneuploid mosaic reconstructed samples (10–90% mosaicism) using both next generation sequencing (NGS) and array-comparative genomic hybridization (array-CGH) techniques. All embryos were cultured to blastocyst stage; trophectoderm biopsy was performed on Day-5 of development or Day6/7 for slow growing embryos. Comprehensive chromosome screening PGS was performed using either NGS or array-CGH methodologies.

Results: Transfers of mosaic embryos with a high percentage of chromosomally abnormal cells (≥50%) resulted in a live birth rate of 16.7% and involved a miscarriage rate of 10%. In contrast, mosaic embryos with a lower aneuploidy percentage (<50%) resulted in a live birth rate of 39.5%, with a miscarriage occurring in 7.0% of the transfers. All pregnancies that went to term were confirmed, through sampling of the chorionic villi and/or amniotic liquid, to have a normal karyotype. A comparison of the clinical outcomes between the groups, with low and high aneuploidy percentage, showed a significantly higher ongoing clinical pregnancy rate/embryo transfer (39.5% vs 16.7%; p=0.036), and baby born rate (41% vs 17%; P=0.027) in embryos with aneuploidy percentage <50% compared to embryos with a mosaicism level >50%. The biochemical pregnancy rate and miscarriage rate were not significantly different between the two groups.

Conclusion: Mosaic embryos with low aneuploidy percentage (<50%) have higher chances to result in healthy babies born compared to embryos with higher mosaicism levels (≥50%). The results of this study further confirm that mosaic embryos can develop into healthy euploid newborns. We demonstrated that the extent of mosaicism affects the IVF success rate. Priority for transfer should be given to mosaic embryos with low mosaicism levels.

OC-09

EMBRYO AND PATERNAL FACTORS ASSOCIATED WITH BLASTOCYST CHROMOSOMAL MOSAICISM.

Lledo Bosch, Belen; Ortiz, Jose A.; Morales, Ruth; Garcia-Hernandez, Eva; Llacer, Joaquin; Ten, Jorge; Bernabeu, Rafael.
Instituto Bernabeu, Alicante, Spain.

INTRODUCTION

There is a high incidence of chromosome abnormalities in human embryos that leads to failure of IVF cycles. The chromosomal mosaicism consists of a mixture of more than one cell line with different karyotype in the same embryo. The main factors that may increase the incidence of embryo mosaicism have not yet been established.

MATERIAL AND METHODS

We retrospectively reanalyzed array-CGH results from trophoectoderm biopsies of day 5 and 6 blastocysts (from January 2013 to January 2017). We analysed 1923 blastocysts from 704 IVF cycles. We considered a mosaic embryo when the percentage of mosaicism, calculated by the log2 ratio, was higher than 25%. We evaluated the relationship between several paternal and maternal factors and embryo mosaicism.

Chromosomal comprehensive screening was performed to couples who attended the Instituto Bernabeu with advanced maternal age, abnormal sperm FISH and/or a clinical history of recurrent miscarriage or implantation failure. Array-CGH analysis was performed using Agilent SurePrint G3 8x60K CGH microarrays with previous whole genome amplification of genomic DNA. The association between variables and mosaicism was evaluated by logistic regression and chi-square (SPSSv20.0).

RESULTS

Trophoectoderm biopsies on day 5 and 6 blastocysts (n=1923) were analysed by array-CGH. We have detected chromosomal mosaicism in 239 blastocysts (12.4%).

There are not statistically significant differences between the maternal ages of mosaic versus non-mosaic embryos (32.56 vs 32.77; p=0.679). On the other hand, the age of the male is higher in the group of mosaic embryos (40.83 years) compare to non-mosaic embryos (39.93 years); (p= 0.037). In fact, the percentage of mosaic embryos for men aged 40 years or older is 14% and 10.7% when the male age is less than 40 years (p = 0.030).

Male factor is not related with blastocysts mosaicism. No significant difference was reported according to semen parameters (WHO 2010 criteria), sperm FISH (7 chromosomes analyzed) and sperm DNA fragmentation (p=0.667, p=0.176 and p=0.292). Finally, the embryo quality is associated with the trophoectoderm mosaicism. A better embryo quality in D+5 is related with a lower percentage of mosaic embryos. Embryos of quality A (Istanbul Criteria) have a mosaic percentage of 8.2%, B of 13.8%, C of 24.1% and finally D of 22.4% (p = 0.000001).

CONCLUSIONS

Mechanisms involve in the development of these mosaic embryos are unknown. Moreover, the knowledge of the parameters that determine the mosaicism are of great relevance so that patients who undergo an IVF cycles know the risk of appearance of mosaic embryos. In our study we have stablished 2 factors associated with mosaicism level: Paternal age and embryo quality. A prospective randomized design should be used in future studies to corroborate the current findings.

OC-10

CUSTOM NGS ALGORITHM FOR CONSISTENT AND ACCURATE DIAGNOSIS OF MOSAICISM IN TROPHECTODERM BIOPSIES.

Vera-Rodriguez, Maria; Navarro, Roser; Lopez, Pilar ; Jimenez, Jorge; Rodrigo, Lorena; Garcia-Pascual, Carmen M.; Riboldi, Marcia; Coprerski, Bruno; Kayali, Refik ; Stankewicz, Tiffany ; Khajuria, Rajni ; Opeyemi, Adedoyin ; Yeh, Christine S. ; Simon, Carlos; Rubio, Carmen .
IGENOMIX, Valencia, Spain.

Introduction

New technologies for the detection of aneuploidies in preimplantation genetic diagnoses for aneuploidy screening (PGD-A) have introduced the possibility to detect mosaicism in embryos at blastocyst stage. Nevertheless, it remains unknown what is the frequency of mosaic embryos and how mosaicism should be diagnosed, as experimental variations or technician training can introduce a bias for a robust diagnosis. Thus, we proposed that a bioinformatic algorithm should be created to decrease variations among observers and experiments.

Material & methods

First, a custom algorithm was developed by the analysis of sequencing data from 72 mosaic samples. Mosaic samples were created by mixing DNA from already diagnosed euploid and aneuploid embryos in order to create 30-50% mosaic-like samples. All libraries were performed using Ion ReproSeq PGS kit and following manufacture instructions (Thermofisher Scientific). Samples were run in groups of 24 on an Ion PGM™ system and, subsequently, data were analyzed to develop an algorithm that included a copy number variation threshold for mosaicism.

Second, a comparative study to detect inter-individual variability in sequencing data interpretation was performed. Eight individuals from the same company blindly analyzed 500 consecutive PGD-A NGS profiles from trophectoderm biopsies (168 cycles) and the results were compared to the mosaicism rates obtained by the algorithm.

Segmental or sex chromosomes aneuploidies were not considered for the study. For the purpose of the study, embryos were labeled as “mosaic" only when a single mosaic chromosome was detected in order to avoid misdiagnoses in noisy profiles. Additionally, when an embryo showed mosaic chromosomes together with aneuploid chromosomes was labeled as “aneuploid”.

Results

The analysis of the 500 trophectoderm biopsies using the custom algorithm resulted in 57% aneuploid, 37% euploid, and 6% mosaic embryos. According to our results per chromosome, we observed a trend to higher proportion of mosaic events in smaller chromosomes, highlighting chromosome 14, 19, 20, and 22; with 6.2%, 5.4%, 5.4% and 5.2% mosaic events, respectively. Whereas, the higher percentage of aneuploid chromosomes was observed for chromosome 15, 16, 21, and 22; with 8.4%, 9.4%, 8.6%, and 12.2% of aneuploid events, respectively. The rates of mosaic embryos found by the different observers ranged from 4.2% to 13.8%. Finally, regarding the accuracy for mosaicism call between each observer and the algorithm, concordance results ranged from 84.0% to 91.8%.

Conclusions

For mosaicism diagnosis, we confirm the potential variability among observers that could have an impact in the estimation of the real incidence of mosaicism when using NGS platforms. This subjectivity could explain the different ranges reported for mosaicism diagnosed in the literature. Thus, a custom algorithm could offer a more accurate and consistent detection of mosaicism in clinical programs.

Session 7: Free communications (related to PGD-A, mosaicism, biopsy) – March 28th 14:00-15:30h.

OC-11

ANEUPLOIDY RATES ARE ASSOCIATED WITH THE BLASOTCYST BIOPSY TECHNIQUE.

Whitney, John B. [1]; Anderson, Robert E. [2]; Garner, Forest [3]; Schiewe, Mitchel C. [1].

[1] Ovation Fertility, Newport Beach, United States; [2] SCCRM, Newport Beach, United States; [3] Fertility Center of Las Vegas, Las Vegas, United States.

Introduction: Our objective was to assess the dynamics of blastocyst biopsying by determining if aneuploidy rates can be affected by factors inherent to the procedure. Does the technique produce equivalent results independent of mechanical variables?

Materials and Methods: Applying a prospective observational cohort design, 1204 blastocysts biopsies were analyzed between July 2015-January 2016. Each embryologist self-reported biopsy parameter variables, including embryo quality, day of development, # of laser pulses applied (≤4,5-9,>10 pulses), biopsy sample size (≤3 or ≥4 cells) and if the resulted biopsy sample appeared intact or primarily lysed. All embryos were laser hatched on Day 3, continued to Day 5/6/7 for trophectoderm biopsy, vitrified using an aseptic closed microSecure system and samples sent for PGS using NGS (VeriSeq™, Illumina). Blastocyst biopsies were performed using manual aspiration and a diode laser set at 600mHz/250 msec pulse duration. For analysis purposes, each blastocyst was classified as having an "ideal" or "difficult biopsy". An ideal biopsy was determined to require <10 laser pulses and yield a single trophectoderm piece containing at least 4 intact cells. Whereas, biopsies containing primarily lysed cells (≥4cells), required ≥10 laser pulses or produced more than one sample piece were classified as a difficult biopsy. Multivariate logistic regression was used to derive models to predict ploidy. Variables were selected for the model and analyzed using forward selection and backward elimination, with $p<0.05$ required to remain in the model. Pearson's chi-square test was used to assess the association of nominal variables (e.g.,difficult biopsies) with observed embryo ploidy.

Results: 85% of all biopsies performed were classified as ideal with 175 blastocysts resulting in a difficult biopsy. Biopsy modelling variables revealed significance for age, embryo grade and the day of blastulation. This model also uniquely identified significant mechanical parameters including number of laser pulses and increased cell lysis as predictors of aneuploidy. A second model based on biopsy mechanics identified cell lysis and the number of resulting biopsy pieces as significant predictors of ploidy. Difficult biopsies resulted in higher ($p<0.05$) aneuploidy rates than ideal biopsies (Table 1). Embryo quality and day of development showed no significance for producing a difficult biopsy. Additionally, no technician effect was observed (n=5 technicians).

Table 1.

	Ideal Biopsy	Difficult Biopsy	
n	1029	175	
#Aneuploid	472	100	
#Euploid	557	75	
%Aneuploid	45.9%*	57.1%*	

* Column values within row are different ($p<0.05$).

Conclusion: Optimal trophectoderm biopsying involves minimal laser pulses and produces a single cellular mass free of appreciable cell lysis. Deviation from this ideal outcome appears to more often produce an aneuploid result. Although no technician effect was identified, the model revealed independent mechanical variables that increased aneuploidy rates. We suggest that the variables identified by these models describe a specific style of biopsy, questioning if technique affects results. These variables appear to be linked, often occurring simultaneously, and in our study were independent of technician, day of biopsy or embryo quality grade. In summary, this study has generated insightful observational data, but cannot delineate if aneuploid embryos are more susceptible to yielding a difficult biopsy.

OC-12

MITOCHONDRIAL DNA COPY NUMBER MEASURED BY MITOSCORE IS ASSOCIATED TO TROPHECTODERM QUALITY.

De Los Santos, Maria José [(1)]; Mercader, Amparo [(1)]; Delgado, Arantzazu [(1)]; Escrich, Laura [(1)]; Buendia, Pilar [(1)]; Rubio, Carmen [(2)]; Diez-Juan, Antonio [(2)].
[(1)] IVI Valencia SL, Valencia, Spain; [(2)] IGENOMIX, Paterna, Spain.

Introduction:

Indirect measurement of mitochondrial DNA copy number has been proposed as a surrogate marker of embryo viability. Embryo with lower implantation potential have higher mitochondrial DNA copy number (Mitoscore) due possibly to an increase in mitochondrial biogenesis induced by stress or other factors. Likewise, it is well known that the ability to implant is influenced by the mitotic index of the throphectoderm cells which relay of mitochondrial fitness to maintain its metabolic activity. Based on this situation we wanted to know whether trophoblast quality is associated with mitochondrial DNA copy number generated after trophectoderm biopsy.

Material & methods:

Retrospective study involving a total of 1572 biopsied blastocysts from 421 PGS cycles (June 2016 – January 2017). Indications for PGS were: advanced maternal age (n=265), repetitive implantation failure (n=51), severe male factor (n=32), recurrent miscarriage (n=36), previous pregnancy with aneuploidy (n=6), monogenic disease (n=22) and others (n=8). Blastocyst quality was based on ASEBIR grading system, where A quality were embryos expanded and beyond with trophectoderm made of many cells forming a joined epithelium, B if trophectoderm cells have fewer cells but still forming and homogeneous epithelium, C when trophectoderm have few cells and D when trophectoderm cells show some signs of degeneration. T Student test was use for patient demographic comparisons whereas Kruskal Wallis non-parametric test was used for Mitoscore comparisons.

Results:

No differences in terms of age (38.9 $\pm$ 3.7, 38.8 $\pm$ 3.9), days of stimulation (10.6 $\pm$ 3.7, 11.1 $\pm$ 3.6), total doses of gonadotrophins (2664 $\pm$ 1444 UI vs 2471 $\pm$ 1584UI), and total number of oocytes retrieved (10.5 $\pm$ 7.8 vs 11.5 $\pm$ 7.9) were observed between patients. From the total number of embryos analysed, 2.48 % were graded as A, 53.18% graded as B, 43.08 % graded a C and 0.25% were graded as D. Mean age values of embryos in each category came from women of similar age ranges (A= 38.9 $\pm$ 3.6, B= 38.7$\pm$ 4.2, C= 38.6 $\pm$ 3.7 and D= 38.6 $\pm$ 3.5). Median values of Mitoscore were as follows, A= 19.02, B= 21.07, C= 21.43; and D= 31.5 (p= 0.0459). As it can be observed embryos with lower trophectoderm quality have a trend to present more elevated Mitoscore.

Conclusions

The relationship between quality of trophectoderm and Mitoscore may indicate that alterations in mitochondrial biogenesis may negatively affect trophoblast proliferation capacity. This situation may therefore impair further trophoblast differentiation which is necessary for the adhesion and invasion steps during implantation.

OC-13
THE INCIDENCE AND ORIGIN OF SEGMENTAL CHROMOSOME ABNORMALITIES IN HUMAN IVF EMBRYOS DETECTED DURING PGD AND PGS.
Hornak, Miroslav; Horak, Jakub; Kubicek, David ; Navratil, Rostislav ; Tauwinklova, Gabriela; Travnik, Pavel; Vesela, Katerina.
Repromeda, Brno, Czech Republic.

Introduction:

With the introduction of genome-wide techniques such as arrayCGH, SNP arrays or NGS into human PGD/PGS, it was clearly observed that relevant proportion of human IVF embryos are detected with sub-chromosomal abnormalities. Such abnormalities mostly consist of gain or loss of part of the chromosome from the distinct breakpoint to telomeric region, but, rarely, more complex intra-chromosomal aberrations might also be detected. The clinical impact of segmental abnormalities is significant, since such chromosome errors very likely lead to decreased embryo implantation or may be a cause of severely affected foetus. We have evaluated the incidence, origin and fate of segmental abnormalities in human IVF embryos based on collated data from our PGD and PGS programme to provide deeper insight into the topic.

Material & methods:

The occurrence of segmental abnormalities was assessed in ~1700 blastomere and ~3600 trophectoderm biopsies of human IVF embryos using 24sure, 24sureV3 and Veriseq NGS platforms (Illumina) during routine PGS. In a small follow up study, 31 embryos detected with segmental chromosome abnormality based on blastomere biopsy were followed up and examined as a whole or separated into inner cell mass (ICM) and trophectoderm (TE) at Day 5. The embryos and their parts were examined using 24sure arrayCGH (Illumina). The incidence and origin of segmental abnormalities were assessed in ~800 embryos using SNP arrays (HumanKaryomap-12 BeadChips, Illumina). Moreover, fifty spermatozoa obtained from a translocation carrier were analysed for all chromosomes using 24sure+ (Illumina) in an independent study.

Results:

Based on analysis of ~1700 blastomere biopsies, we have observed that 11% of the embryos contained one or more segmental abnormalities (oocyte age 34.1 ± 4.8). When assessing ~3600 of Day 5 embryos using trophectoderm biopsy, the frequency of segmental imbalances decreased to 8% (oocyte age 33.4 ± 5.3). We have followed up 31 embryos detected with segmental chromosome abnormality based on blastomere biopsy and observed that 25 (81%) embryos underwent self-correction and were euploid at blastocyst stage. Concerning the origin of segmental abnormalities detected in 794 trophectoderm biopsies using SNP arrays, 36 out of 50 segmental errors were detected in paternal chromosomes in contrast to 14 ones which affected maternal chromosomes. Further, based on arrayCGH, 1 out of 50 examined spermatozoa contained segmental aberration (not related to translocation), which was assessed in an independent study focused on segregation analysis of a male translocation carrier.

Conclusions:

Based on our findings from the small follow up study, the majority of segmental chromosome abnormalities detected in blastomere biopsy do not progress into blastocyst stage. Using SNP arrays, we have observed that segmental errors tend to affect significantly more frequently paternal chromosomes. We assume that segmental abnormalities arise predominantly *de novo* in developing embryos. Following this assumption, we hypothesize that some fertilizing spermatozoa, despite being balanced, contain chromosomal fragments, which are subsequently manifested in unbalanced segmental abnormalities of paternal chromosomes in developing embryos.

OC-14
CLINICAL SIGNIFICANCE OF UNDIAGNOSED MOSAICISM IN IVF EMBRYOS.
Perry, Emma; Beyer, Claire; Willats, Elissa; Lin, Jane; Low, Lee-Yean; Mullen, Jayne; Rombauts, Luk.
Monash IVF, Melbourne, Australia.

Introduction

Preimplantation Genetic Screening (PGS) technologies have advanced rapidly over recent years. Next Generation Sequencing (NGS) is one of the leading technologies for PGS as it is more sensitive in detecting mosaicism in embryo biopsy samples compared with previous PGS techniques such as array-CGH (aCGH). Our clinics data suggests that approximately 25% of IVF embryos are diagnosed as mosaic following PGS using NGS. Until recently, embryos diagnosed as 'mosaic' following PGS at Monash IVF were considered abnormal and not suitable for transfer. Whilst mosaic embryos may be associated with an increased risk of implantation failure and miscarriage, some studies have shown that a proportion of these mosaic embryos may result in a healthy live birth.

The aim of this study was to perform a retrospective analysis of embryos previously diagnosed as 'euploid by aCGH' to determine whether a portion of these were mosaic following reassessment by NGS. NGS results were compared against clinical outcomes to investigate the impact of unintentionally transferring mosaic embryos assessed as 'normal' using the best available technologies at that time. The results of this study will be used to re-evaluate Monash IVF's reporting and transfer guidelines for embryos diagnosed as mosaic following PGS testing using NGS technology.

Material and methods

A retrospective study was performed on stored embryonic DNA samples from embryos that were initially diagnosed as 'euploid by aCGH' between November 2013 and April 2015. All donated embryonic DNA samples were from embryos that had been transferred in a single embryo transfer procedure and had a known clinical outcome. Samples were reanalysed using the 'Veriseq' NGS comprehensive chromosome screening platform (Illumina) and the results were compared to the clinical outcome for that embryo.

Results

55 embryos that were diagnosed as 'euploid by aCGH' and subsequently transferred are being re-analysed by NGS. Of these 'euploid by aCGH' embryos, 41/55 embryos resulted in a healthy live birth, 11/55 embryos resulted in implantation failure and 3/55 embryos resulted in miscarriage. Of the 24 samples re-analysed by NGS to date, only 1 embryo has been shown to be mosaic. We will present completed data.

Conclusion

Whilst retrospective studies may show that the transfer of 'mosaic by NGS' embryos can result in live births, the clinical significance of mosaicism is still unclear. The level of mosaicism present in an embryo and the area of the genome involved may be contributing factors as to why some mosaic abnormalities may result in a live birth whilst others result in implantation failure or miscarriage. The transfer of a mosaic embryo may carry less risk than the transfer of an embryo that has not undergone PGS testing, as these embryos provide couples with a 95% assurance that the embryo does not contain whole chromosome aneuploidy. Further studies are needed to better understand the clinical significance of mosaicism throughout the genome, and the implication this will have on the use of mosaic embryos for transfer.

OC-15

MITOSCORE VALUES ARE NOT AFFECTED BY ATMOSPHERIC OXYGEN CONCENTRATION DURING EMBRYO CULTURE.

Mifsud, Amparo [(1)]; Gámiz, Pilar [(1)]; Grau, Noelia [(1)]; Rubio, Carmen [(2)]; Diez-Juan, Antonio [(2)]; De Los Santos, Maria Jose [(1)].

[(1)] IVI-VLC, Valencia, Spain; [(2)] IGENOMIX, Paterna, Spain.

Introduction: The use atmospheric oxygen has been shown to have negative effects upon the embryo's molecular and cellular physiology, adding stress factors to the culture and hence decreasing embryo viability. Additionally, measurement of mitochondrial DNA copy number has been proposed as a surrogate marker of embryo viability in the way that the higher the copy number the lower the embryo viability. The objective of this study was to assess whether the DNA mitochondria copy number (Mitoscore) may be influenced by the use of atmospheric oxygen concentration during embryo culture.

Material & methods: Retrospective study involving a total of 1499 biopsied blastocyst from 456 PGS cycles (November 2016 – January 2017). Indications for PGS were: advanced maternal age (n=265), repetitive implantation failure (n= 54), severe male factor (n=57), recurrent miscarriage (n= 41), monogenic disease (n= 23), and previous pregnancy with aneuploidy (n=16). A total of 781 embryos from 230 cycles were culture under atmospheric oxygen concentration (Group A) and 718 from 226 PGS cycles under low oxygen concentration (Group B). Embryos from group A were cultured in IVF medium (Vitrolife AB, Kungsbacka, Sweden) until day-3, and then were placed in co-culture medium (CCM) (Vitrolife AB, Kungsbacka, Sweden), whereas embryos from group B were cultured in cleavage medium (Cook Medical, France) until day-3, and then were placed in blastocyst medium (Cook Medical, France). Comparisons between the two groups were performed by using a Mann-Whitney U test.

Results: There were no differences between the two groups A and B in terms of age (38.7 ±2.8 *vs* 38.6 ± 4.0), number of oocytes aspirated (10.3 ± 7.8 *vs* 11.6 ± 8.5) and gonadotrophin doses (2925.0 ± 1124 *vs* 2718 ± 1406). The percentage of good quality blastocysts in both culture conditions were no statistically different, Group A (40.16 %) *vs* Group B (41.82%). When we compared the average of Mitoscore values, no differences were observed between the embryos belonging to Group A: 21.27; 11.94 - 189.37 (median; min-max) and the embryos from Group B: 21.27; 11.50 - 199.15 (median; min-max), p=0.30.

Conclusions: Mammalian pre-implantation embryos are characterized by limited oxidative capacity and great dependence on anaerobic respiration. At blastocyst stage, inner cell mass (ICM) and trophoetoderm (TE) cells have different metabolic activities. While ICM uses glycolitic metabolism to control the generation of free radicals, TE cells require more energy, relaying on mitochondrial oxidative metabolism that could require mitochondrial DNA replication measured by Mitoscore values. Whatever the situation will be, it seems that is not disturbed by using atmospheric oxygen concentration during embryo culture.

OC-16
HOW TO CHOOSE PRENATAL TESTING OPTIONS FOR PREGNANT WOMEN AFTER PREIMPLANTATION GENETIC SCREENING: GENETIC COUNSELING CHALLENGES.
Tamura, Chieko; Arakawa, Hiromi; Kurata, Yoshie; Fujita, Satoko; Nakamura, Yasushi.
FMC Tokyo Clinic, Tokyo, Japan.

Introduction: When women became pregnant after preimplantation genetic screening (PGS) for aneuploidy, it is not clear which prenatal testing options should be offered. In this presentation, we would like to describe prenatal testing options that we have offered to post-PGS pregnant women, and elucidate the rationale and challenges of how to choose these options.

Material & methods: We selected ten typical post-PGS cases who came to our clinic to undergo prenatal testing. These women had PGS with either array-CGH or next-generation sequencing at some different medical centers. Genetic counseling charts of these cases were reviewed to extract prenatal testing options discussed, and to see how testing options were chosen.

Results: Prenatal testing options discussed with post-PGS pregnant women and couples were, first trimester combined screening test with ultrasound and serum markers, cell-free DNA screening, amniocentesis and chorionic villus sampling (CVS) with conventional karyotyping or SNP microarray analysis, and, first and second detailed sonogram for fetal anatomy examination. Regarding non-chromosomal anomalies with their fetuses, people usually chose second trimester detailed sonogram for fetal anatomy scan, with or without first trimester detailed sonogram. When people were worried about fetal chromosomal problems, including false negative PGS results due to blastocyst mosaicism, and/or fetal outcome after transferring embryos with mosaic aneuploidy or segmental mosaicism, selection of prenatal testing options was more complicated, and the rationale discussed was as follows. Supposing that the fetus may have true mosaic aneuploidy and/or true segmental mosaicism, first trimester combined screening test or cell-free DNA screening may be still used as the standard procedure that all pregnant women are offered, but, may not be appropriate to find chromosomal abnormalities that transferred embryos may have, because these options have limitation to detect mosaicism, and, also because they were designed to primarily find trisomy 13, 18 and 21, whereas mosaicism that transferred embryos had were usually with other chromosomes. Amniocentesis seems better than CVS to avoid confusion of placental mosaicism, which could be caused by trophectoderm mosaicism seen with PGS. Detection rate for mosaicism is better with microarray than conventional karyotyping, and SNP microarray is better than array-CGH since only SNP microarray can detect uniparental disomy, which could be caused by trisomy rescue. Therefore, amniocentesis with SNP microarray seems the best, but, this is the most expensive option, and, it still misses low rate mosaicism. Furthermore, people may prefer early procedure to be relieved, whereas amniocentesis is the latest among all options. People also may want to avoid fetal risks that amniocentesis and CVS have, considering the chance that their fetuses have true chromosomal abnormalities after PGS is relatively small.

Conclusion: Selection process of prenatal testing options can be very complicated with post-PGS women and couples. Genetic counseling providers should discuss all factors, including people's preferences, costs, fetal risks, timing of procedures, detection rates of mosaicism, specific chromosome(s) that transferred embryos had mosaicism with, and the chance of true fetal chromosomal abnormalities, to choose options that are medically appropriate as well as suitable for preferences of pregnant women and couples.

Session 9: Free communications (related to new technologies) – March 29th 09:00-10:30h.

OC-17

DETECTION OF SEGMENTAL ANEUPLOIDY AND MOSAICISM IN PREIMPLANTATION EMBRYO MODEL BY NEXT GENERATION SEQUENCING METHODOLOGIES.

Biricik, Anil [(1)]; Cotroneo, Ettore [(1)]; Bono, Sara [(1)]; Surdo, Matteo [(1)]; Minasi, Maria Giulia [(2)]; Cursio, Elisabetta [(2)]; Greco, Ermanno [(2)]; Fiorentino, Francesco [(1)]; Spinella, Francesca [(1)].

[(1)] Genoma Group, Rome, Italy; [(2)] European Hospital, Rome, Italy.

Introduction: Preimplantation genetic screening (PGS), specifically with NGS, for aneuploidy testing on embryos has become a common practice to improve outcomes in patients undergoing treatment for infertility through in vitro fertilization. As technology has evolved, detection of segmental aneuploidy and embryonic mosaicism has become possible. Although possible to diagnose, the limits of detection for segmental aneuploidies and mosaicism between different NGS platforms has not been examined. Here we investigated the capability of MiSeq-based (Veriseq) and personal genome machine (Reproseq)–based next-generation sequencing (NGS) to accurately detect segmental aneuploidy and embryonic mosaicism in trophectoderm cell models. Material & methods: Samples composed of specific euploid/aneuploidy cells ratio (10–90% mosaicism) of 10 (n=54) and 100 (n=54) total cells were obtained and analysed with Miseq Veriseq (Illumina) and PGM ReproSeq (Life technologies) NGS platforms. In addition euploid (n=6) and aneuploid (n=6) cell lines were used as control. Duplicates of eight cell lines with different structural abnormalities (from 4.5Mb to 17Mb) were used for segmental aneuploidy assessment. Mixed of female euploid/male trisomy 21 cell line were used for one set, and a male euploid with either female trisomy 18 or a female trisomy 21 cell line were used for another. Sensitivity and specificity of aneuploidy detection at each level of mosaicism was determined and compared between platforms. CNV analysis was accomplished by current version of BlueFuse software(Veriseq) or IonReporter Software(ReproSeq). Results: In total, 120 samples were assessed for mosaicism detection, 108 chromosomal mosaics (true positive) and 12 aneuploid or euploid (true negative) samples. Sensitivity was 90% (95% confidence interval [95% CI]:83.18% to 94.73%) and 81.82% (95% confidence interval [95% CI]: 74.17% to 87.99%) for Veriseq and Reproseq, respectively. The 12 false negative results obtained with VeriSeq were from samples with 10% mosaicism. The 24 false negative results obtained with ReproSeq were from the samples with <30% mosaicism. Samples with a mosaicism higher than 30% gave an automatic full aneuploidy call on Reproseq. Specificity was 100% (95% confidence interval [95% CI]: 73.54% to 100.00%) for both platforms. This study indicated a different limit of mosaicism detection with a LOD of ≥20% and >30% for VeriSeq and ReproSeq, respectively. Analysis of segmental aneuploidy in the cell lines demonstrated that ReproSeq could identify a segmental imbalance as small as 10 Mb in size, while VeriSeq, using a manual call, identify microdeletion as little as 4.5Mb. No additional segmental imbalances were identified. ReproSeq protocol was more rapid (<15 h) compared to VeriSeq. Conclusions: These findings demonstrate that VeriSeq NGS platform has a much higher resolution for segmental aneuploidies and a higher level of accuracy at a lower level of mosaicism compared to ReproSeq . In addition, the presence of an available algorithm for the manual call of mosaicism on Veriseq allows to easily defining the percentage of mosaicism also at high-level discrimination between mosaic and full aneuploidy samples. This study has been done with the current commercial versions of both NGS platforms, and eventual upgrades on protocols and on software of the systems may change the sensitivity and specificity.

OC-18

EVIDENCE TO SUGGEST A UNIQUE 3D ORGANIZATION OF CHROMOSOMES WITHIN THE SPERM NUCLEUS: IMPLICATIONS FOR FERTILIZATION AND EARLY EMBRYONIC DEVELOPMENT.

Tempest, Helen; Ioannou, Dimitrios.
Florida International University, Miami, United States.

Introduction Genomes are nonrandomly organized within nuclei. Sperm cells are proposed to have a unique hairpin-loop arrangement that has been hypothesized to be critical for the ordered exodus of the paternal genome following fertilization. Early studies suggest centromeres cluster in the nuclear center forming a chromocenter, with p- and q-chromosome arms stretching toward the nuclear periphery forming a hairpin-loop structure. This study has examined whether evidence can be provided to support this model of chromatin organization in sperm using 3D modeling and the implications for fertilization and early embryonic development are discussed.

Materials and Methods This study was approved by the local Institutional Review Board and five normozoospermic sperm donors of proven fertility were included in this study. Three color fluorescence in-situ hybridization targeted the centromere and p- and q-arms of eight different chromosomes (2, 3, 6, 8, 10, 12, 16, and 18). Wide-field fluorescence microscopy and 3D modelling was employed to image and visualize sperm cells and targeted loci in 3D. The radial organization of each probe was assessed by measuring the distance of the geometric center of each loci to the nearest nuclear periphery. Hairpin-loop configurations were determined by the angle created between p- and q-arms. A minimum of 30 cells per subject, per chromosome were studied. Nonrandom radial organization was established using Chi-squared goodness-of-fit test ($p<0.05$).

Results Distinct reproducible chromosome-specific patterns of organization emerged between the different subjects enrolled in this study. All chromosomes possessed nonrandom radial organization ($p<0.05$). Chromosome arms were found to form discrete hairpin-loop configurations. Three categories of hairpin-loops were observed: narrow (<40°: 2, 10, 12, 18), intermediate (>40°<60°: 3, 8), and wide (>60°: 6, 16). Six centromeres (2, 3, 6, 10, 12, 18) were more peripherally localized than their chromosome arms.

Conclusions Reproducible (inter-subject) nonrandom hairpin-loop organization of chromosomes in sperm was observed to support the previously proposed model. However, our findings suggest that this model needs to be further redefined. We do not find evidence to support the existence of a centralized chromocenter with 75% of investigated centromeres being more proximal to the nuclear periphery than their respective chromosome arms. This suggests the sperm nucleus is more segmentally organized. The reproducible segmentally organized nucleus observed in our subject cohort may be of significance for fertilization and early embryogenesis. These findings suggest that specific genomic regions are likely to be exposed, remodeled and activated first following fertilization. The sequential exodus and remodeling of chromatin could impact patterns of gene activation observed within the early embryo, perturbations in which, could negatively impact fertilization and early embryogenesis. Future study objectives involve enrolling patients with male factor infertility who have experienced repeated unexplained fertilization failure and embryonic arrest to determine whether differences in chromosome organization are observed in this cohort compared to proven fertile donors.

OC-19

A NOVEL ALGORITHM FOR DETERMINING THE LEVEL OF MOSAICISM IN PREIMPLANTATION GENETIC SCREENING (PGS) WITH NEXT-GENERATION SEQUENCING (NGS).

Castejon Fernandez, Natalia; Amoros, Diego; Gonzalez-Reig, Santiago; Blanca, Helena; Penacho, Vanessa; Galán, Francisco; Alcaraz, Luís A.
Bioarray SL., Elche, Spain.

Introduction:

Filtering PCR-artifacts, normalization and aberrations calling are common tasks implemented in any software designed for analyzing NGS data. However, most of the algorithms have been developed for post-natal diagnosis, where NGS has recently become a useful but not fully compatible approach for PGS. Previous methods based on whole-genome sequencing (WGS) require high-depth coverage on the whole genome scale and are not cost-efficient. Moreover, they do not determine precisely the level of mosaicism, because this is very uncommon in post-natal diagnosis. In order to accurately identify genome-wide aneuploidies by NGS, we implemented an algorithm that can identify the percentage of aneuploidy for embryo-biopsies. This algorithm is able to improve confidence-value and reduce the risk of misdiagnosis. Less reads are required enabling high-level multiplexing.

Material & methods:

In-silico and *in-vitro* approaches were achieved to evaluate the percentage of mosaicism. *In-silico* mosaic-embryos were designed controlling the percentage of mosaicism from sequencing alignment-data of 25 previous biopsied day-3 and day-5 euploid/aneuploid embryos. *In-vitro* mosaic-embryos were mimetized mixing DNA isolated from aneuploid lymphocyte cell-culture lines showing trisomy18, Turner syndrome and euploid embryos. Biopsied and *in-vitro* embryos were sequenced on Ion-PGM™ platform and analyzed using Ion-ReproSeq™ workflow and our own-designed algorithm. MAPD quality values and ploidy stage per embryo were thoughtfully evaluated. In order to determinate minimum reads number per embryo required for a correct PGS forecast, a random sequence selection within these embryos was carried out with the aim of performing *in-silico* embryos from 100,000 to 5000 reads.

Results:

While Ion-ReproSeq™ workflow needs at least 100.000 reads per embryo for a successful analysis, our algorithm accurately predicted the ploidy stage of submitted embryos with only 10.000 reads. Also, Ion-ReproSeq™ workflow was able to detect the mosaicism stage when it was >40% of aneuploidy; otherwise aneuploidy was hidden and undetected. Our algorithm has been able not only to detect the whole-mosaicism spectrum (from 90 to 10%), but also to indicate the exact percentage of aneuploidy in an analyzed mosaic cell. This has allowed us to be more consistent with the final diagnosis of the embryos submitted. Moreover, our approach would provide the possibility to discover the biological significance of mosaic embryos along IVF cycles and to discuss the convenience of transfer according to its mosaic state. Finally, we can assert that our algorithm is reliable regarding to accuracy improvement, time-consuming and cost-efficiency, which enhances PGS service and allows us to offer it specifically for every couple and cycle. Even, it is limited not only to discover variations within exons, but also on whole genome. Finally, any possible limitation that can difficult diagnosis due to MAPD quality values is semi-controlled by applying our filter-duplicates algorithm that decreases reads-variability while quality of copy number calls increases.

Conclusions:

Our algorithm is more accurate than Ion-ReproSeq™ workflow. It shows the exact ploidy-rate in mosaic-embryos, enabling detection even at very-low mosaicism. This approach could improve implantation chances, giving us a deep-knowledge about the developmental potential of mosaic embryos generated by *in-vitro* fertilization-cycles and biological-implications derived from transferring themselves.

OC-20

COMBINED PGD AND PGS BY NGS ON THE SAME BIOPSY USING A SINGLE INDEX.

Jasper, Melinda; Brockman, Matthew; Hodgson, Bree; Warren, Kimberly.
RHS Ltd, Thebarton, Australia.

Introduction: Whole genome amplification (WGA) and gene specific primers can be successfully multiplexed in a single PCR reaction to allow both aneuploidy detection and limit allele drop-out for preimplantation genetic diagnosis (PGD) from a single cell using DOPlify™. Here we describe a novel approach which allows uncompromised PGS and the increased read depth required for PGD for multiple genes of clinical interest from a single cell using human leukocyte antigen (HLA) as a model for day 3 and day 5 combined PGS and PGD.

Material and methods: Single and 5-cells sorted from aneuploid and euploid cell lines (Coriell Institute) were subjected to WGA using the DOPlify™ TSE (Targeted Sequence Enrichment) protocol (RHS Ltd) with the inclusion of HLA-A specific PCR primers (GenDx) and up to three additional target specific PCR primer sets. Enrichment of target regions was determined using semi-quantitative PCR and NGS. The HLA-A amplicon generated from semi-quantitative PCR was subsequently pooled with the original DOPlify™ TSE WGA amplicons in a 1:10 and 1:20 ratio before library preparation. Kapa Hyperplus DNA libraries (Kapa Biosystems) were prepared from WGA DNA, the HLA-A amplicon alone and the WGA pooled with the HLA-A amplicon with a total of 40 samples multiplexed and sequenced on a MiSeq Platform according to standard 2x75bp protocol (Illumina). The sequencing data was bioinformatically aligned to hg19 then analysed to determine aneuploidy status and targeted sequence coverage.

Results: Correct aneuploidy results were confirmed by NGS for all single cell and multi cell samples with an average of 900,000 mapped reads per sample. In all instances where TSE WGA was performed, there was enrichment of the target site. Read depth was significantly higher for the HLA-A amplicons alone; approximately 829-18,000x (single cell) and 7,621-20,000 (5-cell). When the gene specific amplicon was seeded into the DOPlify™ TSE WGA in a ratio of 1:10 and 1:20, maximum read depths of 267 and 145, respectively were attained. Multiplexing of the HLA-A primers along with 3 other target specific primer sets showed no allele drop-out and did not compromise enrichment or aneuploidy detection.

Conclusions: These data support a novel concurrent WGA and targeted sequence enrichment approach using DOPlify™ that allows concurrent PGS and PGD from single or 5 cells. The system can accommodate the addition of multiple primer sets targeting more than one region allowing numerous sites to be analysed at once. Combined PGD and PGS results can be generated using a variety of different NGS approaches using multiple or single indexes.

OC-21

MATERNAL AGE HAS NO INFLUENCE ON MITOCHONDRIAL DNA (MTDNA) CONTENT IN CHROMOSOMALLY NORMAL EMBRYOS.

Ogur, Cagri; Gultomruk, Meral; Caferler, Julide; Capar, Betul; Findikli, Necati; Bahceci, Mustafa.
Bahceci IVF Center, Istanbul, Turkey.

Introduction: Proper functioning of healthy mitochondria are crucial throughout the embryonic development and later in adult life. The quality and quantity of mitochondria in embryos has previously been found to be associated with aneuploidy and implantation. Here we aimed to present our comparative analysis of mtDNA content with respect to female age in cases undergoing preimplantation genetic screening by next generation sequencing (NGS) technique.

Materials and methods: Between March and December 2016, aneuploidy testing has been performed in 1520 blastocyst-stage embryos obtained from 634 IVF cycles, which have been initiated for the indication of repeated implantation failures and/or advanced maternal age. Next generation sequencing (NGS) technique (Ion PGM, Ion Torrent) was used in order to assess chromosomal status of 23 pairs of chromosomes. Euploid embryos were further analyzed by Ion Reporter software in order to identify the mitochondrial copy number (Mitoscore) in embryos. Cycles with structural chromosomal abnormalities were excluded from the analysis. Biopsy was done at blastocyst stage by removing 4-8 trophectoderm cells. Following biopsy, the blastocycsts were vitrified and transferred in a programmed vitrified-thawed transfer cycle in the presence of normal embryos. The age of "40" was considered as advanced female age. Statistical analysis was performed with using Chi-square test with Yates correction where p=0.01 was considered as significant.

Results & Conclusion: Out of 1520 embryos, 1478 embryos were diagnosed and 716 (48,4%) were found as chromosomally normal. 256 cycles (40,4%) were cancelled as there were no euploid embryos for transfer (Table I). The proportion of euploid embryos were significantly reduced in advanced female age group patients, where in the same group, the rate of transfer cancellation was higher (p<0,001). Neither the female age nor the quality of embryos had an effect on Mitoscore values as there were no significant differences in mitochondrial amounts in normal embryos generated from advanced female age group compared to young age group (20,8 mean vs. 20,1 mean) (ns). Despite not having the data of aneuploid embryos, the present results seem to rule out the influence of female age as a determining factor for mitochondrial content at least in euploid embryos and may indicate that elevated amounts of mitochondria/mtDNA content may be the by-products of a defective energy mechanism. Further investigation is needed to confirm these results.

Table I:

	Total	**<40**	**≥40**
No of cycles	634	392	242
Female age, mean	37	34	42
No of embryos biopsied	1520	1078	442
Diagnosed, n (%)	1478 (97,2)	1049 (97,3)	429 (97)
Normal embryos, n (%)	716 (48,4)	624 (59,5)	92 (21,4)
Cycles with planned embryo transfer*, n (%)	378 (59,6)	310 (79,4)	68 (28,1)
Cycles with cancellation, n (%)	256 (40,4)	81 (20,6)	174 (71,9)

*Cycles with at least one euploid embryo

OC-22

DETECTION LIMIT OF PARTIAL INSERTIONS AND DELETIONS FOR PGS IN TERMS OF NGS BY ANALYZING 242 EMBRYOS OF COUPLES WITH BALANCED TRANSLOCATIONS.

Blanca, Helena; González-Reig, Santiago; Penacho, Vanessa; Castejón-Fernández, Natalia; Amoros, Diego; Galán, Francisco; Alcaraz, Luis A.
Bioarray S.L., Elche, Alicante, Spain.

Introduction: Balanced translocations have a significantly impact in fertility and miscarriage risk. Preimplantation genetic screening (PGS) involves the genetic study of the embryo's genome in order to determine its chromosomal arrangement prior to selection of euploid embryos and desired successful implantation rate after *in vitro* fertilization (IVF). Initial attempts at preimplantation genetic diagnosis were limited by the inability to simultaneously evaluate aneuploidy and unbalanced translocations in all 24 chromosomes. Misdiagnosis reached up to 70% of aneuploidy in chromosomes unrelated to the rearrangement. Next-generation sequencing (NGS) platforms are more accurate and less susceptible to technical errors.

Material and Methods: This retrospective trial involved genetic testing with NGS of 242 embryos from 47 patients with Robertsonian (17) or reciprocal (30) translocations recruited from January 2016 to December 2016. After human oocyte insemination, 242 embryo single-cell or trophoectodermal biopsies were performed on day 3 or 5. Whole genome DNA was amplified by Ion SingleSeq™ Kit. Copy Number Variation (CNV) analysis was performed with Ion Reporter™ Software 5.2., which determined the ploidy status with less than 0.01X read coverage. Aberrations with different sizes and most affected chromosomes (all but chromosomes 16, 19, 20 and X and Y) were analyzed.

Results: As a result of the analysis, we obtained that 179 of the total embryos analyzed (242) were aneuploid: 25 of 179 (14%) presented trisomies, 28 (15.6%) monosomies, 35 (19.6%) full gain and losses and 91 (50.8%) had partial imbalances; 58 (63.7%) of them presented the same imbalance than respective balanced translocation carriers and 33 of them (36.27%) presented others different. A total of 63 from 242 were euploid and suitable for embryo transfer. The implantation rate was 80 % and ongoing pregnancy rate at 20weeks gestation was 60 %. The smallest aberration detected by the software was 10 Mb in size. By manual inspection, we were able to identify aberrations as small as 5 Mb. We found differences depending on the chromosome analyzed. Although reliability of NGS Ion-Torrent™ PGM platform along with low-pass whole genome sequencing for both aneuploidy and unbalanced translocations detection, the smallest fragment detected by NGS was 5 Mb.

Conclusions: PGS coupled to NGS is able to simultaneously identify aneuploidy events and unbalanced translocations, and increases the chances of obtaining a healthy newborn. It's also important to determine the detection limit of this technique, to identify not only the aberration arising from a balanced translocation, but also segmental chromosomal aberrations.

P-01

NEXT GENERATION SEQUENCING (NGS) METHODOLOGY RELIABLE DETECTS SEGMENTAL ANEUPLOIDIES WITH MOSAIC PATTERNS.

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Introduction:Preimplantation genetic screening (PGS) has become a common practice to improve outcomes in patients undergoing in vitro fertilization treatment by identifying and selecting chromosomally normal embryos for transfer. Although, a significant percentage of euploid embryos fail to result in successful deliveries. The effectiveness of PGS is dependent upon the biology of the early embryo and the limitations of the technology. Inaccurate detection of chromosomal mosaicism or segmental aneuploidies in throphectoderm biopsy are possible explanation for IVF failure after transfer of a chromosomally normal embryo. Mixture models of a mosaic trophectoderm biopsy have been useful to establish the detection limits of various CCS platforms for whole chromosome mosaic aneuploidy. However, similar data for detecting de mosaic segmental aneuploidy have yet to be investigated for any platform currently in clinical use. Here we evaluate the ability of next-generation sequencing (NGS) to detect pure and mosaic segmental aneuploidies in trophectoderm biopsies models. Material & methods: Samples composed of specific euploid/aneuploidy cells ratio (10–90% mosaicism) of 10 (n=54) and 100 (n=54) total cells were obtained and analysed with Miseq Veriseq (Illumina) platform. In addition euploid (n=6) and aneuploid (n=6) cell lines were used as control. Duplicates of eight cell lines with different structural abnormalities (from 4.5Mb to 17Mb) were used for segmental aneuploidy assessment. Mixed of two different cell lines with microdeletion (12Mb/17Mb) were used to mimic mosaic segmental aneuploidies (20%, 40%, 60%,and 80% mosaicism). Duplicates of each mix were obtained and analysed. Sensitivity and specificity of aneuploidy detection at each level of mosaicism was determined and compared with cell karyotype. CNV analysis was accomplished by current version of BlueFuse software (Veriseq). Results: In total, 136 samples were assessed for mosaicism detection, 124 chromosomal mosaics (true positive) and 12 aneuploid or euploid (true negative) samples. Sensitivity was 90% (95% confidence interval [95% CI]: 83.18% to 94.73%). The 12 false negative results obtained with VeriSeq were from samples with 10% mosaicism. Specificity was 100% (95% confidence interval [95% CI]: 73.54% to 100.00%). This study indicated a LOD of 20% for both whole and segmental mosaicism. Analysis of segmental aneuploidy in the cell lines demonstrated that VeriSeq identify microdeletion as little as 4.5Mb. No additional segmental imbalances (false positive) were identified and all mosaic microdeletion were detected. Reference curves with defined threshold for each level of mosaicism was determined for whole and segmental chromosomes. Conclusions: These findings demonstrate that VeriSeq NGS platform has high resolution for pure and mosaic segmental aneuploidies with a LOD of 20%. We have validated NGS for segmental aneuploidies with the smallest detectable size of 4.5Mb, with a concordance rate per analyzed chromosome of 100%. Segmental aneuploidy and embryonic mosaicism represent important new areas of research when it comes to determining the cause of failed implantation and delivery when an embryo that has been diagnosed as euploid is transferred. Thus, accurate detection of such aneuploidies in preimplantation embryo might improve selection of the embryos with higher developmental potential.

P-02

NEXT GENERATION SEQUENCING TO DETECT LOW GRADE MOSAICISM AND ITS EFFECT ON THE LIVE BIRTH RATE.

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INTRODUCTION:

Chromosomal aneuploidy is highly prevalent in human embryos. It is a reason for the relatively low success rates of IVF cycles. PGS by aCGH has been used to identify euploid embryos in order to improve the clinical outcome of IVF treatments. However a percentage of morphologically normal euploid blastocysts fail to implant or result in a pregnancy loss. The chromosomal mosaicism has been considered as a feasible explanation for some failures after the transfer of euploid embryos. The incorporation of NGS to PGS has improved the mosaicism detection compared with aCGH. The aim of this work is to assess if PGS by NGS is able to improve the live birth rate compared with aCGH.

MATERIAL AND METHODS

We reanalyzed by NGS trophoectoderm biopsies of day 5 and 6 blastocysts previously analyzed by aCGH (from February 2014 to March 2016). We included 85 euploid blastocysts analyzed by aCGH from patients who didn't achieve pregnancy (n=46) and patients who resulted in live birth (n=39). In all cases only one embryo was transferred. Next-generation sequencing was performed using the Veri-Seq protocol and MiSeq sequencer (Illumina). The analysis was performed using the BlueFuse Multi software (Illumina). The differences between the groups were evaluated using Pearson chi-square and t-student statistical tests (SPSSv20.0).

RESULTS

The DNA samples from trophoectoderm biopsies of day 5 and 6 euploid blastocysts analysed by aCGH (n=85) were reanalysed by NGS. We detected chromosomal mosaicism in 24 blastocysts (28.2%). In all cases the percentage of aneuploid cells was between 20% and 40% (low-grade mosaicism). Within the group of euploid embryos that didn't achieve pregnancy (n=46) we detected chromosomal mosaicism in 15 cases (32.6%), and we detected 9 mosaic embryos (23.1%) in the group of live births (n=39). The percentage of low-grade mosaic embryos seems to be higher among the patients that didn't achieve pregnancy than in those who resulting in live births (32.6% vs 23.1%), however the difference was not statistically significant (p=0.33).

There were no significant differences between the groups (no pregnancy vs live birth) with respect to maternal age (29.5 vs 29.31, p=0.89), paternal age (41.8 vs 39.5, p=0.07), MII oocytes retrieved (10.9 vs 10.7, p=0.79) and percentage of top and good quality embryos transferred (p=0.16).

CONCLUSIONS

The NGS is able to detect low-grade mosaics undetected by aCGH, however the transfer of these embryos does not modify the live birth rate significantly. The NGS is more efficient to detect mosaicism than aCGH. However, the transfer of low-grade mosaics detected by NGS does not affect the live birth rate, according to our data. Therefore, the transfer of these embryos should not be avoided until more data is obtained.

P-03

DEVELOPMENT OF A 5 HOUR PGS PROTOCOL FOR A DAY 5 FRESH TRANSFER.

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Introduction: Chromosomal aneuploidies are the main cause of abnormal development of embryos and implantation failures. Preimplantation genetic screening (PGS) allows the selection of embryos with euploid chromosomal content and increases IVF treatment efficacy. PGS microarrays are traditionally hybridised for a minimum of 3 hours to overnight. The duration of hybridisation impacts on signal intensity, with shorter times typically reducing the array signal. This study aimed to develop a novel hybridisation solution which could significantly decrease protocol duration, enabling same workday results and providing an opportunity for routine fresh transfers of PGS screened embryos.

Material and methods: Cell lysis, whole genome amplification and labelling of single cells of known karyotype (Coriell Institute for Medical Research) and reference (male genomic DNA, Promega) was performed according to the standard EmbryoCellect™ protocol (RHS TDS version 3.2). Labelled test and reference were then co-precipitated and resuspended in one of two different hybridisation solutions; solution A or B, before being competitively hybridised to the RHS EmbryoCellect™ microarray. Hybridisations were performed for incrementally shorter times (1 hour, 30 mins, 20 mins, 10 mins) at different incubation temperatures (37°C and 45°C), and samples were also assessed using the standard overnight EmbryoCellect™ protocol as a control. The microarrays were subsequently washed and scanned using an Axon 4000B scanner (Molecular Devices) according to standard RHS protocols. Average fluorescence and background signals for both the 532 (test) and 635 (reference) laser channels were analysed using the RHS Macro version 6.3.

Results: Correct results were achieved with hybridisation solution A for both 1 hour and 30 mins at 45°C, with signal intensities (F635/532) of 22648/21755 relative fluorescent units (rfu) and 19253/18963 rfu, respectively. These results with the same sample are comparable to those using a standard hybridisation performed overnight at 37°C. Solution B gave poor signal intensity results for all tested hybridisation times and analysis could not be performed on the microarrays.

Conclusions: Results from this study indicate that it is possible to use a novel hybridisation solution to screen for single cell aneuploidy using a 30 minute or 1 hr hybridisation at 45°C. The incorporation of this short hybridisation into a PGS workflow will allow same day result reporting. Further efficiencies are currently being validated with a predicted target being the achievement of a 5 hour PGS protocol.

P-04

VALIDATION OF EMBRYOCELLECT™ WITH SUREPLEX AMPLIFIED EMBRYO BIOPSIES.

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Introduction: In order to validate a PGS test clinically, the same WGA product can be split. The RHS EmbryoCellect™ kit has been extensively validated using cell lines of known ploidy. EmbryoCellect™ uses the RHS DOPlify™ WGA, which is significantly different to SurePlex, the WGA system used for other PGS kits. Validation of EmbryoCellect™ for PGS would therefore require embryo rebiopsy, which is not acceptable as the sample may be impacted by embryo mosaicism. A screening laboratory and an IVF clinic collaborated to develop a protocol for labelling and hybridisation of archival SurePlex products from blastomere and trophectoderm biopsies in order to provide an approach to clinically validating the EmbryoCellect™ microarray from the same WGA product.

Material and methods: Forty-nine SurePlex amplification products derived from blastomere (45) or blastocyst (4) biopsies were labelled using a modified EmbryoCellect™ protocol and hybridised according to standard EmbryoCellect™ protocols. The initial results for 19 samples were tested unblinded to develop and validate the new protocol, then the remaining 30 samples were tested blinded with results provided by the screening laboratory to the IVF clinic for comparison with the original 24Sure results only after the results were shared.

Results: Concordance was reported for both euploid (18/18), whole chromosome aneuploid (9/10), and complex abnormal (7/7) results for single blastomeres. Blastomeres carrying segmental aberrations or translocations (n=10) below the resolution of array were only partially concordant as expected. Concordance was reported for both euploid (2/2) and whole chromosome aneuploid (1/1) results for trophectoderm samples hybridised to the EmbryoCellect™ microarray, with one trophectoderm sample failing RHS QC acceptance criteria.

Conclusions: The compatibility and ability to interchange commercially available WGA protocols and microarray platforms with RHS technologies provides an exceptional level of flexibility to a laboratory. Cross platform protocols allow individual laboratories to tailor validation and screening to suit their need, time frame and required level of resolution.

P-05

DOES THE CHANGE OF TECHNOLOGY FROM ACGH TO NGS IMPACT ANEUPLOIDY RATES?

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Introduction: Recent advances in preimplantation genetic diagnosis for aneuploidy (PGD-A) using next-generation sequencing (NGS) promote a higher throughout and cost-efficient analysis in comparison to array comparative genomic hybridization (aCGH) screening in human embryos from in vitro fertilization (IVF) cycles. In the last years aCGH was mainly performed on day-3 biopsies and with the change of the technology to NGS, there is a current trend to combine trophectoderm biopsies and NGS. Therefore, the objective of this study was to retrospectively analyzed differences in aneuploidy rates using these two technologies with both biopsy strategies.

Material & methods: Patients were selected in Brazilian IVF clinics between January 2015 and December 2016. The average female age was 38.4 years. In total 439 patients underwent day-3 biopsies and 2.784 patients underwent trophectoderm biopsies, either on Blastocyst (day-5 or day-6). The patients were divided according to the technology applied for aneuploidy screening and the biopsy day. Four different groups were established accordingly: group A: aCGH/D3; group B: aCGH/Blastocyst; group C: NGS/D3 and group D: NGS/ Blastocyst.

Results: A total of 3223 patients were included with the following distribution among groups: A:25.8%, B:74.2%; C:11.9% and D:88.1%. And, 8234 embryos were analyzed (A:996; B:2887; C:520; D:3831). The average woman age in each group was: group A:38.8±3.9; group B:38.4±4.1; group C:39.6±3.7; and group D:38.2±3.8, with significant differences only between groups A and C (*P=0.043)*. The comparisons among groups has shown significant differences in aneuploidy rates between groups A and C (82.5% vs. 76.5%; *P=0.006*) and between groups B and D (61.3% vs. 58.1%; *P=0.007*). Segmental aneuploidy rates between A and C biopsied embryos were 0% and 1.7% (*P=0.006*) and between B and D biopsied embryos were 0% and 2.2% (*P=0.008*). For complex aneuploidy (>5 aneuploid chromosomes), a significant decrease was observed with NGS for both D3 (14.5% vs. 7.5%; p=0.0002) and D5 biopsies (2.9% and 1.1%; p<0.0001).

Conclusions: In conclusion, the results achieved in this study has shown a slight but significant decrease in NGS for the detection of whole chromosome aneuploidies. This decrease could be mostly related to the decrease in the percentage of embryos showing a chaotic pattern with NGS compared to aCGH in both biopsy types. However, NGS has shown a higher detection potential for segmental aneuploidies. Follow-up studies of the clinical outcomes and molecular analysis of products of conception would reinforce the potential benefits of NGS for the selection of embryos with the higher implantation potential.

P-06

MATERNAL AGE TRIGGERS THE FORMATION OF CHROMOSOMAL LOSSES MORE THAN GAINS AND/OR SEGMENTAL ANEUPLOIDIES IN PREIMPLANTATION EMBRYOS.

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Introduction: Female age is the most profound effect in the formation of chromosomal aneuploidies in preimplantation embryos.Here we aimed to determine the impact of female age on the formation of specific sub-types of chromosomal abnormalities.

Materials and methods: 23,690 chromosome pairs belonging to 1030 embryos from 411 aneuploidy screening cycles between 2013-2016 were analyzed retrospectively.Cycles with structural chromosomal abnormalities were not included.Biopsy was done at blastocyst stage by removing 4-8 trophectoderm cells. Comprehensive chromosomal screening (CCS) was performed by array comparative genomic hybridization (aCGH) technique.The results of each chromosome-pair were grouped into 4 categories as a-'normal' (no detectable numerical or segmental abnormality in all 23 chromosome-pairs, b-'loss' (chromosomal loss, either nullisomy or monosomy), c-'gain' (chromosomal gain, either trisomy or tetrasomy) and d-'segmental'(deletion and/or duplication of chromosomal segments).In order to assess the impact of maternal age, embryos were grouped into 3 categories according to the maternal age as i.<35 years, ii.35-39, and iii.40-46.Statistical analysis was performed with using Chi-square test with Yates correction where p=0.01 was considered as significant.

Results: The extensive chromosomal assessments showed that chromosomes 21>16>22>20>18 are the ones that were more error-prone among other chromosomes;13.9%,12.5%,11.2%,10.9%,7.5% respectively.A highly noticeable increase in the tendency of each individual chromosome to be abnormal (loss, gain or segmental aneuploidy) begins only after 40 years of female age, but not before (8,6% vs 6% and 5,4%, p<0.0001)(table I).While the probability of a whole chromosome loss/gain are increasing with advancing age (p<0.01) (table I), the proportion of chromosome losses among all sub-types was more profound (p<0.0001), (table I).No noticeable change was observed for segmental aneuploidy with advancing female age.

Conclusion: The effect of maternal age is more recognizable after age 40, which could thereby be used as a more realistic limit to define "advanced female age".As the proportion of chromosomal abnormalities (both gains and losses of individual chromosomes) are increasing with advancing female age, the biggest contribution comes from chromosomal losses (rather than gains) which might suggest a mechanism in the older oocytes favoring the disposal of chromatid(s) to polar bodies instead of keeping them inside after events of non disjunction of chromosomes or premature separation of sister chromatids.Finally, the results showed that the formation of segmental aneuploidies could be caused by a mitotic mechanism rather than meiotic since their presence was unaffected by female age.Data from different cell stages should also be evaluated and compared with the present data in order to draw a more comprehensive conclusion on the complex behavior of chromosomes in the preimplantation period.

Table I:

Maternal age, (min-max)	**21-35**	**35-39**	**40-46**	**p-value**	**Total (21-46)**
no of CPs assessed	10235	7981	5474		23690
Losses per CPs (%)	1,4	1,8	3,3	<0.01	2,0
Gains per CPs (%)	3,1	3,6	4,9	<0.01	3,7
Segmentals per CPs (%)	0,9	0,6	0,4	ns	0,7
Total chromosomal abnormalities per CPs (%)	5,4	6	8,6	<0.0001	6,3
sub-type analysis					
loss/total abnormalities (%)	*26,7*	*29,8*	*38,3*	<0.0001	*31,4*
gain/total abnormalities (%)	*57,3*	*60,3*	*57*	ns	*58,2*
segmental/total abnormalities (%)	*16*	*9,9*	*4,7*	ns	*10,5*

CPs: chromosomal pairs

P-08

CATTLE KARYOMAPPING TO OPTIMISE FOOD PRODUCTION AND DELIVERY OF SUPERIOR GENETICS: THE FIRST LIVEBORN CALVES.

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Introduction

Karyomapping was originally developed as a universal means of detecting monogenic and chromosome disorders simultaneously in human IVF embryo using SNP chips. The major non-human applications of SNP chips however lie in animal breeding. Traditional practice in the UK beef and dairy breeding industry involves the selection of dam (female) and sire (male) lines based on phenotypic progeny testing of live born animals. Genomic estimated breeding values (EBVs) are increasingly identified through SNP chip interrogation strategies, especially for sires.

With continued advancements in multiple ovulation and embryo transfer (MOET), ovum pickup (OPU) and *in vitro* production (IVP) of embryos (analogous to superovulation, egg collection and IVF in humans), the ability to produce a greater number of genetically superior animals has enabled significant improvements to beef and dairy production. However, the combination of inherited parental genotypes is random and therefore the inheritance of specific traits is not guaranteed. Furthermore, live birth rates of IVP embryos remain relatively low, indicating poor ability to select the 'healthiest' embryos using commonly applied morphology grading systems.

Materials and Methods

Combining SNP chip genotyping with IVP technology (generating EBVs from embryos rather than liveborn animals) is a much-needed advance as it significantly increases selection intensity whilst shortening the generation interval, thereby expediting the introduction of new genetics into the supply chain. To this end, we have adapted PGD technology involving IVP of cattle embryos, blastocyst biopsy, whole genome amplification then interrogation of SNP ships for the screening of cattle embryos. Simultaneous genotyping for EBVs is possible, as is aneuploidy screening using karyomapping. This approach complements traditional morphology screening to enable the selection of embryos with the best chance of survival to term, and ensures that resulting calves are proven carriers of desirable traits (e.g. those associated with health, welfare and productivity). Moreover, Karyomapping effectively acts as a genetic fingerprint to confirm the diagnosis of the embryo by matching it with that of the live born calf.

Results

At time of writing, 77 embryos have been genotyped in this way and we have made the first preliminary estimates of aneuploidy rates and high-resolution recombination patterns in cattle. A total of 50 embryo transfers have been made, of which 18 led to pregnancies: 9 of these are ongoing, 4 aborted, 5 were born but 1 died soon after birth of a post-natal infection. More transfers and pregnancies are imminent and we continue to improve protocols. In the most recent run, following transfer of 2 chromosomally normal blastocysts, 2 live births of phenotypically normal Holstein calves ensued.

Conclusions

The results herein presented represent the first examples of the use of karyomapping for non-human purposes and will allow the study of aneuploidy and recombination in a physiologically relevant animal model.

P-09

PREIMPLANTATION GENETIC DIAGNOSIS FOR TRANSLOCATIONS AND INTERCHROMOSOMAL EFFECT ASSESSED BY ARRAY CGH.

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Introduction: Heterozygotic carriers of translocations have a normal phenotype but they have an increased risk of having fertility problems, recurrent miscarriages or offspring with an unbalanced chromosomal rearrangement. This is due to the fact that they produce a high percentage of unbalanced gametes as a result of different types of segregation during the meiosis. Some authors have suggested the possibility that the chromosomes involved in these types of rearrangements could interfere with the correct segregation of other chromosomes not implicated in translocation. This is known as interchromosomal effect (ICE). Several studies on ICE have been published with ccontroversial results. It has been proven that preimplantation genetic diagnosis (PGD) with comprehensive chromosome screening techniques in these patients led to an improvement in their reproductive expectations. This work shows our results of PGD performed with array *CGH* (aCGH).

Material & methods: This retrospective study compiled 300 cycles with a day-3, day-5 or day-6 biopsy. Clinical indications for PGD were the presence of a reciprocal translocation carrier in 122 couples (n=155 cycles) or a Robertsonian tanslocation carrier in 119 couples (n=145 cycles). Whole Genome Amplification (WGA) was performed and samples and control DNA were labeled with Cy3 and Cy5 fluorophores, mixed according to the manufacturer's instruction and hybridized for 6–12 hours on 24sure+ arrays (Illumina, San Diego, CA). The hybridized slides were washed, dried and then scanned with a two-channel laser scanner (Powerscanner, TECAN, Mannedorf, Switzerland). BlueFuse Multi software (Illumina, San Diego, CA) was used for data processing.

Results:

	Robertsonian translocation		Reciprocal translocation	
	D3	D5	D3	D5-6
Cycles	137	8	106	49
Mean female age, years (SD)	34.5 (4.5)	35.9 (5.2)	36.0 (4.1)	35.3 (4.2)
No. of informative embryos (%)	857 (97.2)	46 (100.0)	725 (96.8)	184 (98.9)
No. of abnormal embryos (%)	637 (74.3)[a, c]	27 (58.7)[a, d]	648 (89.4)[b, c]	147 (79.9)[b, d]
No. of aneuploid balanced embryos (%) (ICE)	289 (33.7)[e]	16 (34.8)	157 (21.7)[e]	39 (21.2)
No. of aneuploid unbalanced embryos (%) (ICE)	118 (13.8)[f]	3 (6.5)	212 (29.2)[f]	43 (23.4)
No. of cycles with transference (%)	90 (65.7)	4 (50.0)	42 (39.6)	16 (32.7)
Clinical pregnancy rate/embryo transfer	48.9	75.0	50.0	43.8
Implantation rate	41.8	83.3	47.1	33.3

[a, b, c, d, e, f]: $p<0.05$ *(Chi-square test)*

Conclusions: For both biopsy strategies, we observed a high percentage of abnormal embryos in both types of translocations, significantly higher in reciprocal compared to Robertsonian translocations. A high number of embryos with aneuploidies for chromosomes not involved in the translocations were observed. ICE was significantly higher on day 3 for Robertsonian compared to reciprocal translocations for aneuploid balanced embryos, whereas ICE was significantly higher in reciprocal translocations for aneuploid unbalanced. No differences in ICE were observed on blastocyst biopsies, probably due to the lower number of cases included. Selection of balanced/euploid embryos in these patients resulted in high ongoing pregnancy rates.

P-10

INTRODUCTION OF A NOVEL, UNIVERSAL NGS-BASED RESEARCH METHOD FOR PREIMPLANTATION GENETIC DIAGNOSIS AND SCREENING.

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Introduction

Over the past few years, several new technologies have been developed for the high resolution molecular cytogenetic analysis of embryos prior to implantation. Although technologies such as STR-PCR, SNP arrays and aCGH have been utilized for the detection of mutations associated with Single Gene Disorder (SGD) and/or translocations for Preimplantation Genetic Diagnosis (PGD), significant challenges persist. To overcome these challenges, we have developed a novel NGS-based method coupled with specific algorithms that utilize parental haplotype information to detect SGD-associated mutations and translocations. Our approach can be successfully applied both to single blastomeres and trophectoderm cells, and does not require gene-specific spike-ins, thereby making it highly versatile assay. Furthermore, our approach can concurrently detect aneuploidies, thus combining PGD and Preimplantation Genetic Screening (PGS) in one method.

Material and methods

To evaluate the performance of our method, we processed embryo biopsies harboring SGD-associated mutations and translocations. Biopsies included blastomeres and trophectoderm cells. The data were analyzed using the novel algorithms we developed, and the results were compared to those obtained using a validated technology in a reference lab. Additionally, we also evaluated the performance of our assay using cell lines that harbor aneuploidies.

Results

Our analysis on a diverse set of embryos that included single blastomeres and trophectoderm biopsies showed excellent concordance with results from a validated method from a reference lab. Additionally, we observed excellent concordance for a wide range of chromosome aneuploidies from cell lines. We will present these data as well as incidental findings revealed in the course of our analysis.

Conclusions

Our research method provides a novel, universal end-to-end NGS workflow for PGD and demonstrates the power of combining PGS with PGD. Furthermore, our method can be readily adapted to perform PGS alone using the same underlying workflow.

P-11

VALIDATION OF TWO WHOLE GENOME AMPLIFICATION METHODS FOR PGD ON MONOGENETIC DISEASES AND ANEUPLOIDY SCREENING.

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Introduction: It is increasingly common to perform preimplantation genetic diagnosis (PGD) for both monogenetic disease and aneuploidy screening on a single embryo biopsy sample. However, different whole genome amplification (WGA) methods are adopted for this combined testing. Our experience showed that WGA used in aCGH was suboptimal for PGD by linkage analysis and gap-PCR (unpublished). Recently, next generation sequencing (NGS) has been used in aneuploidy screening as it allows a high throughput at a cost lower than aCGH. Here we compared two WGA methods on performance of linkage analysis and clinical use of the better performed method was validated on aneuploidy screening by NGS with prenatal samples of known karyotypes.

Materials & methods: Prenatal chorionic villi samples (CVS) which has underwent karyotyping (47,XXY) was collected for testing. Single cell was isolated under microscope and amplified by two WGA methods (WGA I - SurePlex DNA amplification system, Illumina; WGA II - Repli-g Single cell, Qiagen). Genomic DNA (gDNA) was extracted from the remaining chorionic villi cells. Linkage analysis was performed on chromosome 11, 14, 15 and X. To further validate WGA II on aneuploidy screening, prenatal placental samples which underwent karyotyping (47,XY,+15; 47,XY,+16; 47,XY,+18) was collected (n=3, 5-cells each) and WGA was performed by WGA II followed by aneuploidy screening on NGS platform (Veriseq, Illumina) accordingly to the manufacturer's protocol.

Results: Linkage analysis on WGA II gave concordant results with gDNA on all 4 panels of microsatellite markers. WGA I showed frequent failure in amplification of microsatellite markers (14/30, 47%) and produced stutter peaks. When applying WGA II on aneuploidy screening, 100% of samples (9/9) showed concordant results on all chromosomes analyzed, when compared with cytogenetic result.

Conclusions: Repli-g Single cell provides good quality linkage analysis results comparable to those from gDNA. It also showed concordant results on aneuploidy screening using NGS. Its use enables integration of the existing linkage analysis panels for PGD on monogenetic diseases with aneuploidy screen by NGS, with no additional bioinformatics manipulation. This protocol has been used in PGD for combined monogenetic diseases detection and aneuploidy screen in 14 treatment cycles on 10 monogenetic diseases and results in an excellent on-going pregnancy rate of 80.0 % (vs 38% with PGD only).

P-12

INCIDENCE OF CHROMOSOMAL ANEUPLOIDIES AT EMBRYONIC LEVEL WITH COMPARISON BASED ON TYPE OF BIOPSY AND MATERNAL AGE: FIRST INDIAN EXPERIENCE.

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Introduction

Aneuploidy is the most frequent type of chromosomal abnormality in human oocytes resulting in early pregnancy loss, miscarriage, stillbirth, and developmental defects in fetuses and are common in preimplantation human embryos. Preimplantation genetic diagnosis for aneuploidy screening (PGD-A) has been available worldwide from many years and it is offered in many IVF centers before transfer of embryos to improve IVF outcome of certain groups of patients as it helps to identify the aneuploid embryos, to improve implantation and decrease miscarriage complications during the pregnancy. PGD-A facility was not available in India before year 2013, so the IVF center were not able to utilize this program. The present study is the first of its kind from India evaluating the incidence of aneuploidies along with the comparing based on maternal age and type of biopsy used in IVF patients after performing PGD-A for 24-chromosomes.

Material & methods

This is a retrospective cohort study of 868 cycles including 825 patients (median age 34.7±4.2 years, range 25-44) undergoing PGD-A for different indication including advanced maternal age, repetitive implantation failure, severe male factor infertility and recurrent miscarriage. Patients were divided in different age groups (25-26, 27-28, 29-30, 31-32, 33-34, 35-36, 37-38, 39-40, 41-42, 43-44). In total 4993 embryos were biopsied after IVF-ICSI, which include 3514 cleavage stage and 1479 blastocyst stage embryos. Blastomere biopsies were performed on Day-3 and trophectoderm biopsies were performed on Day 5 of development or Day 6/7 for slower growing embryos. The method involved whole genome amplification followed by both NGS (Ion torrent PGM (Life technologies) and array-CGH (Illumina) technologies. Copy number variation analysis was accomplished using BlueFuse Multi software and Ion reporter server.

Results

Of those 4993 embryos, 33.6% were euploid, 66.4% were aneuploid and 7.8% were not analyzed. No significant difference was seen in number of informative embryos for cleavage stage (92.3%) and trophectoderm biopsy (91.9%). A significantly larger proportion of embryos were euploid on trophectoderm biopsy (50.0%) compared with cleavage stage biopsy (26.8%, $p<0.05$). Significant difference was seen in euploid embryos identified in trophectoderm biopsies for each age group between 25-42 years as compared to cleavage stage biopsies. No significant differences were observed for the age group of 43-44 years. Out of overall 66.4% aneuploid embryos, 20.8% were complex aneuploid (with more than five aneuploidies). Complex aneuploidies were reported more in cleavage stage (17.9%) than the blastocyst stage (4%) embryo, accounting for the main differences in the percentage of abnormal embryos in the two biopsy types.

Conclusions

Maternal age and type of biopsy do not affect the informative rate of embryos. However, for aneuploidy rates and the percentage of complex aneuploidies, a significantly higher percentage was observed at cleavage stage. Combining trophectoderm biopsy and aneuploidy assessment by PGD-A can provide a more efficient means of achieving euploid pregnancies in IVF.

P-13

NGS-ANALYSIS OF CHORIONIC VILLI OF MISCARRIAGES AND CONFORMING TROPHECTODERM CELLS OF TRANSFERRED BLASTOCYSTS.

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Introduction

Chromosomal abnormalities are the most common cause of first-trimester miscarriages. The purpose of PGS/CCS methods is a selection and transfer of the euploid embryo in order to increase the success rate of IVF cycles. However, the observed pregnancy rate per transfer of normal/balanced embryos diagnosed by aCGH in a group of patients with RIF (N = 633) is 49% with the miscarriage rate of 12% (unpublished own data). Nowadays, the potential role of chromosomal mosaicism (<50%) in miscarriage and implantation failure due to embryo development arrest should be considered. NGS method has the highest sensitivity of mosaicism detection when compared with other PGS/CCS methods. Thus, reexamination of WGA-PGS samples of trophectoderm cells and products of conception of miscarried pregnancies after transfer of euploid embryos diagnosed by aCGH allows studying the role of mosaicism in successful pregnancy achievement.

Material and Methods

We performed NGS reanalysis (VeriSeq, Illumina) of 20 samples of chorionic villi of miscarriages (with normal conventional karyotype) after embryo transfer of euploid blastocysts diagnosed by aCGH followed by NGS reanalysis of the corresponding (N=20) euploid samples (according to aCGH) of WGA-DNA of trophectoderm cells of transferred blastocysts.

Results

- 2 samples (10%) of chorionic villi of miscarriages after transfer of euploid embryos had undetected by conventional cytogenetics mosaicism: one case of mosaic trisomy 13 and one case of mosaic monosomy 20;
- 2 samples (10%) of trophectoderm cells of transferred blastocysts had mosaicism undetected by aCGH: one case of mosaic trisomy 1 and one case of double mosaic monosomies 7 and 8. However, products of conception of miscarriages after transfer of these blastocysts had euploid NGS-profiles without mosaicism;
- profiles of 2 samples (10%) of the euploid WGA-DNA samples, which have been characterized by partial DNA degradation (aCGH), had more indicative “wave” profile in NGS reanalysis;
- in 3 cases (15%) of WGA-DNA samples diagnosed as euploid by aCGH a mosaic form of trisomy 19 was detected by NGS. However, chromosome 19 is the most difficult for normalization due to the high CG-content. Therefore, the observed abnormality may be non-specific due to suboptimal WGA-product and the increase in the noise level.

Conclusions

The reanalysis of trophectoderm cells and products of conception of miscarriages indicates that previously undetected mosaicism may be implicated in pregnancy achievement and carrying. Nevertheless, there is an ambiguous interpretation of mosaicism, which depends on a technique and subjective interpretation of the results. This excludes the possibility of direct comparison of results obtained by different researchers. Mosaicism in trophectoderm cells should further be investigated by appropriately designed studies on larger samples before specification of clinical recommendations regarding tactics of transferring embryos with the mosaic profile and/or DNA degradation.

P-14

RE-ANALYSIS OF ANEUPLOID EMBRYOS AFTER TE-BIOPSY AND ACGH BY NGS ON INNER CELL MASS BIOPSY.

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Introduction: The primary goal of PGS in IVF is to select euploid embryos for transfer to result in a healthy and successful pregnancy. Trophectoderm (TE) biopsy at the blastocyst stage is nowadays the gold standard for sampling, being less invasive compared to blastomere biopsy on cleavage stage embryos and with lower frequency of mosaicism. However, it is still unclear to what extent mosaicism at the blastocyst stage and especially in TE cells is present and whether the genetic information of the (biopsied) TE cells reflect the genetic constitution of the inner cell mass (ICM). Our study aimed to re-analyse TE-biopsied blastocysts tested as aneuploid by aCGH. Re-analysis was performed on cells biopsied at the ICM region and analysed using low-pass next generation sequencing (NGS).

Material & Methods: Our study was approved by the local ethical committee (Etická komise FN a LF UK v Plzni, decision from 06.10.16). Written informed consent to re-analyse aneuploid tested embryos for this study was given by the patients. A total of 12 blastocysts diagnosed with non-mosaic, *de novo* single whole chromosome aberrations by aCGH (24sureV3/24sure+, Illumina) after TE-biopsy (approximately 8 TE cells) were included. For re-biopsy, cells at the region of the ICM were mechanically removed (4-20 cells) and analysed by NGS using VeriSeq platform (Illumina).

Results: We could confirm the previous results analysed on TE cells using aCGH in 8 out of 12 (67 %) ICM samples using NGS. Thereof, 7 ICM samples showed a non-mosaic constitution of the single whole chromosome aberration, whereas in 1 sample the aberration was observed in mosaic (70% cells with the noted aneuploidy). However, in 2 ICM samples (17%), we did not find the original TE detected aberration but a more complex chromosomal rearrangement involving other chromosomes. Further, in 2 other samples (17%) neither the original change nor other *de novo* aberrations were found.

Conclusion: We found full concordance of aneuploidy detected by TE cells analysis using aCGH in 67% of ICM samples analysed by low-pass NGS. However, in 33% of the samples discordant results were obtained, indicating the occurrence of mosaicism in these blastocysts, with unequal distribution of different cell lines within the embryo. Little is still known about concordance between genetic testing in TE and ICM cells and the true prevalence of mosaicism in human blastocysts. The relatively high prevalence of discordant results between TE and ICM cells might on the one hand indicate that part of aneuploid tested embryos by TE-biopsy might develop fully normal as ICM is not affected by the aneuploidy. On the other hand the observed prevalence of mosaicism might be overestimated as we tested only embryos with *de novo* chromosomal changes in TE samples. One could suggest that chromosomal instability might be higher in these embryos. These findings however should be confirmed in a large data set, including a more detailed examination and sampling.

P-16

TRANSFER OF ANEUPLOID EMBRYOS FOLLOWING PREIMPLANTATION GENETIC DIAGNOSIS: THE ADDED VALUE OF A HAPLOTYPING-BASED GENOME-WIDE APPROACH.

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Introduction

Embryo selection for monogenic diseases has been mainly performed using targeted disease-specific assays. Recently we have developed haplarithmisis, which is based on genomic haplotype reconstruction of cell(s) biopsied from embryos. This provides information not only about the inheritance of Mendelian disease alleles, but also about numerical and structural chromosome anomalies and haplotypes genome-wide. Reflections on how to use this information in the diagnostic laboratory are lacking.

Material and methods

Here we present the clinical outcome of 164 embryo transfers following PGD for monogenic and/or chromosomal disorders using haplarithmisis. Given that (1) our main aim is selection against the genetic disorder that occurs in the family and (2) embryo selection is performed on day 3 biopsied material, we opted for a relaxed aneuploidy testing model, according to which only embryos carrying viable trisomies and trisomies of meiotic origin are excluded from transfer. Embryos were approved for transfer based on (1) the haplotype in the region of interest, (2) genome-wide copy number profile and (3) embryo morphology at the blastocyst stage.

Results

From January 2015 until December 2016, 164 embryos had been transferred in 152 single and 6 double embryo transfers. This led to 44 singleton and 2 twin pregnancies which gives a pregnancy rate of 30% per embryo transfer. Interestingly, 38 embryos with one or more aneuploidies had been included in the transfers, 6 of which gave rise to a clinical pregnancy and resulted in the birth of 4 healthy babies and 2 ongoing pregnancies.

Conclusions

Haplarithmisis allows the distinction of meiotic and mitotic trisomies enabling the use of embryos that would have otherwise been discarded as inappropriate for embryo transfer. With regard to pregnancy outcome, our data show an improved pregnancy rate per embryo transfer in case of chromosomally normal embryos (33% versus 15.8%). Importantly, a remarkable 15.8% of the aneuploid embryos seem to be leading to normal pregnancies and life births, stressing the value of correct interpretation of detected aneuploidies on day 3. Both findings underline the added value of genome-wide based haplotyping used in preimplantation genetic diagnosis.

P-17

NGS ANEUPLOIDY SCREENING IN EMBRYO CELLS AND MISCARRIAGE MATERIAL.

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Introduction: NGS is increasingly used for aneuploidy screening of preimplantation embryos (trophectoderm cells). Detection of aneuploid embryos and transfer of only healthy ones allows increasing IVF efficiency and decreasing risk of sick baby birth. Besides, this method allows diagnosing chromosomal pathology in other tissues. In group of companies "Mother and child" (Russia) we also use NGS for aneuploidy detection in miscarriage material (chorion cells). Analysis of miscarriage pregnancies causes gives the possibility to individualize tactics of future pregnancy care.

Materials and methods: "Mother and child" group of companies (31 clinics) uses NGS (next generation sequencing) for PGS beginning from January 2016. The study included 303 women from 22 to 49 years, mean 35,9 years. 910 embryos were analyzed. Also study included 106 samples of chorion cells from miscarriage pregnancies. Mean age of women was 34,6 years (ranged from 18 to 45 years). Mean term of pregnancy termination was 8,6 ± 2,6 weeks (M ± SD).

Results: genetic pathology was found in 567 of 910 trophectoderm samples (62,3%). Aneuploidy of more than one chromosome was higher in women older than 40 years in comparison with women younger than 35 years ($p = 0.0412$). The most often aneuploidy was seen in 15, 16, 21 and 22 chromosome. Mosaicism frequency in women older 40 years old was statistically significantly lower in comparison with women younger than 35 and 35-40 years ($p < 0,01$ for both groups), but trisomy frequency was highest in women older 40 years old.

Chromosome pathology was found in 67 samples of chorion (63,2%). Aneuploidy frequency in women younger 35 years, 35-40 years and older than 40 years was 57,5%, 60,9% and 81,2%, respectively. The most often aneuploidy was seen in 16 and 22 chromosomes (18,2% for both cases).

Conclusion: results of this study suggest that NGS in PGS can be used successfully for prophylaxis of birth of healthy offspring in the ART program as for aneuploidy detection in miscarriage pregnancies.

P-18

WHAT NEXT GENERATION SEQUENCING BRINGS TO PREIMPLANTATION GENETIC TESTING.

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Introduction: Preimplantation genetic testing was a controversial test during an era when a limited number of chromosomes was screened using FISH technology. Advances in technology and availability of comprehensive chromosome screening led to a more widespread use of preimplantation genetic testing in IVF applications thus the test became more reliable and better accepted by IVF groups.

Next generation sequencing technologies which caused a paradigm shift in the field of genetic diagnosis started to have a huge impact in reproductive genetics. Preimplantation genetic testing became more attractive and efficient due to advantages of next generation screening technology over array CGH technology. We have started to use NGS technology more efficiently compared to array technologies in our genetic diagnostics laboratory because of its advantages and in this study we present data of 1245 embryos of 470 patients screened using NGS technology.

Materials & Methods: All single cells were collected in 2µl PBS solution. Whole genome amplification procedure was performed using SurePlex DNA Amplification Kit (Illumina, Inc). Amplified samples were processed with VeriSeq PGS kit (Illumina). Protocol was performed according to instructions of VeriSeq PGS workflow (Illumina, Inc.). The following bioinformatics analysis was accomplished with a pre-release version of BlueFuse Multi for NGS (Illumina, Inc.).

Results: 1245 embryos of 470 patients (which had 4,15 previous IVF trial on average) biopsied at the blastocyst stage were screened. 1202 embryos were intrepeted and resluts revealed that 36.9% of these embryos had normal chromosomal structure, whereas 19.3% were aneuploid and 34.8% were complex aneuploid. Mosaicism was detected in 2.2% of embryos.

In the analysis performed according to the age groups, pregnancy rate per embryo transfer was 62% in the group of patients under 35 years of age, whereas embryo transfer was cancelled in 13% of these patients due to lack of euploid embryos .

Similar pregnancy rate per transfer was achived in patients of 35-39 years. On the other hand and increase to 23 % ET cancellation was observed due to lack of euploid embryos. Pregnancy rate was 53% in the group of patients over 40 years and embryo transfer was cancelled in 46 % of patients.

Conclusion: Results obtained in this study suggest that PGS technology increases the pregnancy rates, especially in the patients with advanced maternal age (>40 years) . It should also be highligted that in this patient group ET cancellation due to lack of euploid embryos is quite high (46%). However, PGS prevents the patient's unnecessary anticipation and the success of pregnancy rate is higher compared to convential IVF group in the cases whare an euploid embryo is present. Mocaicism frequency was reported to be independent of PGS indication and maternal age (Grifo & Munne et al, 2015). It was also reported that NGS technology detects mocaicism better compared to array technologies due to its more dynamic evaluation interval. The results of this study are in agreement with these reports and a better detection of mosaicism was observed.

P-19

IMPLEMENTATION OF THE NEXT GENERATION SEQUENCING BASED PREIMPLANTATION GENETIC SCREENING IN CLINICAL PRACTICE: CHALLENGES AND BENEFITS.

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Introduction: The success of an in vitro fertilization (IVF) is based on variety of factors, mainly blastocyst morphology and the chromosome status of the embryo as well as an adequate endometrial receptivity. The implementation of molecular genetic assays in the assisted reproduction (AR) field worldwide allows preimplantation embryo karyotype examination via preimplantation genetic screening (PGS) techniques. The focus in the PGS/PGD field has now shifted from day 3 blastomere biopsy to day 5/6 trophoectoderm (TE) sampling and utilization of comprehensive chromosome screening technology as microarray Compartive Genomic Hybridization (aCGH) or Next Generation Sequencing (NGS) in order to provide a more accurate assesment of implantation and developmental potential of embryos. The TE as biopsy material and NGS as high throughput genetic screening technique are the logical algorithm in the successful ART strategy. In the present study we report our experience on implementation these methods in the clinical practice.

Material & methods: Whitin the spector of the present study there are eighteen embryos from three couples with clinical history of unsuccessful IVF attempts. The samples were taken vie laser assisted biopsy. Twelve of the embryos were biopsied on day 5 and the rest on the day 6 post ICSI. Whole genome amplification (WGA) with SurePlex kit (Illumina Inc., USA) was performed according to manifacturer's instructions. The amplified DNA was subjected to copy number counting using VeriSeq NGS kit (Illumina, Inc.). Blastocyst were vitrified after biopsy with Cryotop open system method (Kitazato, Japan).

Results: From the eighteen biopsied embryos, five (28%) were with euploid karyotype. The rest were with different chromosome anomalies: eight (45%) of them were aneuploid and four were chaotic. Per chromosome, NGS concordance was 99.20%. In part of the samples mosaicism, subchromosomal shifts with unknown clinical significance and artefacts from the specificity of the assay were observed.

Conclusions: The NGS technology gives us opportunity to detect aneuploidy with more accuracy and it also may have the potential to provide more detailed insight into other aspects of embryo genome. The demonstrated method increased the dynamic range enabling enhanced detection of mosaicism in multicellular samples and the potential automation of the sequencing library preparation to minimize the human factor in the technical performance. Compared to aCGH based PGS, NGS provides not so labor intensive and more accurate solution for karyotyping preimplantation human embryos. More data and analysis are needed in order to have the chance to clarify the new aspects of the generated results based on NGS platforms.

P-20

A SINGLE BLASTOCYST BIOPSY RESULTING IN A MOSAIC AND EUPLOID PROFILE: A CASE REPORT.

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Introduction: Next generation sequencing (NGS) has enabled the determination of genetic mosaicism. However, the subjective interpretation of a mosaic profile, resulting in a non-transferrable embryo, can significantly impact a clinical decision. Published reports of viable pregnancies following the transfer of mosaic embryos questions the clinical significance of mosaic findings. The role of microsurgical manipulations during the biopsy procedure has not yet been considered as a potential source of error creating a mosaic profile. The purpose of this case report is to critically evaluate a specific incidence of mosaicism.

Materials and Methods: Patient elected to have PGS performed. A single hatching blastocyst was biopsied and trophectoderm cells isolated. Upon cell loading, the sample was not visualized in the PCR tube. To insure a result, the embryo was re-biopsied and another, lesser quality, sample taken and successfully visualized when placed into a second PCR tube. Both samples were snap frozen and later tested by NGS (VeriSeq™, Illumina). The blastocyst was vitrified post-biopsy, subsequently warmed, diluted and then transferred (VFET cycle).

Results: Differential findings resulted. The first sample generated a clean profile assessed as euploid. Whereas, the second sample produced a mosaic profile, with the greatest loss being over 20%, but below 40%, for chromosomes 5, 10 & 11. However, a decision was made to transfer the single blastocyst, and an ongoing pregnancy (>10 weeks) with cardiac activity was attained.

Conclusion: Low level mosaicism has been reported as clinically relevant in the literature. This limited report indicates biopsy technique and cellular health as a possible factor in producing mosaic profiles. Furthermore, this case report highlights that technical and tube loading parameters are important variables in PGS testing. We also question if putative low level mosaics are potentially noise resulting from whole genome amplification, and thus should not carry overtly critical clinical significance. This case report gives hope to a better understanding of why mosaicism can appear on NGS profiles. In addition, it not only offers justification for more closely evaluating biopsy and cell transfer technique, but also provides evidence for expected pregnancy outcomes when transferring mosaic embryos.

P-21

MITOCHONDRIAL GENOME COVERAGE FOR COPY NUMBER DETERMINATION AND DETECTION OF DISEASE; THE IMPACT OF WGA.

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Introduction: Mutations in the mitochondrial genome have been linked to diseases such as cancer, diabetes and deafness. Additionally, recent data suggests that mitochondrial genome load can impact implantation potential of euploid embryos. The selection of embryos for IVF transfer using the additional information from mitochondria requires an accurate and high coverage WGA methodology. The mtDNA genome is 16,569bp in length and there are multiple copies per cell, providing a model to evaluate performance metrics of whole genome amplification (WGA) technologies. This study aimed to compare two different commercially available WGA kits; PicoPlex® (Rubicon Genomics) and DOPlify™ (RHS Ltd), evaluating overall mtDNA genome coverage along with coverage of 23 common mitochondrial mutations using NGS of whole genome amplified single cells.

Material and Methods: Single cells sorted from an aneuploid cell line (Coriell Institute for Medical Research) were subjected to WGA using DOPlify™ (n=2) and PicoPlex® (n=2) according to manufacturer's instructions. Nextera libraries (Illumina) were prepared from 50ng WGA DNA with a total of 23 samples subsequently multiplexed and sequenced on a NextSeq platform according to standard 2x150bp protocol (Illumina). The sequencing data was bioinformatically aligned to hg19 then analysed to determine mitochondrial genome coverage.

Results: The number of total reads for each of the four samples was quite variable, with the PicoPlex® samples having considerably more total mapped reads than DOPlify™; 16,200,000 and 9,800,000 vs 6,500,000 and 8,200,000. Overall coverage of the mitochondrial genome for the single cells analysed was on average 33x and 1763x for PicoPlex® and DOPlify™, respectively. NGS reads for the two DOPlify™ samples covered 100% of the mitochondrial genome; with minimum depth of 53 and maximum depth of 3229. The PicoPlex® sequenced reads covered on average 79% of the mitochondrial genome. Evaluation of DOPlify™ amplified cells confirmed coverage at all 23 common mitochondrial mutation sites; with a minimum read depth of 120 and an average depth of 678 reads. For the two PicoPlex® amplified cells, reads mapped to 16/23 and 11/23 of the common mutations, however for only 6/23 and 2/23 mutations the read depth was more than 50 reads, which is within the range recommended for PGD.

Conclusion: DOPlify™ whole genome amplification resulted in significantly greater breadth and depth of coverage over the mitochondrial genome when compared to PicoPlex® and replicated all 23 common mitochondrial genome mutation sites from single cells.

P-22

INVESTIGATION OF THE RELATIONSHIP BETWEEN EMBRYO PLOIDY, NUCLEAR MITOCHONDRIAL MISMATCH AND EMBRYO MORPHOLOGY.

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INTRODUCTION: Chromosomal aneuploidies are a major cause of early reproductive losses and embryonic implantation failure. In addition to chromosomal abnormalities, poor preimplantation embryo development can be caused by maternal factors which include mitochondrial function. Proteins required for mitochondrial function are encoded by both mitochondrial DNA (mtDNA) and the nuclear DNA (nDNA) necessitating the coordination between the two genomes. It has been proposed that mitochondrial-nuclear mismatch may cause embryonic cell death. Changes in ATP synthesis and metabolic reactions in the mitochondrial electron transport chain (ETC) have been linked to female infertility. This study explores mitochondrial haplogroups and nuclear DNA haplotypes of 53 nuclear encoded genes involved in mitochondrial function, by assessing aneuploidy status and preimplantation embryo development.

MATERIALS AND METHODS: 11 fertile couples undergoing PGD for monogenic disorders and their corresponding embryos (n=57, collected on day 6-7 post fertilization) were used in this study. Parental samples were compared to a group of 12 couples with repeated miscarriage (RM) for unknown reasons. Parental mtDNAs were analysed from both groups by Long range PCR and NGS. Each sample was assigned a mitochondrial haplogroup using HaploGrep and confirmed by EMMA software. The selected 53 nuclear genes were sequenced from parental genomic DNA and from embryos following whole genome amplification by MDA using SureSelect QXT (Agilent). Embryonic aneuploidy screening was performed using the GenetiSure pre- screening kit (Agilent). Analysis of the similarity of the embryonic nuclear DNA genotypes to the maternal genotype on 53 mitochondrial related genes was carried out by logistic random effects analysis, recognizing the lack of independence of the embryos from a couple. As mitochondria in embryos are maternally derived, differences in the embryonic combination of SNPs and indels were assessed and compared to the maternal haplotype per family to identify evidence of mitochondrial mismatch in poorly developing embryos.

RESULTS AND CONCLUSION: Several mitochondrial haplogroups were identified. Overall more couples from the repeated miscarriage group (4/ 12) had identical mtDNA haplotypes between partners, compared to the fertile group (1/11). Within the fertile group the presence of the T haplogroup in one of the partners was associated with poor blastocyst formation. Aneuploidy status analysis showed that chromosomally chaotic embryos had poor morphology regardless of the presence of the mitochondrial T haplogroup. Comparison of the percentage of similarities and differences of embryonic genotypes to the maternal haplotype of the selected 53 nuclear encoded mitochondrial genes showed a marginal significant effect on embryo morphology when the indels percentages were different between the embryo and maternal haplotype (OR = 1.03 (95% CI 1.00 to 1.06), p = 0.049). As the indel percentage difference from the mother increases by one, the odds of an embryo to develop to the blastocyst stage increases by 3%. The same analysis was performed for a set of nuclear non-mitochondrial related genes (n=6) which showed no significant effect, OR = 0.998 (95% CI 0.97 to 1.02), p = 0.84. This effect was independent of the chromosomal status. Further analysis of these indels is required to determine any functional effects of these changes.

P-23

THE STRUCTURE AND LOCATION GRADATION OF OOCYTE MEIOTIC SPINDLE AND ITS RELATIONSHIP TO EMBRYOS' QUALITY AND EUPLOIDY.

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Introduction. As generally known it is important to protect the meiotic spindle from damage during intracytoplasmic sperm injection (ICSI). The polarized light microscopy could help to visualize the structure and location of the oocyte meiotic spindle and by that we could predict the outcome of the embryo development after oocyte fertilization as well as foresee its morphology and euploidy.

Material and Methods. From October to December 2016, the investigation was carried out in the Medical Centre IGR. The study involved 443 oocytes obtained from 43 women who were treated with IVF/ICSI cycles, and among them embryos gotten from 23 women (187 oocytes) were investigated using preimplantation genetic screening (PGS). The mean age of women was 30.6±4.1 years. The spindle visualization was performed using Oosight imaging systems (USA). The structure was evaluated and classified as following spindle's grade characteristics: A - compact rhomboid spindle with defined edges, B - modified spindle form with blurred edges, C - weak visualization, D - spindle on the 1st polar body border and cytoplasm (telophase I), E - spindle not visualized. The location was graded as the follows: "a" - 0°-20° due to the 1st polar body,"b"- 21°-45°,"c"- 46° - 90°,"d"- 91° - 180°. Due to spindle structure and location, we evaluated the number of embryos on cleavage stage, AA category blastocyst and amount of euploid and aneuploid embryos. For the PGS on the post-fertilization day three or five embryo biopsy was performed, samples were fixed and diagnosed using FISH on chromosomes 13, 16, 18, 21, 22, X, Y. Statistical analysis was carried out using Shapiro-Wilk test for normality, Spearman correlation and Chi-square test.

Results. Distribution of euploid embryos AA and euploid embryos at all morphological categories depending on the spindle morphology gradation demonstrated statistically significant differences from aneuploid embryos AA ($r=0.04$, $p=0.02$). At the same time 81.5% of euploid embryos AA had good quality spindle morphology -"A" and "B", but aneuploid embryos AA had only 69.4% of this, what could be explained as spermatozoa contribution to embryos' aneuploidy. Besides 2.2% of euploid embryos AA and 11.2% of aneuploid embryos AA had spindle morphology "E".

Spindle morphology of euploid embryo showed a positive correlation with the indication of its location ($r=0.36$, $p=0.05$) - better quality of spindle associated with lower index of location. Aneuploid embryos AA had all possible combinations of spindle morphology and its location parameters.

At the same time the cleavage-cell division rate is not related to the spindle structure and location - the average number of blastomeres in 72 hours of development in euploid and aneuploid embryos AA was 8.2 ± 1.2 and 8.7 ± 1.3 respectively, because this rate depends more on the spermatozoa centrosomal structure.

Conclusions. The results showed that the oocyte spindle evaluation by structure and location could predict the embryo's quality and euploidy. Spindle visualization could be introduced to every day practice in the ART clinics as a useful procedure that helps to rationally use patient's biological material and consumables, but also it makes the truthful projections to meet the patients' expectations.

P-24

ANEUPLOIDY STATUS IS ASSOCIATED WITH THE LENGTH OF TIME THAT THE PRONUCLEI ARE VISIBLE.

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Introduction

Non-invasive selection of viable embryos with the highest chance of implantation, continuing development and normal live birth is an ultimate aim of any IVF clinic. While PGS allows the screening for chromosome abnormalities, the inherent need for prior biopsy makes the technique time consuming and invasive to the embryo. Establishing morphokinetic criteria that are indicative of successful euploid future development is therefore a priority. Time-lapse imaging is a technique that allows visualisation of developing embryos, with minimal disruption to culture conditions however few morphokinetic criteria however few criteria have been associated with levels of aneuploidy.

Materials and Methods

In this study we used Embryoscope® (Vitrolife), a combined incubator and camera unit that captures images through different focal planes at 20 minute intervals. Images supplied by The Bridge Centre (London, UK) were retrospectively annotated against pre-set morphological markers; including extrusion of the second polar body, pronuclear events, cleavage checks and the start of cell compaction leading to blastulation. The embryos were from PGS cycles that had been biopsied at blastocyst stage and assayed for chromosome copy number using array Comparative Genomic Hybridisation (aCGH). Aneuploidy testing and morphokinetic profiling were completed “blond” of one another and by different individuals to avoid observer bias. Mann Whitney U tests were performed to determine any statistical significance between morphokinetics and cytogenetics.

Results

The results of this study showed a statistically significant association between the length of time the pronuclei (PN) were visible and the ploidy status of the embryo (p=0.01). That is, the PN were visible in aneuploid embryos for longer time periods, and showed more variance in the time visible, in comparison to their euploid counterparts. This study also determined that there were no statistically significant differences between the timing of the first cell cycle ($t3 - t2$), the second cell cycle ($t4 - t3$), the third cell cycle ($t5 - t3$) and the genetic status of the embryo (p=0.36, p=0.488 and p=0.453 respectively). Furthermore, no statistically significant relationship was determined between the timing of blastulation (*tB-tSB*) and genetic status of the embryo (p=0.412).

Conclusions

The results of this study indicate a statistically significant association between the ploidy status of an embryo and length of time the PN are visible, but no other morphokinetic markers studied. Length of time at which PN are visible is thus a candidate marker of the ploidy status of the embryo, however the degree of overlap between aneuploid and euploid embryos for this criterion suggest that this is not diagnostic of embryo ploidy. The use of time-lapse in clinical IVF is widely debated and more research needs be conducted, from a wide variety of clinics, to answer fully whether time-lapse is a useful tool for the selection of viable embryos. Time that PN are visible thus may be a promising avenue for further investigation.

P-25

EVALUATION OF REPRODUCTIVE LABORATORIAL PARAMETERS OF PATIENTS WITH X CHROMOSOME MOSAIC KARYOTYPE.

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Introduction: The diagnosis of chromosomal alterations in infertile individuals is performed with the assistance of karyotype. It is common in literature the association between chromosomal alterations involving X chromosome and decrease of ovarian reserve and premature ovarian insufficiency. Preimplantation genetic diagnosis (*PGD) is highly recommended to these patients* in order to avoid pregnancies of embryos carrying consequent chromosomal aberration. *PGD is a procedure performed by embryonic genetic content's analyses, which is obtained by an embryonic biopsy, preferable on day 5. So, it depends on a good lab performance in order to obtain good embryos. The present study aimed to evaluate the reproductive results get by couples* with X chromosome mosaicism in karyotype.

Material and Methods: Retrospective study that analyzed medical records of 17 patients with mosaic karyotype evolving X chromosome that underwent in vitro fertilization (IVF) in 2015-2016. The patients were divided in two groups according to the number of cell lineages affected: two (G1) and three lineages (G2). The laboratorial variables evaluated were: oocyte maturation, fertilization, cleavage, blastocyst formation and aneuploidy. The statistical analysis was performed using the Chi-square test to evaluate qualitative outcomes between the groups and logistic regression to estimate the laboratorial closure. The significance level used was $p \leq 0.05$.

Results: Comparing the reproductive profile among patients in G1 (n=09) and G2 (n=08), it was possible to observe a low range of fertilization at the G1 (p=0.039). The frequency distribution's analysis also showed higher possibilities of the unsuccessful oocyte fertilization at this same group (p=0.043). No difference was found to the other laboratorial variables. Those patients with two and three lineages involving the X chromosome obtained 63% and 60% genetically normal embryos, respectively.

Each patient concluded at least one cycle of ovarian stimulation. From them were punctured 128 mature oocytes, 99 oocytes were fertilized and 96 were cleaved. However, only 13 patients obtained blastocysts (n=34). Considering D3 biopsies 6 were euploid (6/8). Considering D5, 18 biopsies were performed and 10 returned euploid results. Ten genetically normal embryos and three non-biopsy embryos were transferred to eight patients, resulting in three positive pregnancies, being one of them, a gemelar pregnancy. Until this moment, only one patient gave birth. Two patients had only aneuploid embryos, two patients didn't have embryos to the biopsy and four patients haven't transferred embryos yet.

Conclusion: The karyotype with X chromosome mosaicism presents negative impact on fertilization outcomes and embryonic genetic analyses. Our experience analyzing these patients shows the complexity in obtain viable embryos and the pregnancy success.

P-26

MITOCHONDRIAL ASSESSMENT IN DAY 3 BIOPSY, A PROGNOSTIC FACTOR FOR POSITIVE PREGNANCY.

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Introduction

Mitochondria are involved in the regulation of multiple essential cellular processes and are considered as the principal cellular power houses providing the adequate energy to ensure the embryo viability. The mitochondrial content of mammalian cells ranges from a few hundred to thousands, determined by the cell's volume and energy needs. The aim of this study was to evaluate the potential of mitochondrial DNA analysis as a viability score in blastomeres obtained from Day 3 embryos and try to establish a cutoff according to the mtDNA content in those embryos and its potential implantation rate.

Material and Methods

A total of 198 euploid embryos at cleavage stage are included in this study. Embryos had undergone biopsy procedure at Day 3. Only patients with single embryo transfer or double embryo transfer but both embryos included on the same group, were used for this study. Comprehensive chromosome analisys and analysis of mitochondrial DNA (mtDNA) were carried out by next Next Generation Sequencing. Mitochondria Viability ratios (mtV) and Multiple of Mean (MoM) values were compared between embryos which established pregnancy and those which failed to establish pregnancy.

Results

Using our mtV score, 30 embyos of this study group(15%) were classified as Grade B (mtV score <2.7) and 168 (85%)were classified as Grade A (mtV score >2.7). Grade B Embryos have showed an implantation rate of 30% (n=9) while 92 of Grade A embryos established pregnancy showing an implantation rate of 54.8%. A statistically significant increase ($p<0.05$) in implantation rates was observed when the amount of mitochondrial DNA was also elevated.

Conclusion

Elevated amount of mitochondria DNA in euploid embryos may be an indicative of increased implantation rate. Of clinical importance, we were able to establish a threshold of mtDNA content above which the implantation rate for an euploid embryo is almost the double (30% vs 54.8%). This study strongly suggest that the clinical potential of pre-implantation assessment mitochondrial DNA quantitation, in biopsied blastomeres, can be used as a prognosticator of pregnancy potential.

P-27

THE LIKELIHOOD OF TRANSFERRING A EUPLOID EMBRYO AFTER PGD-ANEUPLOIDY CYCLES DEPENDS NOT ONLY ON FEMALE AGE BUT ALSO ON THE NUMBER OF OOCYTES COLLECTED.

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Introduction: Aneuploidy is the most common type of chromosome abnormality and the leading cause of implantation failure, miscarriage and congenital abnormalities. Three RCTs have already demonstrated higher clinical pregnancy rates in young good prognosis patients with single embryo transfer, but investigations in other groups are limited to observational studies. Although scientific principles of comprehensive aneuploidy screening are widely accepted, controversy remains about the clinical and, particularly, economic effectiveness of this approach (Lee et al., 2014). Thus, patients and especially advanced maternal age cases need to be informed properly regarding their realistic chances of producing euploid embryos in different clinical scenarios.

Material & methods: This retrospective study comprised a period between August 2011 and December 2016. A total of 1229 patients were subjected to aneuploidy screening, due to advanced maternal age (≥38), recurrent pregnancy loss (≥2) and repeated implantation failure (≥3). Patients were grouped according to female age (<35: n=247; 35-37: n=151; 38-40: n=329; 41-42: n=309 and ≥43: n=193). They were also analyzed regarding the number of cumulus oocyte complexes (COC) (<5: n=289; 5-9: n=455; 10-14: n=243; ≥15: n=331). Statistical significance between groups was tested with chi-square. Trophectoderm biopsy samples were analyzed with array CGH.

Results & Conclusions: The likelihood of finding at least one euploid blastocyst (LFLOEB) was not statistically different in women <35 years of age, as for <5; 5-9; 10-14 and ≥15 COC categories LFLOEB was calculated as 56.3%, 68.5%, 66.1% and 80.2%, respectively (p=0.08). Mainly, five groups exist and the highest LFLOEB was found for females between 35-37 years of age with ≥15 COC (86.2%). LFLOEB was elevated for patients between 35-37 with 10-14 COCs and females between 38-40 with 10-14 and ≥15 COCs (75.0%, 75.4% and 69.0% respectively). Likewise, the LFLOEB was similar for females between 35-37 and 38-40 years of age with 5-9 COCs, and for the 41-42 age category with ≥15 COCs (53.4%, 54.3% and 54.0%, respectively). A diminished LFLOEB was obtained for females between 35-37 and 38-40 years of age with <5COCs, but also for patients between 41-42 and ≥43 years of age with ≥15COCs (46.4%, 49.4%, 44.2% and 45.8%, respectively). The worst scenario was for patients of 41-42 years of age with ≤5 COCs and ≥43 years of age with ≤5 or 5-9 COCs (21.1%, 17.1% and 18.2%, respectively). Overall, the increase in LFLOEB with higher number of COCs was statistically significant for the age categories 35-37, 38-40, 41-42 and ≥43 (p=0.022; p=0.0033; p=0.0006 and p=0.0072, respectively).

In conclusion, until 35 years of age a good ovarian reserve providing a higher number of COC does not make a statistical difference regarding the likelihood of finding at least one euploid blastocyst. However, above 35 years of age, the number of COC can compensate for the age effect leading to aneuploidy. Thus, when counselling patients for PGD-A, maternal age and ovarian reserve should be considered together to inform the couple about their realistic chances of achieving a viable pregnancy.

P-28

VALIDATING PGS BY PROBING THE KARYOTYPIC CONCORDANCE BETWEEN ICM AND TE.

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Introduction

Clinical trials continue to assess the validity of comprehensive chromosome screening (CCS) for increasing implantation rates in IVF, with increasing evidence justifying the routine use of preimplantation genetic screening (PGS). At the blastocyst stage, an embryo's karyotype is determined from a 5-10 cell biopsy collected from the trophectoderm (TE), a precursor tissue to the placenta. Interestingly, a considerable number of embryos deemed euploid do not result in pregnancies; conversely, there are spurious reports of aneuploid embryos that implant and lead to births of healthy, chromosomally normal babies. One rationale for these phenomena is that the TE biopsy collected from blastocysts is not always representative of the inner cell mass (ICM), the precursor of all tissues in the fetus. This study investigates the karyotypic concordance between ICM and TE.

Materials and methods

Aneuploid blastocysts as determined by routine PGS (Illumina's Veriseq NGS platform) were selected for re-analysis. An ICM biopsy, as well as a second TE biopsy were captured for each embryo and subjected to PGS. Three analysts blindly interpreted all resulting karyotype profiles independently.

Results

Within a sample population of 29 aneuploid embryos determined by routine PGS, two embryos (~7%) had euploid ICMs. A further three embryos (~10%) had ICMs that contained the aneuploidy detected in the initial TE biopsy, but had additional minor karyotypic discrepancies with the original profile. For these three embryos the second TE biopsy was concordant with the original profile, suggesting that the entire TE had a different karyotypic makeup than the ICM. The remaining 24 embryos (~83%) showed perfect karyotypic concordance between ICM and TE.

Conclusions

If the observed trends hold true in our currently expanding sample size we surmise that routine PGS has a ~7% 'biological false negative' rate.

P-29

PATIENTS UNDERGOING PREIMPLANTATION GENETIC SCREENING MAY BENEFIT FROM ARTIFICIAL OOCYTE ACTIVATION.

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Introduction: At present, artificial oocyte activation (AOA) is widely used in IVF to overcome the reduced or failed ICSI fertilization. Meanwhile, it is still considered as an experimental approach and the data on impact of AOA on obtained embryos ploidy is still scarce. In this study we were investigating the possible effect of calcium ionophore on aneuploidy rate of obtained embryos.

Material & methods: This retrospective study includes 174 embryos of 49 patients with preimplantation genetic screening by aCGH analysis (24sureV3, BlueGnome, UK) after AOA. From these, for 133 embryos of 37 patients the aCGH data from conventional ICSI cycles was also known. Indications for AOA were the previous ICSI with poor fertilization, cleavage stage arrest or severe teratozoospermia. Average female patient age was 36.4 years. The AOA was done after ICSI by placing oocytes for 15 minutes in the medium containing 10μmol/l calcimycin (A23187, Sigma). Data collection was performed between April 2015 and January 2017.

Results: The euploidy rate in the AOA group was 39.6%, which is lower than can be expected for the patient's average age. Still, the lack of effect of AOA on ploidy was confirmed when the subgroup of patients having "sibling" (conventional ICSI-aCGH) control cycles was compared. Here, the euploidy rate after AOA was 36.1% compared to 32.5% in control, with no statistically significant difference. This comparison shows that the reduced euploidy rate is characteristic to selected patients group and not influenced by application of AOA. Surprising was that after AOA the number of blastocysts available for trophectoderm biopsy increased significantly, with 3.6 biopsied blastocysts per AOA cycle compared to 2.7 in control group. Among other parameters the fertilization rate was significantly higher in AOA group compared to control (71.2% vs 63.1%). With 43 embryo transfers 21 clinical pregnancies were achieved in AOA group, with average of 1.3 embryos per transfer.

Conclusions: With our data we did not found any evidence of increase in segregation errors in the second meiotic division after ICSI with AOA. Moreover, when AOA is used according to indications it can significantly increase the number of embryos available for thophectoderm biopsy.

P-30
EXCELLENT ONGOING PREGNANCY RATE WITH COMBINED USE OF PCR FOR MONOGENIC DISEASE AND NGS FOR ANEUPLOIDY SCREENING.

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Introduction:

Preimplantation genetic diagnosis (PGD) with polymerase chain reaction (PCR) is used for monogenic diseases. However, the use of PCR cannot detect embryos with aneuploidies and these aneuploid embryos can cause implantation failure or miscarriages. Combined use of next generation screening (NGS) for aneuploidy screening and PCR for PGD was implemented in the last quarter of 2015.

Material & methods:

This is a retrospective analysis on the pregnancy outcome after the above implementation. All these cycles performed in 2016 were retrieved. Trophectoderm biopsy was employed in all cycles. Vitrification after biopsy was performed and genetically transferable euploid blastocyst(s) would be replaced in subsequent frozen-thawed embryo transfer (FET) cycles.

Results:

There was 14 cycles started in 2016. Table 1 listed the indications for PGD. Mean age of women was 33.7 years (range 29-38 years). Mean number of oocyte was 15.7 (range 6-31). Mean number of blastocyst for biopsy was 5.6 (range 1-12). After PGD with PCR for monogenic diseases, there was 3.7 genetically transferrable blastocysts (either normal or carrier blastocysts) (range 1-11). However, 2 women have no euploid blastocyst, one was 29 years old and another was 36. Nine women with total 10 FET were done already. One woman had two FET cycles with the first one ending in miscarriage and second FET resulting an ongoing pregnancy. Seven women got pregnant in their first FET cycle and the pregnancies are ongoing. The ongoing pregnancy rate (OPR) was 80% (8/10) per transfer and 8/9 (88.9%) per couple. The miscarriage rate was 10% (1/10).

Conclusions:

Combined use of polymerase chain reaction for monogenic disease and next generation screening for aneuploidy screening leads to an excellent onging pregnancy. Such information is useful to counsel the couple undergoing PGD.

Table 1. List of indications for PGD.

Indications	***Number of women***
Adult onset polycystic kidney disease	1
Congenital adrenal hyperplasia	1
Familial adenomatous polyposis	2
Hereditary multiple exostosis	2
Nemaline myopathy	1
Shwachman-Diamond-Bodian Syndrome	1
Spinal muscular atrophy	1
Spinocerebellar ataxia	1
Thalassaemia	3
Tuberous sclerosis	1

P-31

THE EFFECTS OF LASER MANIPULATION ON BIOPSY KARYOTYPE IN PGS.

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Introduction

Universally applied preimplantation genetic screening (PGS) protocols for blastocysts rely on the isolation of a trophectoderm (TE) biopsy, with an expectation that the biopsied TE karyotype is representative of the entire embryo. It is imperative for the biopsy collection method not to introduce genetic artefacts in the separated cells, as this could lead to failed tests or false positive/negative interpretations. Current blastocyst biopsy procedures routinely include the use of lasers of infrared or near infrared wavelengths to aid the separation of a cohort of TE cells from the embryo mass. Laser pulses are aimed at interfaces of neighboring cells that are mechanically being pulled apart through suction forces and the instant degradation of cell junctions permits the group of targeted TE cells to fully separate and be recovered for chromosomal analysis. It has been reported that infrared lasers have little to no impact in embryo development and downstream potential. However, recent studies in the fields of dermatology and oncology have shown that infrared and near infrared wavelengths can induce DNA degradation in human cells.

Hence, we set out to determine whether laser intensity and pulse number during TE biopsy affects PGS results.

Materials and methods

Aneuploid blastocysts as determined by PGS (Illumina's Veriseq NGS platform) were used for re-analysis. TE biopsies were collected using methods standard to the industry. The Laser (Hamilton Thorne Lykos v5.12) intensity varied between 400-850 µs and the number of pulses varied between 4-10 pulses. The biopsies were subsequently processed for PGS by NGS.

Results

All TE pieces analyzed to date (n=12) yielded NGS results of similar quality regardless of laser strength and pulse number used at biopsy collection. All parameters used to assess NGS data quality (resulting genetic profile, DLR, read number pre- and post bioinformatic filtering) suggested insignificant DNA degradation across our tested samples.

Conclusions

From our current data we infer that laser strength and pulse number has negligible impact on PGS results due to degraded DNA. In instances of difficult biopsy isolation, stronger laser strength or repeat pulses may be used without compromising downstream NGS data. The impact of laser intensity and pulse number on subsequent embryo viability is yet unknown.

P-32

SINGLE EMBRYO TRANSFER AS AN IMPERATIVE CHOICE FOR PATIENTS OVER 38 YEARS OLD IN AUTOLOGOUS IVF PGS CYCLES.

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Introduction: In recent years comprehensive chromosomal screening has been proven as the best option to increase clinical outcomes in autologous IVF cycles for advanced maternal age patients. The high efficiency of preimplantation genetic screening (PGS) allows clinics to maintain high pregnancy rates while transferring fewer embryos in the younger patient population. Efficiency of single embryo transfers in older patient population (≥38 y.o.) remains controversial. The objective of this study was to evaluate clinical pregnancy rates after single and double embryo transfer in a group of patients 38-41 y.o. and over 41 y.o. in autologous IVF PGS cycles.

Material & methods: A retrospective comparative study was performed between January 2013 and January 2017. 751 cycles (627 patients, average age - 36.06±3.98) of IVF treatment with SNP PGS were included in the study: 409 autologous cycles with maternal age under 38 y.o. and 342 autologous cycles with maternal age over 38 y.o.. All embryos were vitrified after biopsy, and selected embryos were subsequently thawed for a hormone replacement frozen embryo transfer cycle. In a group of patients over 38 y.o. 307 single euploid embryo transfers (SET) and 35 double euploid embryo transfers (DET) were included in the study. Single embryo transfer was promoted by the clinic but not required and some double embryo transfer were performed by patient and individual physician request.Clinical pregnancy rate was defined by the presence of a fetal heartbeat at 6-7 weeks of pregnancy. The cumulative probability of a clinical pregnancy was accessed by non-parametric Kaplan-Meier product limit estimation.

Results: Our data demonstrated no statistically significant difference in ongoing clinical pregnancy rates after single euploid embryo transfer between all age groups (≤34 y.o., 35-37 y.o., 38-40 y.o., and ≥41 y.o.): 58.47%, 63.16%, 62.38%, and 59.22%, respectively (χ^2= 0.815, p=0.3667). Clinical pregnancy rates were higher after elective single embryo transfer versus non-elective single embryo transfer but did not reach statistical significance even in a group of patients ≥41 y.o: 61.70% (29/47) and 57.14% (32/56), respectively (χ^2= 0.21995, p=0.6391). Clinical pregnancy rates were significantly higher after double embryo transfer versus single embryo transfer in a group of patients over 38 y.o.: 80.00% (28/35) and 61.24 (188/307), respectively (χ^2= 4.753, p<0.05). In the same age group, multiple gestation rate after DET was considerably higher than after SET: 0.33% (1/307) and 45.71% (16/35), respectively (χ^2= 16.715, p<0.05). The cumulative probability of a clinical pregnancy after two consecutive single euploid embryo transfers in a group of patients over 38 y.o. was 81.3% and after three consecutive single euploid embryo transfers – 93.2%.

Conclusions: This study offers evidence that clinical pregnancy rate after single embryo transfer is age independent in IVF PGS cycles. With single euploid embryo transfer, high clinical pregnancy rates and low multiple gestation rates can be achieved even in older patient population. Therefore, in IVF PGS cycles single embryo transfer should be imperative for advanced maternal age patients.

Study funding/competing interest(s): None.

P-33

EUPLOIDY RATE OF DAY 7 BLASTOCYSTS DERIVED FROM IN VITRO FERTILISATION (IVF).

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Introduction:

Generally human embryos are cultured up to Day 6 and embryos that do not develop into utilisable blastocysts (assessed with Gardner's grading system) are discarded at the end of Day 6. Occasionally, blastocyst culture is extended to Day 7 to further observe the development of slow growing blastocysts. These Day 7 blastocysts with at least fair graded inner cell mass and trophectoderm may be chromosomally normal and may result in pregnancy. In Alpha Fertility Centre (AFC), we practice blastocyst culture until Day 7 for patients who do not have utilisable blastocysts on Day 5 and/or Day 6. This is a retrospective study to assess the euploidy rate of Day 7 blastocysts at Alpha Fertility Centre, Malaysia from August 2015 to December 2016.

Material & methods:

Twelve patients (age ranged 19 – 41, mean age 36.0) who underwent IVF had their embryos successfully cultured to Day 7. Of these, 2 patients had utilisable blastocysts on both Day 6 and 7 while the remaining 10 patients had only utilisable blastocysts on Day 7. Only Day 7 blastocysts were included in this study. All fifteen (15) Day 7 blastocysts were biopsied and proceeded with pre-implantation genetic screening (PGS) by either using Micro-array Comperative Genomic Hybridisation (BlueGnome, UK) or Next Generation Sequencing (Ion Torrent, USA) according to manufacturer's specifications. All Day 7 blastocysts were vitrified after biopsy.

Results:

Of the 15 Day 7 blastocysts, 7 were found to be euploid and 8 were aneuploid. The euploidy rate of the Day 7 blastocysts was 46.7% and the aneuploidy rate was 53.3%.

Conclusion:

Our preliminary results showed that Day 7 blastocysts can be chromosomally normal and should be considered for embryo transfer.

P-34

THE USE OF LOW OXYGEN CONCENTRATION DURING EMBRYO CULTURE SEEMS NOT TO AFFECT BLASTOCYST ANEUPLOIDY RATE.

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Introduction:

Trofoectoderm biopsy requires extended embryo culture until blastocyst stage. Culture conditions such as using low oxygen concentrations (~5%) can be determinant for embryo developmental viability.

The objective of this study was to assess both blastocyst formation rate and aneuploidy rate from our PGS program depending on two type of culture conditions: low O_2 concentrations (group A) and atmospheric O_2 concentrations (group B).

Material & methods:

Retrospective study involving a total of 362 PGS cycles (November 2016 – January 2017). Indications for PGS were: advanced maternal age (n=227), repetitive implantation failure (n=49), severe male factor (n=44), recurrent miscarriage (n=35) and previous pregnancy with aneuploidy (n=7). Group A: 176 cycles (mean age: 38.7 years; SD: 4.0) with 586 analyzed embryos, Group B: 186 cycles (mean age: 39.4 years; SD: 3.3) with 643 analyzed embryos. Embryos from group A were cultured in cleavage medium (Cook Medical, France) until day-3, and then were placed in blastocyst medium (Cook Medical, France) until day-5 using low O_2 concentration. Embryos from group B were cultured in IVF medium (Vitrolife AB, Kungsbacka, Sweden) until day-3, and then were placed in co-culture medium (CCM) (Vitrolife AB, Kungsbacka, Sweden) until day-5 using atmospheric O_2 concentration. Trophectoderm biopsy was possible in 1,229 embryos (48.0%) from 2,558 fertilized oocytes. Biopsied blastocysts were vitrified within the hour following biopsy. Analysis by aCGH was performed. Statistical comparisons were performed using Fisher's exact test and t-Student test ($p<0.05$).

Results:

No statistical differences were found between group A vs group B in terms of maternal age (38.7 vs 39.4; p=0.6926), days of stimulation (10.8 vs 10.9; p=0.5427), number of oocytes retrieved (11.2 vs 11.8; p=0.4247) and MII obtained (8.9 vs 9.4; p=0.6926). Only fertilization rates have statistically higher values in group B (73.9 vs 76.5; $p<0.0001$).

With respect to blastocyst formation ability, statistical differences were found in favor of group B (56.3 vs 60.9; $p<0.05$). However, no statistical differences were observed between groups when we compared biopsied blastocyst rate (48.9 vs. 47.8; p=0.5104).

Additionally, aneuploidy rate did not decrease when embryos were cultured under high oxygen concentration (46.1 vs 44.3; p=0.053).

Conclusions

Contrary to our expectation, the use of low oxygen tension during culture did not improve the blastocyst formation rate neither the percentage of chromosomal abnormal embryos. We speculate that certain culture media like CCM containing hormones such as insulin, progesterone and estradiol, may overcome the negative effect on embryo development reported by others when high oxygen concentration is used for blastocyst culture.

P-35

PGS DIAGNOSIS INCREASES IMPLANTATION AND CLINICAL PREGNANCY RATE.

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Introduction

This study was designed to compare the clinical outcome of PGS frozen euploid embryo transfer (ET) versus frozen embryo transfers after freeze-all cycles (FET). The impact of trophoectoderm biopsy on implantational potential was also assesed.

Materials & Methods

This is a retrospective cohort study performed in an academic private ART center. Patients who underwent blastocyst frozen embryo transfer from January 2016 to December 2016 were included. The outcome of 21 euploid ET cycles after trofoectoderm biopsy and PGS (PGS group) was compared with a control group (CG) of 25 FET cycles. Variables such as maternal age, number of embryos transferred, day of embryo transfer, number of previous cycles, oocyte donation, and diagnosis of infertility were controlled. A total of 120 embryos were biopsied and analyzed, with 92% diagnosed. PGS diagnosis indication included recurrent miscarriage (33%), advanced maternal age (3%), gender selection (14%), docyte donation (10%), implantation failure (5%) and male factor (5%). Percentage of normal embryos in the maternal age ranges were 59% (≤35 years), 49% (36 to 39 years), and 35% (≥40 years).

Main outcome measures were: IR (sac/embryo transferred), CPR (Presence of Fetal Heart beat by 6th week), Miscarriage Rate (miscarriage number/CPR number).

A descriptive analysis was performed according to the nature of the variables. Qualitative variables were analyzed as measures of frequency and percentage. Quantitative variables were studied taking into account the measures of central tendency as mean and of dispersion standard deviation. To evaluate the association between cohorts and outcomes, the Pearson chi2 proportions test and the estimated difference of rates (RD) were used, with 95% confidence interval (CI). Statistical tests evaluated at a significance level of 5% ($p < 0.05$), using the program SPSS vs23 was used.

Results

The groups were homogeneous for the variables controlled, where no statistically significant differences were found. Differences were significant in recurrent miscarriage rates (P: 0,046), and average number of previous miscarriages (P: 0,032).

IR and CPR showed a significant improvement in the PGS compared with the FET group, 62% vs. 43% RD 0,702 (0.35 – 1.42); 67% vs. 56% RD 0,84 (0,39 – 1,79). However, there was no association between the use of PGS and the miscarriage rate.

Conclusions

This study suggests that frozen PGS embryos have a higher implantation potential and better CPR. This findings may contribute to improve clinical outcomes in patients with selected indications such as advanced maternal age and greater risk of miscarrage. This results do not allow to state that all the patients should undergo PGS, given the percentage of cancelled cycles in this group (12%) compared with the control group (6%). The trophoectoderm biopsy does not seem to impair embryo implantation potential.

P-36

ASEPTIC VITRIFICATION OF BLASTOCYSTS AFTER TROPHECTODERM (TE) BIOPSY.

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Introduction: Vitrification is the standard freezing technique in ART, guarantying optimal survival rates. However, open vitrification carrier systems are still commonly used, implying direct contact of the embryo with liquid nitrogen during freezing and storage. Thereby, the embryo is at risk of contamination with pathogens and of cellular damage by reactive chemical compounds. Whereas we see a trend towards the application of closed (aseptic) carrier devices for routine oocyte and blastocyst vitrification, we lack protocols for aseptic vitrification of blastocysts after trophectoderm (TE)-biopsy. Due to the opening of the Zona pellucida and hatching of biopsied blastocysts, specific vitrification protocols have to be applied, which are different from e.g. protocols for expanded blastocysts. With the increasing demand of PGT, the vitrification of biopsied blastocysts in a safe and reliable manner gains importance. PGT is still a time consuming, costly and elaborate technique. Thus, the freezing techniques should be sophisticated, optimized, and preferable aseptically.

Material & methods: We retrospectively analyzed data collected in three IVF-centers in course of a multi-center study. During a period of 3 years (2014-2016) all warming cycles with aseptically vitrified blastocysts after TE-biopsy for PGT were included. Reasons for PGT were: monogenetic diseases (34 patients), parental structural chromosomal aberrations (43), recurrent implantation failure (53), recurrent miscarriage (63), advanced maternal age (15), previous fetal chromosomal abnormalities (17) or severe male factor (2). The applied aseptic vitrification protocol was specifically adapted on basis of experiences with laser-hatched non-biopsied blastocysts, and collapsed blastocysts. TE-biopsy took place either on day 5 or on day 6. Approximately 5 TE cells were biopsied. Vitrification was performed 30 minutes to 2 hours after biopsy, using aseptic VitriSafe devices and vitrification solutions containing ethylene glycol and DMSO using a protocol specifically optimized for biopsied blastocysts in aseptic conditions. Warming was performed in sucrose solutions 3 hours prior embryo transfer (ET).

Results: In total 418 aseptically vitrified biopsied blastocysts were warmed. Before biopsy, 192 blastocysts showed top-morphology according to the Gardner criteria (AA, AB, or BA grading for inner cell mass (ICM)) and TE, 209 revealed good/moderate quality (BB, BC, CB) and 12 were of low quality (CC). 332 blastocysts were biopsied and vitrified on day 5 and 86 blastocysts on day 6. As primary endpoint survival rate after vitrification/warming was assessed: 94.7% of the blastocysts were classified as fully intact and re-expanding. Out of the 22 blastocysts that did not survive 7 were day 6 blastocysts, and 2 were low quality blastocysts. The secondary endpoint was implantation rate after ET. A total of 394 aseptically vitrified blastocysts were transferred (2 were re-vitrified). We observed an implantation rate of 42.9% after ET.

Conclusions: Aseptic vitrification protects blastocysts from direct contract with liquid nitrogen, thereby preventing them from cross-contamination with pathogens and cellular damages by reactive chemical compounds during cryopreservation, storage and warming. As PGT and TE biopsy is an elaborately process and still expensive, aseptic vitrification of those embryos should be the procedure of choice to guarantee an optimal IVF outcome.

P-37

ADMINISTRATION OF CANESTAN PRIOR TO FROZEN EMBRYO TRANSFER (FET) OF EUPLOID BLASTOCYSTS MAY IMPROVE CLINICAL OUTCOMEADMINISTRATION OF CANESTAN PRIOR TO FROZEN EMBRYO TRANSFER (FET) OF EUPLOID BLASTOCYSTS MAY IMPROVE CLINICAL OUTCOME.

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Introduction:

Embryo transfer (ET) in IVF involves putting embryo(s) in transfer catheters which goes through the cervical os and into the uterine cavity. There is a risk of picking up bacteria and fungus from various vaginal-cervical microorganisms during the process. It has been reported that bacterial and *Candida* infection in women going through IVF can negatively affect embryonic implantation (Wittemer et al., 2004). To our knowledge, this is the only report which alludes to a possible relationship between vaginal yeast infection and implantation following ET. We hypothesize that a preventative treatment such as with clotrimazole (Canestan) to avoid candida infection may improve clinical outcomes. This is an empirical study comparing clinical outcomes between FET patients prescribed with Canesten pessaries and those who were not prior to the transfer of euploid blastocysts. High vaginal swabs (HVS) were not routinely done in this study since the results were not sensitive and accurate enough based on our experience with the outsourced laboratories we work with.

Material and Methods:

A total of 100 patients with blastocysts screened using Preimplantation Genetic Screening (PGS) underwent FET cycles in Alpha Fertility Centre, Malaysia between June 2016 and October 2016. These patients were divided into 2 groups: 57 patients with 69 euploid blastocysts transferred in the treatment group (Group A), and 43 patients with 59 euploid blastocysts transferred in the control group (Group B). All patients were administered with oestrogen (Progynova) and progesterone (Cyclogest or Crinone 8%) for endometrium preparation. In Group A, patients were given Canesten 500 mg pessary 7 days prior to embryo transfer. The mean age of patients in Group A was 33.4 while the mean age of patients in Group B was 31.4 ($p>0.05$). All thawed blastocysts survived with morphologically intact inner cell masses and trophectoderm cells. The mean number of blastocysts transferred for Group A and B was 1.2 and 1.4 respectively. Clinical pregnancy and number of gestational sacs were determined by ultrasound.

Results:

Table 1: Clinical pregnancy rate and implantation rate between the treatment and control groups.

	Group A (Treated with Canestan)	**Group B (Control)**
No. of cases thawed	57	43
No. of cases reached ET	57	43
No. of euploid embryos transferred	69	59
No. of sacs	44	33
Mean age of patients	33.4	31.4
Mean number of blastocysts transferred	1.2	1.4
Clinical Pregnancy Rate per cases reached ET	64.9% (37/57)	62.8% (27/43)
Implantation Rate	63.8% (44/69)	55.9% (33/59)

Overall, the implantation rate appears to be improved in patients treated with Canestan (63.8%) as opposed to those who were not (55.9%), despite the older age of patients in the treatment group. However, this difference was not significant ($p>0.05$). The clinical pregnancy rate in the treatment group (64.9%) is as good as the control group (62.8%), despite the treatment group being older and having less embryos transferred.

Conclusion:

This preliminary study suggests that the use of Canesten pessaries in FET cycles may improve implantation rate. Further studies which are controlled needs to be undertaken to confirm the above.

P-38

ARE METABOLIC DISORDER CARRIES SUBJECT TO POOR OVARIAN RESPONSE IN PGD CYCLES?

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INTRODUCTION

Controlled ovarian hyperstimulation (COH) and expected outcome of IVF in single gene disorder patients varies significantly among women. One of our previous patient with heterozygous mutation on her GALT gene whom responded poorly to COH treatment in her repeated unsuccessful IVF attempts. She had improved results after a 3 months long galactose restricted diet which resulted in live birth. This case triggered carrying out this study with a purpose to examine the effect of being a carrier for the single gene disorder on controlled ovarian hyper stimulation (COH) treatments and the pregnancy success rate.

MATERIAL METHOD

In this study, between September 2011 and May 2016, we included 107 IVF cases where PGD for single gene disorders has been performed. 17 of these cases were metabolic disorder carriers and 90 cases were carries of other single gene disorders. 100 cases that had IVF treatment due to unexplained infertility reason were enrolled in the control group. Also, the ages of the both PGD and the control group were approximate to each other. The number of mature and immature oocytes collected per cycle and successful pregnancy rates were used as evaluation criteria.

RESULT

It was observed that when compared to the control group, the PGD group responded poorly to ovulation induction and had lower pregnancy rates. Another significant result was obtained when metabolic disorder carriers were compared to other single gene disorder carriers. The results revealed that 35% of metabolic disorder carriers respond poorly to COH compared to 25% in other single gene disorder carriers.

CONCLUSION

It is possible that the reason for poor response to ovulation induction in the PGD group could be related to the negative impact of heterozygous mutation on the ovaries. However, it must be kept in mind that there is a possibility that the low rate of pregnancy in the PGD group may have occurred because the affected embryos weren't transferred. The fact that especially metabolic disorder carriers responded even more poorly to the treatment supports this hypothesis. As previously mentioned the results of these patients can be improved with appropriate diet before COH stimulation.

P-39

ACCESSIBILITY TO THE PREIMPLANTATION GENETIC SCREENING.

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Introduction Even though preimplantation genetic screening (PGS) for advanced maternal age it is not accepted universally it has been proposed to reduce the rate of implantation failure, rate of miscarriage and child birth rate with chromosome abnormalities or even to reduce the time/cost to give birth a healthy child. However, PGS is not only producing good results, it is also important to take into account the accessibility of the technique. Some of the requisites to offer an accessible programme are an improvement in the cost-efficiency of the technique and a reduction in the uncertainty and stress for patients. The objective of this study is to describe the result of improving the accessibility of PGS for women of advanced maternal age (>37).

Material and methods. Retrospective and observational study performed from January to December 2016. Until July 2016 our clinic (Clinic A) was a small clinic which offers PGS but with the inconveniences of being a small and isolated clinic on an island inside the Spanish state. In July 2016 our clinic became part of a bigger institution (Clinic B) which provides a wide variety of services, PGS among others. As a Clinic A the comprehensive chromosome screening (CCS) was not integrated in the IVF package and the biopsy was performed depending on the number and the quality of embryos on day 3. If the number and the quality were not sufficient, the embryos were vitrified until we achieved the sufficient number of blastocysts on subsequent IVF cycles. As a Clinic B the CCS was included in the in vitro fertilization budget regardless the number of biopsied blastocyst, reducing the stress of deciding if to accumulate embryos or not for the patient and the clinician.

In both cases the patients were precisely informed by the clinician prior to the ovum pick up.

In 2016, 149 infertile couples have undergone an IVF cycle with or without PGS due to advanced maternal age (>37 years old). In both cases all embryos were cultured until blastocyst stage in a single step medium system. In all PGS cycles embryo biopsy was performed in blastocyst stage. Chi-squared test was used for the statistical analysis.

Results As a clinic A 10,8% (8/74) of the patients will accept to undergone CCS, since being part of the clinic B 60% (45/75) of the patients will accept for CCS ($p<0,05$) Implantation, pregnancy and miscarriage rate were not reliable as 18 cases are still waiting for frozen embryo transfer.

Conclusions In our hands, with the same information and PGS indication we have seen an increase in the number of PGS performed due to advanced maternal age. Uncertainty and stress are some of the consequences inherent to the IVF cycles. The more we do to decrease them the better. In conclusion, it is important to offer an accessible, simple and integrated IVF programme to guide the patient towards the best treatment.

P-40

MORPHOLOGICAL BLASTOCYST GRADE IS NOT A DETERMINANT FOR IMPLANTATION OF EUPLOID EMBRYOS.

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Introduction

The trend of single embryo transfer is increasing the need of reliable and unbiased methods for selection of an embryo with the best potential of development into viable pregnancy. Conventional blastocyst grading system is based on morphologic assessment that evaluates the degree of blastocoel expansion and quality of inner cell mass (ICM) and trophectoderm cells (TE). Preimplantation Genetic Screening (PGS) have shown substantial improvement in pregnancy outcomes of In-Vitro Fertilization (IVF) by selecting euploid embryos for transfer. Traditionally embryos with superior morphology are selected for transfer. Several studies have shown that morphology parameters could be ambiguous predictors of ploidy, implantation and live birth rates after fresh and frozen embryo transfer (FET) cycles. We aimed to evaluate the relationship between morphological grade and chromosomal content of the embryo and to determine its importance in prioritization of euploid blastocysts analysed by PGS for FET.

Material and Methods

Trophectoderm biopsies of D5/D6 blastocysts (n=1710) were tested by either NGS (n=992) (VeriSeq kit, Illumina Inc., USA) or aCGH (n=718) (24Sure-kit, Illumina Inc., USA) for PGS at Create Fertility Centre. Blastocysts were clustered into 3 groups based on their morphological grading before biopsy: good n=328 (1AA, 1AB, 1BA, 2AA, 2AB, 2BA), average n=710 (1BB, 2BB) and poor n=672 (1AC, 1CA, 1BC, 1CB, 2AC, 2CA, 2BC, 2CB). The resolution for detection and reporting of chromosomal aberrations is ³10Mb, with lower limit of mosaicism ³30% for NGS and ³50% for aCGH tested embryos. Pregnancy outcome data from 335 single-FETs of euploid embryos were available for analysis. Statistical data is reported as odds ratios (OR) with 95% confidence intervals (CI).

Results

PGS analysis detected aneuploidy in 28.7%, mosaicism in 13.9% and euploidy in 57.4% of the 1710 tested TE-biopsies. PGS showed significantly higher euploidy rates in good graded embryos of 67.4% than in average grade embryos (58.3%) p=0.005, OR=1.48, CI:1.122-1.944 and among embryos with poor morphology grade (51.6%) p=2.0E-6, OR=1.934, CI:1.468-2.549. Frequency of aneuploidy was significantly lower among good quality embryos (19.2%) than in embryos with average grade (28.2%) p=0.002, OR=0.606, CI:0.44-0.835, and in poor grade embryos (33.9%) p=2.0E-6, OR=0.463, 95% CI:0.337-0.636. The higher prevalence of euploidy in good vs poor grade embryos remains significant after stratification by patient's age: donors (20-30 years) (p=0.005), patients <35 years (p=0.01) and 35-39 years (p=0.03). In patients over 40 years euploidy was lower and with similar frequency in blastocysts of all morphological grades. There was a trend of lower euploidy rates for average graded blastocysts compared to good graded embryos in all age groups.

From 335 transferred euploid embryos, 30.7% were with good morphology, 42.1% with average morphology and 27.2% with poor morphology. The implantation and ongoing early pregnancy rates were similar for all embryos regardless of their morphology. Further analysis based on patient's age (ovum donors 20-30years, <35 years, 35-39 years and ≥40 years) revealed no correlation between implantation and ongoing early pregnancy rates and embryo morphology in all age groups. Embryos graded as poor were not associated with higher miscarriage rates than embryos graded as average, or good (9.5%, 14% and 19% respectively).

Conclusions

Embryos with good morphology have 2-fold higher chances to be euploid than embryos with poor morphology in women younger than 40 years. However, when PGS (aCGH and NGS) analysis is used for selection of euploid embryo for FETs, morphological grade was not a reliable predictor of implantation or early pregnancy outcome. This study provides an important insight for better patient management and supports the inclusive approach for PGS testing and transferring of all available embryos. In addition it emphasises the need for identification of novel predictors of the most viable euploid embryo.

P-41

MEIOTIC OUTCOME IN TWO CARRIERS OF Y AUTOSOME RECIPROCAL TRANSLOCATIONS: SELECTIVE ELIMINATION OF CERTAIN SEGREGANTS.

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Introduction: Reciprocal Y autosome translocations are rare but frequently associated with male infertility. In particular translocations involving breaks in the Yq12 heterochromatic region frequently lead to infertility associated with oligozoospermia or azoospermia. The association of the autosome with the XY-body that is formed during normal male meiosis in carriers is thought to be responsible for meiotic arrest during spermatogenesis. In the present study, meiotic outcome in embryos from two carriers of reciprocal Y-autosome translocations ascertained after a total of six cycles of preimplantation genetic diagnosis (PGD) was studied. The data provides evidence for the selective elimination of certain gametic segregants.

Materials and Methods: Meiotic segregation patterns were determined in a total of 27 embryos fathered by two males with the karyotypes 46,X,t(Y;4)(q12;p15.32)(Couple A) and 46,X,t(Y;16)(q12;q13)(Couple B). The two couples underwent PGD enabling determination of the segregation types that were compatible with fertilization and preimplantation embryo development. Both PGD and follow up analysis were carried out via fluorescence in situ hybridization (FISH) or array comparative genomic hybridization (aCGH).

Results: Interestingly, it was seen that the number of female embryos resulting from alternate segregation with the chromosome combination of X and the autosome from the carrier gamete differed from the corresponding balanced males with derivative Y and the derivative autosome by a ratio of 7:1 in each case (P=0.003) while from the adjacent-1 mode of segregation, the unbalanced male embryos with the combination of der Y and the autosome were seen in all embryos from couple A and in couple B with the exception of one embryo only that had the other chromosome combination of X and derivative autosome (P=0.011). In both cases the deficit groups have in common the der autosome chromosome that includes the segment Yq12 to qter.

Conclusion: The most likely explanation may be that this chromosome is associated with the X chromosome at PAR2 (pseudoautosomal region 2) in the sex-body leading to inactivation of genes on the autosomal segment that are required for the meiotic process and that this has led to degeneration of this class of spermatocytes during meiosis. The data also reflects that the exclusion of certain types of segregants increased the chance of having an embryo with normal XX chromosomes. During clinical PGD, only transferring embryos with a normal female chromosome complement may be expected to minimise the risk of offspring with clinical abnormality and future infertility, but considering the transfer of chromosomally balanced male embryos is likely to increase the couple's chance of achieving an ongoing pregnancy.

P-42

HIGHER THAN EXPECTED REPRODUCTIVE RISK FOR AUTOSOMAL PARACENTRIC INVERSION CARRIERS.

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Introduction

Autosomal paracentric inversions are generally considered to be harmless without phenotypic effect in carriers, but recombination can occur during gametogenesis causing genetically unbalanced gametes and embryos. If a recombinant gamete is formed following a crossover in the inverted segment, the chromosome would be either acentric or dicentric. The resulting embryo will be unbalanced. This could lead to repeated IVF failures or repeated pregnancy loss, frequently seen among inversion carriers as well as a possible affected child. Previous studies performed in sperm of autosomal paracentric inversion carriers show recombination is scarcely ever seen (0-0.81% of recombinants in 5 cases) with the exception of one sperm FISH study showing 28% of recombinant gametes. The main goal of this study is to retrospective analyze the results of PGD performed in autosomal paracentric inversion carriers, in order to see the number and frequency of unbalanced embryos and its possible relationship with the inversion size.

Material and Methods

A total of 65 embryos from 7 IVF-PGD cycles from 6 different couples carrying an autosomal paracentric inversion have been analyzed retrospectively. The abnormal karyotypes included in the study were the following: 46,XY,inv(3)(q13.3q27), 46,XX,inv(1)(q23q32.1), 46,XY,inv(5)(q13.1q21), 46,XY,inv(8)(q11.2q24.1), 46,XX,inv(7)(p13p22), 46,XX,inv(10)(q11.2q22.3). Autosomal paracentric inversion sizes ranged between 43 and 82 Mpb, and the inverted segment involved between 21% and 56% of the total chromosome size. Maternal age mean was 33.71 yo. One blastomere was biopsied from each embryo on D+3 of development. Biopsied samples were amplified and processed for array CGH analysis (Illumina) following the standard protocol. The method of analysis allowed the simultaneous detection of gains and losses related to the inversion and aneuploidies affecting other chromosomes not involved in the rearrangement.

Results

Diagnosis was obtained in 60 out of 65 embryos (92.3%): 3 samples had amplification failure and 2 had no result due to a degraded DNA profile.

7 embryos were found to be unbalanced due to an inversion recombinant (11.67%) and 6 of them were also aneuploid. 17 embryos were euploid (normal/balanced) (28.33%) and 36 had chromosomal abnormalities not related to the abnormal karyotype (60%). Unbalanced embryos were detected in half of the studied patients (3/6). No correlation between the percentage of unbalanced embryos and the inversion size was found, as previously suggested in the literature. For example: in this study, the PGD cycle with more unbalanced embryos detected corresponded to a small autosomal paracentric inversion, 36 Mpb - 26.7% of the whole chromosome size.

Follow up was obtained in 6 cycles. Pregnancy rate was 42.8% per cycle and 60% per transfer.

Conclusions

In this study, autosomal paracentric inversion carriers produce 11% of unbalanced embryos. This frequency is higher than previously described. This study suggests that autosomal paracentric inversion carriers are at risk of producing unbalanced embryos, independently of the inversion size. More attention should be paid to these patients, especially when they are involved in an assisted reproductive treatment due to sterility. An IVF-PGD cycle for inversion analysis should be considered.

P-43

OFF THE STREET PHASING (OTSP): FREE NO HASSLE HAPLOTYPE PHASING FOR MOLECULAR PGD APPLICATIONS.

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Introduction: Phasing of parental mutation-flanking haplotypes is an essential pre-requisite for preimplantation genetic diagnosis (PGD) of monogenic disorders. Classically, haplotype phasing is determined experimentally by genotyping multiple gene-proximal polymorphic markers in both parents and at least one first degree relative. However, the process of marker selection is generally time-consuming and laborious. In addition, immediate family members are not always available to provide DNA samples for the haplotype analysis. In this regard, recent advances in high throughput genotyping technologies are beginning to offer promising solutions to these issues, particularly given the wealth of whole genome datasets that are already available to facilitate accurate population-based haplotype prediction. Accordingly, the aim of this study was to validate population-assisted haplotype derivation in a pre-clinical setting.

Materials and Methods: Targeted sequencing of common *CFTR* variants and ~1,700 gene-flanking highly polymorphic SNPs (+/-2Mb distance from *CFTR*) in 38 Jewish individuals from 9 different PGD families was performed on MiSeq or NextSeq 500 instruments at the Shaare Zedek Medical Center PGD lab. Heterozygous genotype calls were both trio-phased to obtain ground-truth haplotypes, and also population-phased using Shapeit software. Reference panels for population phasing were derived from either the 1000 Genomes sequencing project or from whole genome sequences of 128 or 574 Ashkenazi Jewish samples (kindly provided by The Ashkenazi Genome Consortium). The accuracy of the resulting haplotypes was benchmarked against the trio-phased haplotypes. The study population consisted of 4 subgroups of Jewish individuals: a) Non-Ashkenazi (NA; 10 negative controls); b) Full Ashkenazi (FA; 10 individuals); c) Partial Ashkenazi (13 individuals featuring at least 1 Ashkenazi and 1 non-Ashkenazi ancestor) without Ashkenazi *CFTR* founder mutation carriage (PANM); and d) Partial Ashkenazi (5 individuals) with W1282X CFTR Ashkenazi founder mutation carriage (PAWM).

Results: The 1000 Genomes reference phased an average of 544.5 to 741.2 heterozygous SNPs per sample among the 4 study subgroups. However, the phasing accuracy (ranging from 65.2%+/-6.4% to 73.1%+/-4.3%) was too low for clinical application. Likewise, the 128 Ashkenazi genome reference was not suitable for phasing the NA (591.9+/-57.3 heterozygous SNPs phased with 72.7%+/-4.2% accuracy) and PANM (484.9+/-32.8 SNPs; 78.9%+/-4.9% accuracy) subgroups. Nonetheless, a marked improvement was seen in the phasing of FA (559.4+/-42.0 SNPs; 88.4%+/-5.5% accuracy) and PAWM (665.6+/-40.3 SNPs; 94.3%+/-2.2% accuracy) subgroups using the same reference. In all subgroups, the 574 Ashkenazi genome reference panel was the most accurate. Near perfect phasing was observed for FA (621.4+/-47.4 SNPs, 98.2%+/-1.1% accuracy); almost perfect phasing for PAWM (732.8+/-47.0 SNPs; 91.4%+/-5.3% accuracy); and improved phasing of NA and PANM, respectively (579.4+/-62.3 SNPs; 84.9%+/-3.3% accuracy; 547.8+/-34.7 SNPs; 86.7%+/-5.2% accuracy). Importantly, virtually all errors in the FA group (and in the PAWM group, along a subregion of 3Mb) were traceable to sequencing errors in the ground truth, mostly due to low coverage.

Discussion: These striking results indicate that it may soon be possible to replace experimental haplotype phasing with clinical “OTSP”, population-based phasing, provided that one has access to an appropriate population-matched reference dataset of sufficient size.

P-44

NEW ALL-IN-ONE PROTOCOL FOR 24-CHROMOSOME ANEUPLOIDIES AND MONOGENIC DISEASES DETECTION BY NEXT-GENERATION SEQUENCING: FIRST-YEAR EXPERIENCE.

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Introduction:

Over 4000 human diseases are caused by single-gene defects and the global prevalence of them at birth is approximately 10/1000. The solution for couples carrying some of these monogenic disorders is to perform a PGD analysis along an *in vitro* fertilization (IVF) cycle; a long and stressful way coupled to the common problems that arise during a conventional reproductive cycle. We aim to describe a retrospective study of our PGD cycles performed during 2016, based on application of our own-developed protocol in terms of next-generation sequencing (NGS). Some couples were benefited from this method, which combine simultaneously PGD and aneuploidy screening (PGS) in the same NGS reaction.

Material and Methods:

This study comprises 30 couples performing IVF cycles along the past year, where one or both members were carrying a single-gene disorders as cystic fibrosis (CFTR), spinal-muscular atrophy (SMA), autosomal dominant or recessive polycystic kidney disease (PKD1/PKHD1), Huntington disease (HD), or X-fragile syndrome, among others. Informativity testing prior to each IVF cycle was less than a month and results from PGS/PGD study were available in 48 hours at most. For each individual case, samples from both patients and at least one member of the family were required. We performed our analysis developing an individual workflow for each monogenic disease combining multiplex PCR technology (Ion-Ampliseq™, Thermo Fisher Scientific) for PGD, and a specially designed protocol for single-cell amplification and PGS analysis (Ion Reproseq™), all performed on Ion PGM™ platform. The required material for multiplex PCR would be used for several couples with the same single-gene disorder in the future.

Results:

A total of 30 couples carrying 20 different monogenic disorders were benefited from our combined method. More than 30% of them (7/23) were able to select one or even more embryos for transfer. Implantation rate was 68% among all of the successful cycles. We were able to offer a quick and cost-effective method that integrates two preimplantational techniques, PGS and PGD, which increases the reproductive chances. Both were prepared in the same sequencing process and the results were available in a very short time. Some embryo transfers were carried out in the same cycle without the need to cryopreservation, if required, achieving similar pregnancy results as those obtained with embryos transferred in the next reproductive cycle. Furthermore, our method reduces the conventional turnaround-time for whole PGS/PGD processing.

Conclusions:

Current PGD protocols can delay the start of IVF cycles up to several months due to not enough information of disease-causing mutation is achieved. Our method provides the required information during the usual hormone stimulation period, reporting results within the same IVF cycle period. First-year experience applying our quick and cost-effective protocol gives us a live-birth/healthy-child percentage higher than expected.

P-45

SUCCESSFUL PREIMPLANTATION GENETIC DIAGNOSIS OF A-AND B-DOUBLE THALASSEMIA COMBINED WITH HLA TYPING BY NEXT GENERATION SEQUENCING.

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Introduction: The thalassemia is a group of hereditary anaemias characterized by reduced or even absent production of one of the globin chains of hemoglobin (Hb),which is prevalent in the Mediterranean region and Southeast Asia. In Southeast China, α-and β-thalassemia constitute the majority of monogenetic disorders with the average carrier rates as high as 10.3% and 8.5%, respectively. Patients with severe thalassemia need to be treated with regular blood transfusion. Stem cell transplantation is still the only radical cure method. PGD in combination with HLA typing offers a feasible choice for families design to preselect unaffected embryos that are HLA antigen compatible with a sibling needing cord blood transplantation. NGS is the latest breakthrough encouraging method for PGD/PGS, offering reliability, higher throughput and personalized assays.

Method: A couple, the 31-years-old female partner is a carrier of α- thalassemia(-α^{sea}) and β-thalassemia (CD41-42), the 31-years-old male partner is a carrier of α- thalassemia(-$\alpha^{3.7}$)and β-thalassemia (CD17).Their daughter was born 5 years ago, who was suffered from severe patients with severe β-thalassemia(CD17 and CD41-42) and also a carrier of α-thalassemia(-$\alpha^{3.7}$).Pedigree analysis was determined by both the parents and their daughter using NGS in preliminary study.The couple was counselled and standard ICSI-IVF protocols were applied. Trophectoderm biopsy was performed and cells from biopsy were processed for multiple permutation amplification (MDA).The blastocysts were vitrified following biopsy. MDA products were detected by NGS sequencing platform (Detection of α-thalassemia, β-thalassemia, HLA typing and PGS simultaneously), and HLA-matched embryos with normal genotype were selected for transfer in the frozen-thawed cycle.

Result: Two oocyte retrieval cycles were performed. For the first cycle,four oocytes were retrieved. Only one was metaphase II oocytes and antagonist protocol in the second cycle.18 oocytes were retrieved and 13 oocytes were fertilized by ICSI.7 blastocysts suitable for biopsy were obtained. MDA was successful in all 7 blastocysts, and all samples got diagnostic results. For α- thalassemia, one was found to be affected and two were carriers of α- thalassemia(-$\alpha^{3.7}$), and the other four were absent of α-thalassemia mutation. For β-thalassemia, five were carriers of β-thalassemia (CD41-42) and the other two were carriers of β-thalassemia (CD17). The results of HLA typing showed than 4 embryos were marched and the PGS results of these four marched embryos were euploidy. PGS results of the two HLA no-marched embryos showed that one was mosaic on chromosome 19,and the other one was segmental aneuploidy on chromosome 14.Over all, four euploidy embryos with normal genotype were suitable for transferred. One euploidy embryo, normal of α-thalassemia but carrier of β-thalassemia (CD41-42), was transferred in the frozen-thawed cycle,and achieved singleton pregnancy. The result of prenatal diagnosis was consistent with the NGS-PGD/PGS result. The pregnancy is still on-going with a gestation of 17 weeks.

Conclusion: The NGS platform can simultaneously detect a-and β-thalassemia, as well as HLA typing and PGS. Moreover, The genetic status of individual embryos can be determined by analysis of linked SNP alleles inherited along with the mutant gene,which can reduce the risk of misdiagnosis caused by allele drop out or contamination.

P-46

PREIMPLANTATION GENETIC DIAGNOSIS OF A- AND B-DOUBLE THALASSEMIA COMBINED WITH ANEUPLOIDY SCREENING BY NEXT GENERATION SEQUENCING.

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Introduction: Thalassemias are a group of autosomal recessive disorders caused by the reduction or absent production of one or more globin chains that constitute hemo-globin (Hb) tetramers, which are prevalent in the Mediterranean region and Southeast Asia. α-thalassemia and β-thalassemia are two main types, constitute the majority of monogenetic disorders in Southeast China with the average carrier rates as high as 10.3% and 8.5%, respectively. NGS technology enables an all-in-one informativity testing for analysis of Single Nucleotide Polymorphisms (SNPs) linked to gene regions involved by mutation, which can reduce the risk of misdiagnosis caused by allele drop out(ADO) or contamination. Here, we report a successful NGS-based method for PGD/PGS of α- and b-double thalassemia.

Method: A total of five couples, all of them were carriers or patients of both α-thalassemia and β-thalassemia. They requested PGD treatment in our center after counselled. Blastocysts with at least average grade (Gardner, 1999) were biopsied and vitrified post-biopsy. The biopsied samples underwent whole genome amplification（WGA）using the multiple displacement amplification(MDA) approach and the amplified product were used to perform NGS (Ion TorrentTM) for PGD of α-and β-thalassemia and PGS.For α-thalassemia, the SEA deletion(21kb) was selected as the target region. For β-thalassemia, the HBB(1.6kb) was selected as the target region. A total of 120 high density and closely linked SNPs (60 SNPs for each target region) mapped 1Mb at both upstream and downstream of the genes were used as genetic marker. Primers were designed on the ION AMPLISEQTM DESGNER website. DNA purification, cDNA library construction and sequencing using the Ion Torrent Personal Genome Machine were performed in order. Haplotypes were established by a selection of several informative SNPs. PGS was performed only on unaffected embryos. Standard procedures for thaw of unaffected euploidy embryos were performed and blastocysts transferred in a subsequent medicated TET cycle.

Result:8 ovum pick-up cycles(5 PGD/PGS cycles) were performed for the 5 couples. A total of 104 oocytes were collected and 73 were normal fertilized by ICSI. Trophectoderm biopsy and NGS were performed on 37 embryos.36(97.3%,36/37) embryos gave conclusive PGD results. 13(36.1%,13/36) of the embryos were found to be unaffected of both a-and b-thalassemia. PGS results of these 13 embryos showed that 3 were aneuploidy and one was unable to interpret. Thus 9(24.3%,9/37) embryos were suitable for transfer. One family had no embryo available for transfer. For the other four couples, one embryo was transferred in TET cycle for each couple. Three of them reached clinical pregnancy, with on-going pregnancies of 9 weeks,27 weeks and 33 weeks, respectively. One family failed to conceive. The ongoing pregnancy rate is 75%(3/4) per ET cycle. Two families has had prenatal diagnosis and the result of prenatal diagnosis were consistent with the NGS-PGD/PGS results.

Conclusion: This data suggests that NGS can be used as a reliable and accurate method for the simultaneous detection of α-and β-thalassemia. This eliminates the need to biopsy the same blastocyst twice, avoiding potential damage to the blastocyst and allows the selection of euploid blastocyst for transfer.

P-47

PREIMPLANTATION GENETIC DIAGNOSIS ALLOWS TO CORRECT KARYOTYPE OF A PATIENT AND TO CHANGE RISK CALCULATION.

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Introduction: Karyotyping of IVF patients before IVF cycle is an important predictor of the risk of chromosomal abnormalities in the offspring. During preimplantation genetic diagnosis (PGD) profile of chromosomal changes in embryos should correspond to parental karyotype. Occasionally PGD findings in embryos make the patient's karyotype questionable. We report the case in which chromosomal abnormalities in embryos allowed to suspect in a patient's karyotype different chromosomal rearrangement. This assumption was confirmed by FISH-analysis on maternal blood.

Material & methods: PGD chromosomal abnormalities of embryos was performed by aCGH on microarrays 24sure+ (Illumina). Couple was referred to IVF-PGD due to balanced reciprocal translocation in patient. Analysis of patient's karyotype was performed in two independent laboratories and produced same result: 46,XX,t(4;13)(p14;q34). Two IVF cycles were performed and nine embryos were biopsied for PGD. The trophectoderm biopsy was performed at 5-6 day of embryo development.

Results: Analysis of chromosomal profile in embryos revealed segmental rearrangements in chromosome 4 with pattern of malsegregation not typical for translocation. Also small number of normal embryos in both IVF cycles was noted. These findings allowed to question the accuracy of karyotype. FISH-analysis with subtelomeric and WCP probes for chromosomes 4 and 13 was performed on maternal cultured lymphocytes and confirmed that patient had an interchromosomal insertion of part of chromosome 4 in q-arm of chromosome 13. Revision of patient's karyotype allowed to estimate balanced insertion - 46,XX,ins(13;4)(q34;p14p15.31).

Conclusions: This case shows that PGD is not only an accurate method of chromosomal screening, but also has a capacity to improve diagnostics for the couple. Increased precision of karyotyping provides more accurate prognosis as in this case, because insertions are among rearrangements implying the highest reproductive risk. It is extremely important to draw attention to pattern of embryo chromosomal rearrangements during PGD.

P-48

PREIMPLANTATION GENETIC DIAGNOSIS IN FAMILIES WITH HIGH RISK FOR SPINAL MUSCULAR ATROPHY (SMA).

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Introduction

Spinal muscular atrophy (SMA) is the autosomal recessive neuromuscular disorder caused by mutations in the survival of motor neuron (*SMN1*) with incidence 1 in 10,000 live births. SMA is the main genetic cause of infant mortality. Molecular diagnosis of SMA is complicated by the presence of pseudogene *SMN2*. Unfortunately, widely used MLPA method for diagnosis of *SMN1* copy number is not applicable for embryo biopsy. In families where both parents are carriers of SMA, preimplantation genetic diagnosis (PGD) is an option to avoid transferring of an affected embryo during the IVF. For this purpose we designed a test-system.

Material & methods:

The system combines direct diagnosis of the *SMN1* gene presence by PCR-RFLP and linkage analysis of highly heterozygous STRs. The nested PCR was used for DNA amplification from different sources: a single cell biopsy on 3rd day of embryo development, whole genome amplification products (SurePlex kit, Illumina) of day 5th trophectoderm biopsy is case of combination with chromosomal screening by aCGH (24sure, Illumina) as well as chorionic villus sample (CVS). The developed test system was applied for diagnosis of SMA in 4 families whose children died from the disease. Embryos for PGD were obtained by IVF-ICSI procedure.

Results:

PGD was performed for 3 families of the 4 referred to the laboratory and for one family - only prenatal diagnosis.

Family 1: One embryo was obtained. PGD revealed carrier status, the results of chromosome analysis is normal karyotype. The embryo was recommended for transfer. The pregnancy outcome is healthy child.

Family 2: 5 embryos were obtained and biopsy was done on the 3rd day of development. PGD was performed in the fresh cycle without PGS according with family desire. Amplification was failed for one sample. Four other embryos were analyzed. PGD revealed carrier status for three embryos, one was homozygous for the SMN1 deletion.

Family 3: 6 embryos were obtained and 5 were successfully amplified by WGA. PGD revealed homozygous *SMN1* deletion for one embryo, carrier status for another one and normal homozygotes for the other two. The remaining three embryos had normal karyotype. For one embryo we could identify only one paternal haplotype, this embryo was chaotic by chromosome analysis. Three embryos were transferred but pregnancy was not achieved. Later family got pregnant naturally. CVS was carried out and prenatal diagnosis was performed by MLPA and indirect diagnostics of STR loci. The fetus got two healthy chromosomes with *SMN1*. The results were similar for both methods. Thus, the test system was validated by the MLPA results.

Family 4 had natural pregnancy. Combined prenatal diagnosis revealed carrier status for the fetus.

Conclusions

The described test system can be used in different situations (day 3 and 5 embryo biopsy, direct amplification of single cells and pre-WGA for further chromosomal abnormalities analysis) and as well as an additional method for prenatal diagnosis in combination with MLPA.

P-49

PREIMPLANTATION GENETIC DIAGNOSIS FOR ACHONDROPLASIA BY TWO HAPLOTYPING SYSTEM:SHORT TANDEM REPEATS(STRS) AND SINGLE NUCLEOTIDE POLYMORPHISM(SNP).

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Introduction: Achondroplasia (ACH) is the most common genetic form of dwarfism. Most cases occur spontaneously, and these heterozygous individuals may pass the condition on to their offspring as an autosomal dominant trait. In order to reduce the misdiagnosis caused by allelic drop out (ADO) and contamination, a number of haplotyping methods including short tandem repeats (STRs)-based linkage analysis, SNP markers-based Karyomapping and next generation sequencing (NGS) have been successfully applied to improve the accuracy of PGD. We used two different haplotype methods to establish reliable and accurate preimplantation genetic diagnosis methods for the prevention of birth defects in a family with ACH.

Material & methods: A couple, the male has typical clinical symptoms of ACH and genetic test showed FGFR3 (c.1138G> A, p.G380R) . The family had two pregnancies by natural conception but the prenatal diagnosis showed that both were ACH-affected fetus, thus they requested PGD treatment in our center after twice induction of labor.
STRs Method: Pedigree analysis of ACH was performed using 8 STRs closely linked to the fibroblast growth factor receptor 3 (FGFR3) gene(five STRs were found to be informative) . Embryo biopsy was performed on day-3 (cleavage stage), one blastomere was biopsied from each embryo, and then whole genome amplification(WGA) was performed using multiple displacement amplification (MDA). The MDA products were analyzed using the five informative STR loci and PCR-restriction enzyme digestion of FGFR3 gene.

NGS method: A single trophectoderm biopsy was performed and cells from biopsy were processed for MDA.The blastocysts were vitrified following biopsy. MDA products were detected by NGS(Ion PGM™) sequencing platform.The FGFR3 gene region was selected as the target region. A total of 110 high density and closely linked SNPs mapped 2Mb at the upstream and downstream of the genes were selected. DNA purification, cDNA library construction and sequencing using the Ion Torrent Personal Genome Machine were performed in order. Haplotypes were established by a selection of several informative SNPs. PGS was performed on embryos with normal FGFR3 genotype.

Result: Two different systems were adopted for performing one cycle of PGD for this family, respectively. Five embryos were diagnosed at the first cycle (STR). One failed in MDA and four embryos were diagnosed. Two of them were normal embryos (one of which degenerated in the culture process), one embryo was transferred at TET cycle and achieved singleton pregnancy, and delivered a healthy girl at full term. Nine embryos were diagnosed at the second cycle (SNP-NGS) and all of them had diagnosis results. Two were detected to be normal and euploidy, and the other embryos were ACH-affected.One embryo was transferred at TET cycle but failed to conceive. Another embryo was transplanted at the other TET cycle and singleton pregnancy was achieved. The result of prenatal diagnosis was consistent with the NGS-PGD/PGS result. The pregnancy is still on-going with a gestation of 24 weeks.

Conclusion: Preimplantation genetic haplotyping system can be established by using STRs and SNP systems. While NGS-SNP system can detect more sites and simultaneously perform PGS with automated operation.

P-50

PGD FOR DE NOVO MUTATION: WHEN MOSAICISM PREVENTS PGD SETUP AND LEADS TO GENETIC COUNSELLING REVISION.

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Introduction: In our centre, due to legal restrictions precluding the use of whole genome analyses, PGD for monogenic disorders with de novo mutation is based on multiplex PCR combining direct and indirect diagnosis of single blastomeres. Haplotypes can be either deduced from single sperm, or polar bodies, or during embryo analysis. For clivage-stage PGD, setup is performed and validated on series of single cells. In case of mosaicism, at risk haplotype is present in cells with, but also without, mutation and allele drop out (ADO) cannot be properly evaluated. Due to ADO possibility, only embryos with the normal haplotype are considered for transfer. We present issues and consequences of PGD setup in a case referred for tuberous sclerosis with mosaicism in the male partner.

Material & methods: A 36-year-old woman and her 39-year-old husband were referred for PGD to avoid paternal transmission of tuberous sclerosis due to a c.2250G>A de novo mutation in exon 18 of TSC1 gene. The patient was mildly affected and somatic mosaicism was detected in DNA from his lymphocytes and buccal cells with a low but undetermined mutation level. Transmission risk was probably lower than 50%, but could not be properly evaluated and PGD was proposed. In order to choose the markers to be co-amplified with the mutation, DNA from both partners and from the male's parents were analysed. A 4-plex PCR was set up on normal control cells and single lymphoblasts from the patient. Single sperm was tested in order to determine at risk haplotypes and to better evaluate the mosaicism.

Results: The 4-plex PCR was successful on 12 normal cells. The mutation was detectable in 2 out of 54 single lymphoblasts of the patient, whereas the markers were properly amplified. These cells being diploid, it was not possible to distinguish between ADO and absence of mutation. Haplotyping was performed on 108 sperm cells with an amplification rate of 98%. Three haplotypes were identified: the normal one (51), at risk haplotype with the mutation (only 1), and at risk haplotype without the mutation (53). Mutated alleles had a weaker signal than the normal one in single lymphoblasts and in sperm cells but the lack of mutated cells prevented technical optimization of the direct test. It was thus impossible to validate the setup on series of mutated cells. These results show that the mosaic is probably lower in sperm and that transmission risk might be much lower than initially suspected. In that case, PGD based on linkage would lead to eviction of too many normal embryos, which was considered as unacceptable by the local multidisciplinary committee. PGD procedure was thus stopped. Genetic counselling was revised and the couple was oriented towards spontaneous pregnancy with a possible prenatal diagnosis.

Conclusions: PGD setup for this paternal de novo mutation was compromised because of low mosaicism but it's results could help to better evaluate transmission risk and genetic counselling.

P-51

PGD BY FISH FOR A RECIPROCAL TRANSLOCATION-FIRST BABY FROM SOUTHINDIA.

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Introduction:

Chromosomal abnormalities plays a major role in early pregnancy loss and can also cause infertility. Balanced translocations carriers have high risk of having an abnormal pregnancy . The balanced translocation status remains undetectable unless they reproduce or have first trimester pregnancy loss . Earlier the only option left to couples carrying a genetic or chromosomal abnormality was a donor option if the couple fails to conceive a healthy pregnancy naturally. Preimplantation genetic diagnosis (PGD) is a widely accepted and practiced technology which helps to select and transfer an euploid or genetically normal embryo thereby avoiding the use of donor gametes. There are many technologies available to screen and diagnose chromosomal abnormalities in embryos. Fluorescent in situ hybridization (FISH) technique is the gold standard applied to detect unbalanced chromosomal defects due to balanced translocations. The aim of this study was to prove the efficacy of the FISH technique to detect the aneuploidies on embryos resulting from balanced translocation and whether a single euploid embryo transfer can result in a pregnancy .

Materials and Methods:

At the time of IVF female partner was 33 years old, with karyotype 46,XX,t(15;16)(q22.;p13.1). Her husband was karyotypically normal. History of three previous spontaneous abortions from natural conception, with no genetic evaluation of the aborted fetuses. The male partner was normospermic.The IVF treatment was conducted in our fertility hospital. From the first round 10 embryos were obtained. These embryos were frozen on day 2 . In the next cycle around 8 embryos were obtained and frozen on the same day as of the first cycle. In the next cycle only after PGD on all embryos was carried out and patient's endometrium was prepared with medications

Subtelomere and centromere probes for chromosomes 15 and 16 which were commercially available were used in this study. These probes were first checked both in the metaphases and interphase cells of the mother prior to applying on embryos.

All the 18 embryos survived post thawing and a single blastomere was biopsied and fixed on a glass slide. Slides were processed for FISH and probes were applied and analysed.

Results: Out of the 18 embryos analysed. 3 embryos were found to be normal. Rest were abnormal and two of them showed inconclusive results. The day 3 embryos post biopsy were allowed to grow till blastocyst stage. A single normal blast embryo transfer resulted in a live birth

Conclusion : FISH technique though assess limited number of chromosomes, yet proves to be a gold standard effective, robust in providing a diagnosis to help select a normal euploid embryo . Majority of females with a balanced translocation are not infertile and only experience recurrent pregnancy loss, hence the chances are more if a single euploid embryo free from the indicated defect is transferred. However NGS technology is now enabled and optimized to screen all 23 pairs of chromosomes and has shown to improve pregnancy rates even higher .

P-52

PRE-IMPLANTATION GENETIC SCREENING FOR DISCRIMINATION OF CARRIER AND NON-CARRIER RECIPROCAL TRANSLOCATION WITH ARRAY-CGH AND PGD.

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Introduction:

A reciprocal translocation is an unusual arrangement of chromosome, typically an exchange of two chromosomal segments from two non-homologous chromosomes, leading to less embryos of normal euploid status, increased failed implantations and recurrent miscarriages, and some liveborn with serious health problems. Pre-implantation genetic screening (PGS) with array-Comparative Genomic Hybridization (CGH) is currently in clinical use for detecting reciprocal translocations. However, CGH cannot differentiate between euploid embryos carrying and not carrying the balanced. Pre-implantation genetic diagnosis (PGD) using molecular techniques was performed post-CGH on whole genomic amplification (WGA) material to further distinguish euploid embryos as carriers or non-carriers of the balanced reciprocal translocation.

Objective:

To use PGS-CGH and PGD-polymerase chain reaction (PCR) in combination on embryos from couples who carry a balanced chromosomal rearrangements to see if pregnancy rates, and the chance for a successful live birth, could be increased.

Material & Methods:

Following an IVF cycle, a single biopsy of Day5-6 trophectoderm was subjected to whole genomic amplification (WGA) and PGS-CGH/TL using 24 Sure^{+} (Illumina) to detect the precise breakpoints using chromosomally unbalanced translocation embryo(s) and screen aneuploidy at the same time. Post-CGH PGD-PCR analysis was performed on the WGA using informative short tandem repeat (STR) polymorphic markers close to and straddling the breakpoints.

Results:

Two couples presented where the first couple having a son carrying a maternally inherited balanced translocation t(7;8)(q11.23;q21.2), and the second couple having a son with a gain in 10q26 arising from a paternally inherited balanced translocation t(10;12)(q26;p11.2). In all 12 embryos were analyzed with 8 (67%) revealing an unbalanced pattern. STR markers close to each breakpoint were identified and PGD-PCR performed on WGA from the 4 balanced translocation embryos (2 embryos/family). Two embryos (50%) were found to be carriers and 2 embryos (50%) to be non-carriers (1 carrier and 1 non-carrier from each family). The first family chose to transfer the carrier embryo leading to the birth of a healthy carrier female. The second family transferred a non-carrier embryo, but pregnancy was not achieved. The second family chose to only transfer non-carrier embryos and consequently underwent a further two IVF/PGD treatment cycles with 6 embryos having PGD-CGH analysis. All were unbalanced for the reciprocal translocation and therefore not suitable for transfer.

Discussion and Conclusion:

The results indicated that for carrying a reciprocal translocation family the option of PGS-CGH/TL using 24 sure^{+} can accurately evaluate the breakpoint to allow further diagnosis by PGD-PCR. It is a feasible approach to obtain embryos with a normal chromosome complement and increased the chance for a successful live birth. However, this technique still has limitations in some families.

P-53

IMPACT OF APPLICATION OF NEXT GENERATION TECHNOLOGIES ON THE OUTCOME OF PREIMPLANTATION GENETIC TESTING FOR STRUCTURAL REARRANGEMENTS (PGT-SR).

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Introduction: PGT-SR is presently performed by the application of next generation technologies expected to significantly improve the effectiveness of the procedure and the outcome of PGT cycles. The objective of this report is to analyze our experience of PGT-SR over the last six years to provide the resulting data for genetic counseling of couples with balanced translocations.

Methods& Materials: Analyzed data included 34 blastomere biopsies and 1035 trophectoderm biopsies, obtained from 193 IVF cycles from June 2011 to January 2017. Of these cycles, 154 (822 samples) were analyzed by array Comparative Genomic Hybridization (aCGH), and 39 (247 samples) using Next Generation Sequencing (NGS). The rate of unbalanced (UB) embryos, normal/balanced (NB) embryos and embryos suitable for transfer (SFT; normal/balanced and euploid) was calculated in each scenario based on PGT results for each translocation.

Results: Average age of female partner was 33.01 years (SD 3.8 years). Overall, the rate of SFT embryos for reciprocal (30.9%; 567/821) vs. Robertsonian translocations (43.1%; 141/248), differed significantly based on translocation type ($p < 0.02$). The rate of SFT embryos based on paternal or maternal carrier of translocation did not differ significantly ($p > 0.05$). In reciprocal translocation carriers the rate of UB, NB and SFT embryos did not differ significantly between maternal and paternal translocation carriers. [UB total: 56.1% (461/821); Maternal UB: 58.6%(246/420); Paternal UB: 53.6% (215/401); $p > 0.05$] [NB total: 43.8% (360/821); Maternal NB: 41.4%(174/420); Paternal NB: 46.4% (131/401); $p > 0.05$] [SFT total: 30.9% (254/821); Maternal SFT: 29.3% (297/420); Paternal SFT: 32.7% (270/401); $p > 0.05$]. In Robertsonian translocation carriers the rate of UB, NB and SFT embryos did not differ significantly between maternal and paternal translocation carriers. [UB total: 30.6% (76/248); Maternal UB: 33.8% (47/139); Paternal UB: 26.6% (29/109); $p > 0.05$] [NB total: 69.3% (172/248); Maternal NB: 66.2% (92/139); Paternal NB: 73.9% (80/109); $p > 0.05$] [SFT total: 43.2% (107/248); Maternal SFT: 38.1% (254/821); Paternal SFT: 49.5% (54/109); $p > 0.05$].

Conclusions: This study shows that Robertsonian translocation carrier couples have a higher rate of embryos suitable for transfer following PGD testing, compared to reciprocal translocation carrier couples. The rate of embryos suitable for transfer does not differ significantly based on maternal or paternal parental carrier of the translocation. This information is useful for pre-PGT-SR counseling for translocation carrier couples when offering them the application of the next generation technologies.

P-54

ANEUPLOIDY RATES OF NEXT-GENERATION SEQUENCING IN PREIMPLANTATION GENETIC DIAGNOSIS FOR BALANCED TRANSLOCATION CARRIERS.

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Introduction:

There is an elevated risk of meiosis nondisjunction in carriers of balanced chromosomal rearrangements. The mispairing of translocated chromosomes during the first meiotic division causes different forms of segregation, which can result in aneuploidy of the translocated chromosomes. Carries of balanced translocation carriers are subject to elevated risk of chromosomally abnormal gametes, resulting in high rates of miscarriage and/or children with congenital abnormalities. Previous studies reported that the presence of chromosome rearrangements may also cause an increase in aneuploidy affecting structurally normal chromosomes due to disruption of chromosome alignment on the spindle or disturbance of other factors related to meiotic chromosome segregation and this situation is known as inter-chromosomal effect.

The aim of this study was to present our cases using preimplantation genetic diagnosis after the identification of a parental balanced translocation. Preimplantation genetic diagnosis for a known translocation and aneuploidy screening were studied for all cases. It was also aimed to evaluate the effect of interchromosomal effect on pregnancy success rates by using NGS array.

Method:

All single cells were collected in 2 µl PBS solution. Whole genome amplification procedure was perfrmed with SurePlex DNA Amplification Kit (Illumina, Inc).. Amplified samples for NGS were processed with VeriSeq PGS kit(Illumina,Inc). Whole procedure was performed according to VeriSeq PGS workflow. The following bioinformatics analysis was accomplished with prerelease version of BlueFuse Multi for NGS (Illumina,Inc).

Results:

39 couples with balanced translocations were included in this study. Average tested embryo number for each case was 3.61. Results of 141 embryos revealed 30% euploidy rate . Segmental translocation imbalances (Partial gain or loss of chromosomal segments) rate was % 27 of total embryos. 68% embryos were designated as aneuploid. 21% of aneuploid embryos were accompanied by unbalanced translocations. 28% of aneuploid embryos had only aneuploidy in chromosomes that are not included in balanced chromosomal rearrangements. 2 % amplification failure was observed.

Conclusion:

The statistical analyses showed that NGS-based pre-implantation genetic diagnosis provides an evaluation on clinical outcomes and pregnancy rates for translocation carriers.

Our results demonstrated that NGS provides an accurate approach to detect embryonic imbalanced segmental rearrangements and aneuploidies for translocation carriers. Our report showed that NGS-based pre-implantation genetic diagnosis increases IVF success due to analysing segmental imbalances and aneuploidies simultaneously.

P-55

STRATEGIES AND CLINICAL OUTCOME OF PREIMPLANTATION GENETIC DIAGNOSIS FOR POLYCYSTIC KIDNEY DISEASE.

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Introduction: Polycystic kidney disease (PKD) comprises a group of monogenic disorders that result in renal cyst development. In this study, we describe the strategies and clinical outcome of a large cohort of preimplantation genetic diagnosis (PGD) cycles for PKD performed at the Centre for Medical Genetics of the Universitair Ziekenhuis Brussel.

Material & methods: Between 2005 and 2015, pre-clinical PGD workup was performed for 48 couples at risk for PKD. Thirty-three couples underwent 73 PGD cycles for PKD (24 couples for polycystic kidney disease 1 (PKD1), 1 couple for polycystic kidney disease 2 (PKD2) and 8 couples for autosomal recessive polycystic kidney disease (ARPKD)).

Results: Fifteen single cell clinical tests for PKD based on multiplex PCR of short tandem repeat (STR) markers, with or without a specific mutation (analysed directly by fragment length difference or by minisequencing) were developed. Blastomere biopsy was performed on 450 day 3 cleavage stage embryos. Genetic analysis resulted in 428 embryos (95.1%) with a diagnostic result of which 174 (38.7%) were genetically transferable. Transfer of 68 embryos in 48 fresh cycles resulted in a live birth delivery rate of 39.6% per embryo transfer with 17 singleton and 2 twin live births. Transfer of 21 cryopreserved embryos in 20 frozen embryo transfer (FET) cycles resulted in a live birth delivery rate of 45.0% per FET with 9 singleton live births. Twenty-five embryos still remain cryopreserved. The observed cumulative live birth rate was 62.9% per couple with a maximum of five PGD cycles.

Conclusions: The application of single cell clinical PCR tests containing at least six STR markers for each of the three PKD genes increases the diagnostic accuracy and reliability because it allows a robust detection of allele drop-out, contamination and recombination. Our data represent a large cohort of PGD cycles for PKD with good reproductive outcome for both fresh and frozen embryo transfers and can be a valuable tool for counselling PKD patients about their reproductive options.

P-56

UNIPARENTAL DISOMY TESTING IN ROBERTSONIAN TRANSLOCATION CARRIERS.

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Introduction: Carriers of the most common balanced structural rearrangements Robertsonian translocations (ROBs) are at increased risk of producing offspring with aneuploidy or uniparental disomy (UPD). Estimated incidence of UPD of any chromosome is 1:3,500 live births, around 50% of these cases in the UPD databases are associated with acrocentric chromosomes and over 10% of these acrocentric derived UPDs involve Robertsonian translocation. Among the acrocentric chromosomes, 14 and 15 have been established as imprinted with defined clinical phenotype. We report prenatal case of paternal UPD14 in an offspring of the der(13;14) carrier.

Material & methods: Foetal chorionic villi sample (CVS), amniotic fluid (AF), parental peripheral blood (PB) samples and embryos trophectoderm (TE) cells were examined by following methods: array CGH (SurePrint ISCA 8x60K Agilent, GenetiSure Prescreen Kit 8x60K Agilent), karyotype and QF-PCR.

Results: We investigated a CVS sample of foetus with ultrasound abnormalities in the 12th week of gestation. We discovered mosaic trisomy 14 using array CGH and confirmed it by classical karyotyping. Next, parental PB and AF sampling were indicated. We found Robertsonian translocation der(13;14) in father's PB and in euploid AF by karyotyping. Using QF-PCR targeted to STR markers we confirmed paternal UPD14 as a consequence of a trisomy rescue and the pregnancy was terminated. The consequences of the paternal translocation were discussed with the pair and PGD recommended. Then, we employed PGD array combined with QF-PCR UPD14 exclusion in TE cells of 16 embryos to identify 3 euploid non-UPD embryos. Consequently, these embryos were recommended for the embryo-transfer.

Conclusions: Carriers of Robertsonian translocation involving acrocentric chromosomes are at risk of having a child with aneuploidy or UPD. Given the relevance of the imprinted chromosomes 14 and 15, genetic testing must be complemented with UPD analysis in all couples carrying ROB involving 14 or 15 and undergoing PGD.

P-57

PRENATALIS® NIPT: ACCREDITED HIGH RESOLUTION NON-INVASIVE PRENATAL TESTING BY USING MASSIVE PARALLEL ULTRA-DEEP SEQUENCING.

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Introduction: Prenatalis® is a non-invasive prenatal test (NIPT) based on Illumina´s Verifi technology that is the first NIPT in Europe with a DIN EN ISO 15189 accreditation obtained by the German accreditation body DAkkS. The Prenatalis® NIPT provides an *adjusted* specific risk for trisomy 13, 18 and 21 and is recommended for pregnancies with a medium sum risk score (1:50 – 1:1000) following first trimester screening or maternal age above 35 y. Prenatalis® is utilizing a up to 3-fold higher sequencing coverage (20 Mio reads) compared to e.g. targeted enrichment based test procedures, resulting in an improved performance in samples with low fetal fraction (FF), e.g. FF < 4%.

Material and Methods: Here we report the results of 2586 analyses performed at a gestational age from 10th to 26th week. The fetal fraction averaged at 9,4% (SD: 4,2%) and included 8,6% of samples with low fetal fraction (2,7%<FF< 4%). A Z score-based NCV (normalized chromosome value) was calculated for each chromosome tested after normalization of the sequence read number to a sample internal reference chromosome set. This algorithm allows a high dynamic range of NCVs and therefore facilitates the classification regarding the presence of a numerical aberration of chromosome 13, 18 and 21.

Results: The specificity of the test equals or is higher than 99,9% for all chromosomes tested. The sensitivity for the detection of trisomy 21, 18 and 13 was 99,1%, 98,3% and 98,1% respectively. The positive predictive value (ppv) for trisomy 21 (trisomy 18) tested in a high risk cohort was 99,4% (91,0%). In a low risk cohort, with a lower trisomy prevalence, the test demonstrated a 10x-fold increased ppv above first trimester screening (45,5% vs. 4,2%).

Conclusions: Current experience with Prenatalis® reinforces that the test is highly accurate for fetal aneuploidy detection in a general obstetric population. According to German law, NIPT requires *a priori* genetic counseling and should be carried out under the supervision of a physician. Due to potential serious consequences of prenatal diagnostic results, we strongly recommend embedding NIPT in a holistic, prenatal environment including professional medical interpretation and counseling. The Prenatalis® consortium (www.prenatalis.de) is a network initiative offering a platform for information exchange and continuous medical education for prenatal medicine professionals aiming at highest quality standards in patient care.

P-58

SPERM DNA QUALITY CORRELATES WITH PGD RESULTS.

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Introduction. Almost 60% of blastocysts formed in IVF are aneuploid. Besides that, spontaneous miscarriage occurs in 10–15% of clinical pregnancies in the normal fertile population but the rate is known to be higher in subfertile couples. Increased sperm DNA damage has been associated with poor embryo quality and pregnancy loss. For the purposes of this study we investigated the correlation of sperm DNA fragmentation index with the PGD results after NGS.

Materials and methods. The size of study was 15 couples with the male factor of infertility undergoing the ART cycles. Sperm samples were analyzed following WHO 2010 indications. DFI was measured by the method of sperm chromatin dispersion (SCD) following the protocol of HaloSperm, Halotech (Spain). Totally 45 high quality blastocysts were biopsied on the 5th and 6th days of culture and outsourced to commercial reference laboratory for next generation sequencing (NGS) test. Correlation of observed DFI with the number of aneuploid blastocysts was examined with Spearman coefficient.

Results. We evaluated the possible relationship between DFI value and PGD results. The significant positive correlation of the sperm DNA fragmentation index with the number of aneuploid blastocysts has been observed (r_S = 0.68, P<0.01). There were no correlations of DFI with the total number of formed blastocysts and the number of the high quality blastocysts. The average age of patients' was 44.1±5.7 years old. No dependence of DFI on the male age was found in mentioned group. Nevertheless we have shown that DFI increases in men after 35 years old in our previous studies.

Conclusion. Existing data regarding the relationship between sperm DNA integrity and fertilization and pregnancy rates are conflicting. Results of the present study point to a significant relationship of sperm DNA fragmentation with the chromosomal abnormalities in preimplantation embryos. The results of our work show that DFI does not influence the blastocyst formation rates but could be the reason of embryo implantation failures and pregnancy loss because of the relationship with embryonic genome.

P-60

DIFFERENCES IN EUPLOIDY RATES BETWEEN THE MAIN INDICATIONS FOR PGS.

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Introduction

Currently, the indications for PGS are: advanced maternal age (AMA), repeated fail implantation, recurrent miscarriage, and severe male factor. In all these groups of patients an increased embryo aneuploidy rate is presumed. PGS has been applied for the past 15 years to test the chromosome content of IVF embryos to improve the reproductive outcome of specific groups of patients. However, the beneficial effect of PGS has not been proved yet in a properly designed RCT in any of these groups.

Materials and Methods

This is a retrospective cohort study with data from 2016, realized at Fertilitat Clinic – Center of Medicine Reproductive, in Porto Alegre – Brazil, A total of 229 cycles were analyzed, of which 140 biopsies were realized for advanced maternal age (> 40 years; AMA), 46 for severe male factor, and 43 for translocation (man or woman). The embryo biopsy was performed on day 3 of embryo development followed by blastocyst culture. Whole genome amplification and 24-chromosomal analysis was performed by CGH/NGS. Statistical analysis was realized by the chi-square heterogeneity test ($p<0.01$) and odds ratio was calculated on the BioEstat 5.3 software.

Results

In the group with AMA indication, 476 embryos were analyzed and 12% were euploids. When the indication was the male factor, 173 embryos were analyzed, with an euploidy rate of 23.1%. The AMA + male factor group had a 3% rate of euploids in 58 analyzed embryos. In patients who underwent the biopsy due to translocation, an euploidy rate of 19% was observed from 192 embryos analyzed. The statistical analysis revealed that when the biopsy is performed due to male factor there is a 2.21x higher chance of having a normal embryo when compared with that patients with indication of AMA for PGS. ($P= 0.0007$). Our data also show that there is a trend of greater chance of having a normal embryo when the embryos are biopsied due translocation, although not being statistically significant.

Conclusions

Thereby, our data showed that when PGS is performed due to SMF there is an increased chance of having a normal embryo, compared to when the PGS was indicated for AMA. There is a lack of scientific data on the use of PGS applied for SMF and so RCTs are required for this indication.

P-61

CHROMOSOMAL POLYMORPHIC VARIATIONS IN FEMALES SIGNIFICANTLY AFFECTS CLINICAL OUTCOMES FOLLOWING AN IN *VITRO* FERTILIZATION CYCLE.

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Introduction Chromosomal polymorphic variation has been considered normal for a long period. This retrospective study comprehensively analyzed the correlation, in infertile couples, between chromosome polymorphic variants and the outcomes of IVF treatments.

Material and methods Retrospective study performed in 11 private clinics belonging to IVI group from January 2012 to December 2016. We included 2652 couples in which in 29.6% (n=784) the male was carrier of a polymorphism; in 18.3% (n=486) the female was the carrier, in 2.0% (n=54) both members were carriers of a chromosome polymorphic variant; and in the remaining 50.1% (n=1328) of the couples both members had normal karyotype. All couples underwent a fresh autologous ICSI cycle. Statistical analysis was performed by ANOVA and chi-square where applicable. The cytogenetic study was performed by culture of peripheral blood lymphocytes stimulated with phytohemagglutinin and subsequent staining with trypsin-Giemsa (GTG bands). 15 metaphases were evaluated for each case and the banding resolution was 400–550 bands per haploid set. Polymorphisms were included only when their size was greater or smaller at least twice the size on the other homologue.

Results. Basal data showed statistical differences. For age, data showed 35.6±0.2 years for couples with a male carrier; 36.2±0.2 years for couples with a female carrier; 35.2±1.2 years for couples with both members affected; and 37.3±0.3 years for control couples, p<0.001. For total sperm, data revealed 96.3±10 million for couples with a male carrier; 93.2±15 million for couples with a female carrier; 95.4±30.0 million for couples with both members affected; and 117.1±5.0 million for control couples, p=0.001. For total progressive sperm, data exposed 46.5±6.1 million for couples with a male carrier; 41.9±17.3 million for couples with a female carrier; 44.6±18.0 million for couples with both members affected; and 54.5±2.7 million for control couples, p=0.020.

Implantation rates were as follows: 35.6% in male carriers; 30.7% in female carriers; 36.8% for couples with both members affected; and 39.6% for control group, p<0.001. Clinical pregnancy rate was 47.0% for male carriers; 41.1% for female carriers; 45.2% for couples with both members affected; and 55.6% for control group, p<0.001. Miscarriage rate was 18.1% for male carriers; 17.2% for female carriers; 27.8% for couples with both members affected; and 10.9% for control group, p=0.034. Finally, ongoing pregnancy rate showed the following values: 38.9% for couples with a male carrier; 35.6% for couples with a female carrier; 34.8% for couples with both members affected; and 42.3% for control group, p=0.027.

Conclusions. Our study reveals that the presence of chromosomal polymorphic variations in women significantly affects the results of an in *vitro* fertilization cycle irrespectively of the karyotype of the male partner. These findings suggest that women carrying a polymorphic variant could have a higher risk of chromosomal abnormalities in their embryos. Therefore, these couples may have worse reproductive prognosis. Despite being overrepresented in infertility couples compared to normal population, the role of polymorphic chromosomal variations continues to be an intriguing question. Screening prospective couples for chromosome variants may help enhance the success of an assisted reproductive treatment.

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