ABSTRACTS

15th International Conference on Preimplantation Genetic Diagnosis

Bologna, Italy

8th-11th May 2016

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Luca Gianaroli, Conference Chairman
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Welcome to the 15th International Conference on Preimplantation Genetic Diagnosis

Dear Colleagues,

On behalf of the Scientific Committee, it is our great pleasure to welcome you to Bologna and to the 15th International Conference on Preimplantation Diagnosis, organized by the Preimplantation Genetic Diagnosis International Society (PGDIS).

Preimplantation Genetic Diagnosis is undergoing a tremendous evolution at a rapid pace and the Congress aims to provide the unique opportunity to keep everyone updated on all of the latest advancements in this field.

We are excited that so many distinguished clinicians and scientists have joined the faculty and will take part in the Congress this year. They will be presenting on groundbreaking studies and innovative data to an audience composed of specialists from more than 35 countries around the world!

Our committee is also thrilled that 85 abstracts were submitted for the Congress by laboratories and clinics from across the globe. A panel of international specialists evaluated this impressive amount of scientific work and these studies will be presented as oral presentations or posters.

We hope that you will enjoy the Congress and that interaction with colleagues from many different countries will stimulate a creative exchange of ideas and will be professionally rewarding. We also hope and trust that you will enjoy the lovely city of Bologna and all the beauty it has to offer.

Yours sincerely,

Luca Gianaroli, MD
Chairman of Local Organizing Committee

Svetlana Rechitsky, PhD
President of PGDIS

PGDIS

SCIENTIFIC PROGRAM

PRE – CONFERENCE COURSES
Sunday 8th May 2016

From 10.00 to 14.00

PCC 3: "Preimplantation Genetics – The Illumina Difference" (organized by Illumina) – Sala Verde

- Reproductive and Genetic Health Solutions - The Illumina Difference, (A. Thornhill)
- Next Generation Sequencing solutions for aneuploidy in embryos and non-invasive prenatal testing (T. Gordon)
- Cromosomal aneuploidy and ovarian stimulation (T. Hardy)
- Validation and implementation of Veriseq PGS for aneuploidy screening (A. Hellani)
- Cllinical evidence and benefits for PGS (S. Munné)
- Carrier screening and karyomapping to prevent inherited disease (A. Bisignano)
- What's on the horizon for embryo testing?(A. Handyside)

From 15.00 to 18.00

PCC 1: "Clinical aspects of PGD/PGS" – Sala Rossa

- Ovarian stimulation and aneuploidy: any correlations? (D. Griffin)
- Ovarian stimulation and endometrial receptivity: the body of evidence (N. Macklon)

Ovarian stimulation for PGD/PGS cycles

- The Role of Ovarian Reserve in the Possibility of Finding Euploid Embryos (S. Kahraman)
- Milder stimulations to bank multiple embryos for analysis (B. Fauser)

- Transfer of euploid embryos: fresh or frozen? (S. Giulini)
- Present and future role of PGS according to different clinical indications (A.P. Ferraretti)

PCC 2: "Embryo biopsy troubleshooting" – Sala Bianca

- Poor embryo development: biopsy or not biopsy? (A. Pomante)
- Failed DNA amplification (A. Biricik)
- Re-Biopsy (M.C. Magli)
- How to avoid misdiagnosis in PGD (S. Rechitsky)
- The misdiagnosis: PGS (S. Munné)
- Mosaic embryo: to transfer or not to transfer? (F. Fiorentino)

18.00 – 19.30 PGDIS Board Meeting (Sala Rossa)

MAIN SCIENTIFIC PROGRAM

DAY 1 - Monday 9th May 2016

08.45 Welcome

Session 1 - The impact of genetic diseases on humankind
Chairpersons:D. Griffin – A. Kuliev

09.00 – 09.20 Impact of PGD on humankind - Making better babies: pros and cons (R. Sparrow)
09.20 – 09.30 Discussion
09.30 – 09.50 Genetic counselling before and after PGD (L. Black)
09.50 – 10.00 Discussion
10.00 – 10.20 NIPT of foetal aneuploidies: a dream become reality (P. Paterlini)
10.20 – 10.30 Discussion
10.30 – 10.50 Germline genetic modification: what is wrong with it? (J. Cohen)
10.50 – 11.00 Discussion

11.00 – 11.20 Coffee Break

11.20 – 12.00*Roundtable:* Interpretation of trials and data analysis for the introduction of new techniques.
CHAIRMAN: J. Cohen
DISCUSSANTS: B. Fauser –C. Barratt– N. Treff

12.00 – 13.00Selected oral communications
Chairperson:J. Cohen

12.00 – 12.10 Chromosomal analysis on spermatozoa from infertile couples (A. Crippa)
12.10 – 12.15 Discussion
12.15 – 12.25 Reproducible segmental organization of telomeres and centromeres in sperm: implications for early embryonic development (D. Ioannou)
12.25 – 12.30 Discussion
12.30 – 12.40 Accurate recombination risk prediction for Preimplantation Genetic Diagnosis of Monogenic disorders (T. Harasim)
12.40 – 12.45 Discussion
12.45 - 12.55 Whole genome sequencing of a single human cell by Linear Amplification via Transposon Insertion (LIANTI) (S. Xie Xiaoliang)
12.55 - 13.00 Discussion

13.00 – 14.00 Lunch and posters session

Session 2. Novelties in PGD
Chairpersons: D. Wells – F. Fiorentino

14.00 – 14.20 Introduction of new technologies in the lab: when and how? (D. Griffin)
14.20 – 14.30 Discussion
14.30 – 14.50NGS – Next Generation Sequencing (D. Wells)
14.50 – 15.00 Discussion
15.00 – 15.20 Potential relevance of segmental aneuploidies in PGD(J. Vermeesch)
15.20 – 15.30 Discussion
15.30 – 15.50 Simultaneous detection of monogenic and chromosomal disorders (S. Rechitsky)
15.50 – 16.00 Discussion
16.00 – 16.20 Karyomapping (A. Handyside)
16.20 – 16.30 Discussion
16.30 – 16.50 Blastocentesis: innovation in embryo biopsy (L. Gianaroli)
16.50 – 17.00 Discussion

DAY 2 - Tuesday 10th May 2016

Session 3. Clinical impact of PGD for aneuploidy
Chairpersons:S. Kahraman – A. Kuliev

08.30 – 08.50PGD and Ovarian Reserve: Which Patients Benefit More? (S. Kahraman)
08.50 – 09.00 Discussion
09.00 – 09.20 Preimplantation Aneuploidy Testing: when it's necessary? (K. Vesela)
09.20 – 09.30 Discussion
09.30 – 09.50 Lifestyle and aneuploidy: is there a correlation? (H. Tempest)
09.50 – 10.00 Discussion
10.00 – 10.20 New perspectives on embryo biopsy: not how, but when and why (K. Xu)
10.20 – 10.30 Discussion

10.30 – 11.10*Debate:* Preimplantation Aneuploidy Testing: on day 3 embryos or on blastocysts?
DISCUSSANTS: C. Rubio and N. Treff

11.10 – 11.30 Coffee break

11.30 – 13.00Selected oral communications
Chairpersons:C. Rubio - N. Treff

11.30 – 11.40 Impact of mosaicism detection on PGS clinical outcome (J. Horak)
11.40 – 11.45 Discussion
11.45 – 11.55 Improved detection of chromosomal mosaicism by Next generation Sequencing (NGS)-based preimplantation genetic screening (F. Spinella)
11.55 – 12.00 Discussion
12.00 – 12.10 Mosaic embryos, detected by NGS, are mostly classified as euploid by aCGH (L. Ribustello)
12.10 – 12.15 Discussion
12.15 – 12.25 Mosaicism rates in embryos resulting in live birth or miscarriage (R. Capaldi)
12.25 – 12.30 Discussion
12.30 – 12.40 Mosaicism of single segmental chromosomal changes in trophectoderm biopsy (R. Vlckova)
12.40 – 12.45 Discussion
12.45 – 12.55 Combined time-lapse imaging and Preimplantation Genetic Screening (PGS): a valuable strategy for embryo selection (E. Rocafort)
12.55 – 13.00 Discussion

13.00 – 14.00 Lunch and posters session

14.00 – 14.30Selected oral communications
Chairpersons: N. Treff –D. Cram

14.00 – 14.10 Single cell testing versus amniocentesis/NIPT: ultra-high resolution preimplantation screening for microduplication/microdeletion syndromes (D. A. Zeevi)
14.10 – 14.15 Discussion
14.15 – 14.25 PGD for variants of unknown significance (VUS): perform or not to perform? (S. Aktuna)
14.25 – 14.30 Discussion

Session 4. PGD as a tool for a better understanding of Embryology/Reproductive Biology
Chairpersons: N. Treff –D. Cram

14.30 – 14.50 Sperm contribution to aneuploidy (C. Barratt)
14.50 – 15.00 Discussion
15.00 - 15.20 Oocyte contribution to aneuploidy (C. Ottolini)

15.20 – 15.30 Discussion
15.30 – 15.50 Developmental potential of mosaic embryos (E. Fragouli)
15.50 – 16.00 Discussion
16.00 – 16.20 Accepting mosaicism in PGS: a new paradigm shift (S. Munné)
16.20 – 16.30 Discussion

16.30 – 17.30 ***Back-to-back lecture***
Aneuploidies: the embryos' point of view (M. C. Magli)
Aneuploidies: the uterus' point of view (N. Macklon)

18.00 PGDIS BUSINESS MEETING

DAY 3 - Wednesday 11th May 2016

Session 5. From non invasive PGD to NIPT
Chairpersons: J.L. Simpson – V. Bianchi

08.30 – 08.50 Analysis of the cumulus cells (S.Hamamah)
08.50 – 09.00 Discussion
09.00 – 09.20 DNA in culture media (A. Sunde)
09.20 – 09.30 Discussion
09.30 – 09.50 The impact of low fetal fraction on NIPT results (F. Fiorentino)
09.50 – 10.00 Discussion
10.00 – 10.20 How to improve availability and affordability in the private and public sectors (T. Gordon)
10.20 – 10.30 Discussion
10.30 – 10.50 Medical supervision for genetic tests (J.L. Simpson)
10.50 – 11.00 Discussion

11.00 – 11.20 Coffee break

Session 6. Translocations and segmental abnormalities
Chairpersons:A. Handyside –D. Cram

11.20 – 11.40 Evolution of PGD for translocations (A. Kuliev)
11.40 – 11.50 Discussion
11.50 – 12.10 Interchromosomal effects (T. Escudero)
12.10 – 12.20 Discussion
12.20 – 12.40 MPS for translocations (F. Van Nieuwerburgh)
12.40 – 12.50 Discussion

13.00 – 14.00 Lunch an posters session
Session 7. Mitochondria and beyond
Chairpersons:E. Fragouli – A. Veiga

14.00 – 14.20 Mitochondria: the engine of the oocytes (U. Eichenlaub Ritter)
14.20 – 14.30 Discussion
14.30 – 14.50 Preferential segregation of mitochondrial DNA mutation in human oocytes (G. Romeo)
14.50 – 15.00 Discussion
15.00 – 15.20 Levels of Mitochondrial DNA and embryonic implantation potential (E. Fragouli)
15.20 – 15.30 Discussion
15.30 – 15.50 The predictive value of mitochondrial DNA (B. Smeets)
15.50 – 16.00 Discussion
16.00 – 16.20 Mitochondrial replacement: from ART to stem cells (A. Veiga)
16.20 – 16.30 Discussion

CLOSING LECTURE
Chairpersons: S. Rechitsky – L. Gianaroli

16.30 – 17.0095% success rate in human ART (A. Trounson)

17.00 – 17.15 Closing Remarks

Speaker's Abstracts

Impact of PGD on humankind
Sparrow R
Philosophy Program, Monash University, Australia

A number of influential bioethicists and philosophers are now arguing that the proper goal of medicine should include "human enhancement" – the quest to make people "better than well". In particular, Professor Julian Savulescu, at Oxford University, and Professor John Harris, at the University of Manchester, have argued that we are morally obligated to use technologies of genetic selection and genetic modification to have "the best children possible". In this presentation I will explain and evaluate this claim. Using a linked set of hypothetical cases, I will demonstrate that the implications of their argumentsfor both parents and society are much more radical and disturbing than these authors acknowledge.

Genetic Counseling Before and After PGD
Black LD
Pacific Fertility Center, San Francisco, USA

This presentation will discuss the salient points of genetic counseling for preimplantation genetic diagnosis (PGD) before and after testing. Outlined in the discussion will be the non-directive nature of genetic counseling, the main points of informed consent for PGD, various methods of patient education, the need for consistent messaging to patients regarding confirmatory prenatal testing, and a description of the currently available prenatal testing options.

Advances in the use of trophoblastic cells for prenatal non-invasive diagnostics of genetic disorders
Paterlini Bréchot P
University Paris Descartes, INSERM INEM UMR-S1151

Next Generation Sequencing of cell-free fetal DNA from maternal plasma, also known as non-invasive prenatal testing (NIPT), has enabled accurate prenatal diagnosis of aneuploidies, in particular trisomy 21, and other prevalent aneuploidies (e.g., trisomy 18, trisomy 13) gaining clinical acceptance, as it significantly reduces the necessity of invasive diagnostic procedures. However, these aneuploidies have been estimated to account for approximately two-thirds of prenatal abnormal karyotypes.

Although NIPT technology is evolving, current available tests provide no -or limited- information about other less common chromosome anomalies, and monogenic disorders that can be identified by invasive diagnostics.

Circulating fetal erythroid cells would be the ideal target to develop an alternative non invasive prenatal diagnostics. However, they are very inconsistently found because of their short life and frequent apoptosis.

Our work has explored the potential of Circulating Fetal Trophoblastic Cells (CFTC) collected non-invasively from maternal blood and from maternal cervix to perform genetic diagnosis of monogenic disorders, using Cystic Fibrosis (CF) and Spinal Muscular Atrophy (SMA) as models.
CFTC from 63 mothers at risk of having a fetus with either CF or SMA were isolated from blood by ISET, STR-genotyped andblindly assessed for mutation analysis. Diagnostic results were compared with those obtained by CVS showing that all 14 affected fetus were correctly identified as well as non-affected pregnancies.

We then developed a method to recover trophoblastic cells from the cervix through a completely non-invasive approach and applied it to 21 pregnant women including 6 at risk of having a child affected by CF or SMA. Trophoblastic cells were identified by single cell laser microdissection and STR genotyping in all the 21 cases. Successful non invasive prenatal diagnosis was also blindly performed in the 6 mothers at risk of having an affected fetus.

We further developed an approach based on single cell NGS analysis to detect aneuploidy, chromosomal abnormalities and single gene mutations through the analysis of circulating trophoblastic cells.

We will discuss the implication of these results for new developments in non-invasive prenatal diagnostics.

Germ-line genetic modification: what is wrong with it?
Cohen J
Reprogenetics, Life Global and Althea Science, Livingston, NJ, USA

Genetic modification in the human is being investigated for different goals, including: (1) the potential to study gene function, (2) cure or treat existing disease through gene therapy, (3) prevent disease in current or future generations through pre-implantation manipulation, and (4) the addition of a particular function or desirable characteristic to the genome either temporarily or permanently not necessarily related to illness of current or future generations. Each of these aims, though related, must be considered separately on not just scientific grounds, but also ethically, economically and politically. Genetic modification can target either somatic or germ-line cells, but there is a slippery slope when technology is applied to somatic cells. One potential effect of somatic gene modification is 'new' sequences may also integrate into the reproductive cells and be passed to future generations without further screening to prevent presumably permanent genetic changes.

Germ-line genetic modification has been used successfully in mice and other animal models. Human germ-line genetic modification could be used, at least in theory, to effect permanent heritable genetic change in an individual's genetic code. Modification of germ cells or early stage embryos could permit a functional gene to be incorporated into every cell of the body. A new generation of genetic engineering techniques, known as "gene editing" or "genome editing," has prompted speculation about their use in human embryos or gametes. Researchers in several countries have been studying spare human embryos with the inexpensive, relatively easy-to-use gene-editing tool system referred to as CRISPR. In April 2015, a research team at Sun Yat-sen University in China published a report of an experiment in which they used CRISPR to edit a gene associated with the blood disease beta-thalassemia in non-viable human embryos. This pilot study showed that the technique may be inefficient or needs optimizing for routine use in pre-implantation embryos. However, that conclusion is not all that different from the one drawn in 1978 shortly after the birth of Louise Brown. Human ingenuity in bioscience and engineering has been shown effective time and again particularly the ability of scientists to improve and optimize once limited proof of principal has been established. Now is the time to consider and debate the advantages and risks associated with the application of gene editing for prevention and transmission of genetic disease. Single gene mutations are probably a good starting point, particularly if they have have been studied for many years. With improved precision and efficiency, editing could be exploited in other areas such as mitochondrial diseases and polygenic disorders. It is paramount that this revolutionary technology be evaluated in conjunction with health authorities and the public at large.

How and when should new technologies be introduced into the clinic?
Griffin DK, Sheldon S
Centre for Interdisciplinary Studies of Reproduction (CISoR), University of Kent, Canterbury CT2 7NJ, UK

Reproductive medicine is relatively unique: it is one of the few occasions in which patients undergo radical therapies with an intention other than to benefit to their own health. It is the only medical discipline in which the physiologies of two individuals combine (even if the two parties do not meet, e.g. sperm donation) for the sole purpose of producing a third (or fourth, or fifth …). Also there can be few fields in which imperceptible "good gardening" skills of a combination of different academic disciplines (including clinical medicine, anatomy, physiology, cell biology, genetics, biochemistry, physics, endocrinology etc.) can have such a profound effect on success.

One view of evidence-based medicine generally is that a therapy should only be introduced into the clinic after a double blind randomised placebo controlled clinical trial is performed. For many standard

pharmacopeia this is entirely appropriate and it is thus perhaps not unreasonable to suggest that novel IVF treatments should be governed by equivalent strictures. Indeed, in reproductive medicine, RCTs are considered a gold standard for examining the efficacy of procedures such as ICSI, oocyte preservation, sperm DNA damage testing, metabolomic analysis and development of new culture media. Problems arise however with the classic evidenced-based model: placebos are not usually relevant, the skill of the operator (or lack of it) can negate any beneficial effect of the treatment and any randomization can thus be rendered semi-meaningless. e.g. in a trial on the efficacy of ICSI (where standard IVF is the control) it is hard to imagine that operators are blinded to the fact that they are injecting the embryo.

Perhaps more than in any other area of medicine therefore, evidence of the efficacy of a protocol change (e.g. a small change in culture medium) relies, in part, on anecdote and retrospective single centre analysis as much as multi-centre meta-analysis and prospective RCT data. Clinics (especially private clinics) depend, for their survival and the employment of their staff, on their ability to innovate quickly. Indeed it has been suggested that ICSI for instance would never have been introduced if it had to have been subject to the rigours of a randomised trial before being licenced. We can also assume that many new variants on IVF culture media would not be introduced if subjected to such a degree of scrutiny. Moreover, when randomised trails are designed (e.g. current ones assessing the efficacy of screening for sperm DNA damage) they can often take several years to obtain funding and perform the trial, while the benefits of the treatment may already be apparent without them or appetite to perform the trial may have waned.

Conversely, motivated by the need to be seen to be innovating (and the income associated with charging patients for “the latest” therapy) could, in some cases, be a sufficient impetus for clinics to continue to offer new treatments, regardless of convincing evidence supporting their efficacy. Perhaps the most controversial treatment regimen in this regard is PGS (AKA PGD-A). On the one hand, the theory behind PGS is sound - we know that a large proportion of embryos are aneuploid (and would either not develop or produce an affected conceptus) and we have, for some years, had the technology to detect aneuploidy in single cells. On the other hand, problems arose with convincing randomised trail data suggesting it was ineffective or even harmful. With the benefit of hindsight, the 10 year + era of cleavage stage biopsy followed by FISH diagnosis might have been handled better had sufficient evidence (including single centre retrospective studies, meta analyses and randomised trials) been presented earlier and accepted more readily. Nonetheless, the correct lessons need to be learned, namely that, while most analyses suggest that FISH with only 5-7 probes had a high false positive rate and did not have sufficient power to make a demonstrable difference to clinical pregnancy rates, sub-optimal biopsy procedures (and ultimately the wrong choice of developmental stage) also negated (or reversed) any beneficial effect. A switch to trophectoderm biopsy and to more sophisticated analysis protocols (array CGH, NGS) seems to show more positive results in retrospective and prospective (randomised) trials.

In this study we argue that, while PGS should continue in the light of this evidence, much work still needs to be done: We need to stratify the patient groups that would ultimately benefit from PGS, consider the role of meiotic vs. post-zygotic errors, understand mosaicism better, introduce a proper EQA scheme and consider appropriate “staged” introduction protocols for new innovations such as blastocentesis and karyomapping. For the purpose of this study we also consider the two “camps of opinion” that have formed around the prospect of whether or not PGS should be discontinued. By creating arguments using a “straw man” approach (termed “Jacob” and “Giuseppe”) we address the effect that new evidence has on the other’s point of view. We conclude by considering the evidence base that supports IVF innovations in general (and PGS in particular) in its unique setting considering the relative value of anecdotal and retrospective studies and the possible pitfalls surrounding relying on randomised trials alone. We also consider, from an ethico-legal perspective, the implications of not implementing PGS, including e.g. the harm caused to a patient who has an adverse outcome (e.g. trisomic conceptus), assuming that they could, and would, have chosen to avoid this, had PGS been offered.

Next generation sequencing

Wells D
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Reprogenetics UK, Institute of Reproductive Sciences, Oxford Business Park North, Oxford, OX4 2HW, UK

In recent years, preimplantation genetic diagnosis of aneuploidy, often referred to as preimplantation genetic screening (PGS), has undergone a remarkable evolution. Within the space of seven years the technology used for aneuploidy detection has progressed from fluorescence in situ hybridisation (FISH), through metaphase comparative genomic hybridisation (CGH) and microarray CGH (aCGH) to next generation sequencing (NGS). Methods such as quantitative PCR and single nucleotide polymorphism (SNP) microarrays have also been part of this story of innovation and successful clinical application. While all the methods used for PGS have different advantages and drawbacks, it could be argued that best technology currently available is NGS.

Next generation sequencing (NGS) is not a single method, but rather refers to a variety of technologies that all have in common the ability to provide large amounts of DNA sequence data, relatively rapidly and at comparatively low cost. Although NGS is often used for the sequencing of entire genomes, in the context of PGS it is usually applied in a different way, providing very little data concerning the sequence of individual genes, but allowing accurate determination of the copy number of each chromosome. The advantages of NGS over other cytogenetic methods employed for PGS are several. Firstly, the 'per sample' cost of NGS is lower than achieved with alternative techniques. Secondly, resolution (the ability to detect losses and gains of small pieces of a chromosome) is high, at least as good as obtained using aCGH and probably superior. Thirdly, NGS can reveal when a trophectoderm biopsy sample contains a mixture of normal and aneuploid cells (i.e. when the sample is mosaic).

Strategies used for NGS can be divided into high-resolution and low-resolution. High-resolution NGS still only sequences a small fraction of the genome, generally in a random manner, and provides virtually no information of the sequence of specific genes. Nonetheless, enough of the genome is sequenced to allow detection of segmental abnormalities and imbalances associated with translocations down to about 3Mb in size. Sensitive detection of mosaicism is also provided revealing instances where only 10-20% of the cells in the specimen are aneuploid. Low-resolution NGS usually involves a targeted amplification of the genome, either focusing on a number of specific sequences on each chromosome or interspersed repeat sequences present in a large number of copies scattered throughout the genome, followed by sequencing. In some cases segmental abnormalities and mosaicism may be less readily detected using low-resolution approaches, but such methods may have some cost advantages. There has been a rapid adoption of NGS for preimplantation aneuploidy detection and the method has displaced aCGH as the most frequently used method, at least in the larger reference laboratories. Although utilisation of NGS should theoretically have a positive effect on IVF cycle outcome, similar to that observed for other PGS methods involving comprehensive chromosome screening, well designed trials to conclusively demonstrate efficacy are needed. The STAR study, a large, multicentre, international trial, supported by Illumina, has been examining the capacity of NGS to improve the identification of viable embryos and results are expected before the end of the year. If a benefit of NGS is shown, it is likely that the application of this method will expand further still. Future prospects for NGS are likely to include further reductions in the cost of analysis, workflows that are less time consuming and less laborious and deeper sequencing of the genome, providing more information about the nuclear and mitochondrial genomes, including an insight into the status of individual genes.

In the context of preimplantation genetic diagnosis (PGD), NGS has quickly transitioned from a research technique to a frontline clinical methodology, employed for the detection of aneuploidy in human preimplantation embryos. The rapid speed of adoption has been driven by a desire to lower the costs of comprehensive chromosome screening and by the perception that NGS offers the best chance of facilitating this of any method currently available. Additionally, NGS has the potential to provide a number of extra benefits in the future, such as revealing the DNA sequence of individual genes. At present several variant NGS methods are available, utilizing different sequencing platforms and/or alternative DNA amplification strategies. Each method has its own combination of benefits and drawbacks compared with the others. Depending on the technique, there may be differences in cost per sample, throughput, speed, quantity and quality of the DNA sequence data produced.

To date, NGS techniques intended for aneuploidy screening have typically focused on 'low-pass' methods, in which a small fraction of the genome is sequenced at random, or targeted methods involving the sequencing of specified DNA fragments generated in large multiplex PCR amplifications. While these strategies may be ideal for aneuploidy detection, allowing many samples to be analysed simultaneously and consequently reducing the cost of screening, there is also interest in approaches that provide deeper sequencing. Such methods have the potential to permit the detection mutations in individual genes and, taken to their logical conclusion, could provide total genome sequencing for individual embryos. However, there are significant technical and economic barriers to routine utilization, not to mention considerable ethical questions concerning the possible use of genome sequencing for the purpose of embryo selection.

There is no doubt that NGS represents a great opportunity for PGD laboratories to improve patient care. On the one hand, excitement surrounding this new technology needs to be tempered by a realistic assessment of current technical capabilities. While on the other hand, discussion of the ethical implications of future developments such as routine whole genome sequencing, which may technically feasible sooner than many imagine, should be initiated as a matter of urgency.

Potential relevance of segmental aneuploidies in PGD

Vermeesch JR
University of Leuven, Belgium - Joris.vermeesch@uzleuven.be

Recent developments in preimplantation genetic testing have implemented genome wide screening tools such as array CGH or sequencing and are interrogating copy number variation across the genome. Those methods aimed for the identification of embryos or cells carrying whole chromosomal imbalances. Meiotic aneuploidy rate increases with advanced maternal age.

In addition, a high incidence of mitotic chromosomal missegragations has been demonstrated to occur during the cleavage stages of embryo development. Hence, selection of embryos without chromosomal imbalances may well increase the IVF success rate. Duringthose analyses, we discovered, unexpectedly, not only a high incidence of whole chromosomal aneuploidies but also of segmental chromosomal segmental deletions, duplications and amplifications that were reciprocal in sister blastomeres were detected. We demonstrated since that chromosome breakages and fusions occur frequently in cleavage stage human embryos and instigate subsequent breakage-fusion-bridge cycles. The clinical relevance of those segmental imbalances remains unclear. In this presentation, I will show data mapping the frequency and charting the landscape of segmental imbalances observed during PGT. I will provide direct and indirect evidence that the embryo is the cradle of chromosomal disorders.

Impact of 24-chromosome aneuploidy testing on the outcome of PGD for monogenic disorders

Rechitsky S[1], Kuliev A[1]
(1) Reproductive Genetics Innovations, 2910 MacArthur Blvd, Northbrook, IL, USA

INTRODUCTION
Preimplantation 24-cromosomes aneuploidy testing (24-AT) is presently becoming an acceptable practice in PGD for monogenic disorders with or without HLA typing in couples of advanced reproductive age. The objective of this report is the analysis of clinical impact after introduction of 24-AT in our experience of PGD for single gene disorders (SGD), representing the largest PGD series for SGD in one Center.

MATERIAL AND METHODS
PGD for SGD was performed following the whole genome amplification (WGA) product, obtained in 24-AT procedure, allowing both PGD for SGD and concomitant 24-AT in the same biopsy material. A combined testing was performed in 762 (21.7%) cycles of a total of 4501 PGD cycles performed for SGD and HLA typing by the present time. This involved PGD for SGD combined with 24-AT for more than a hundred different conditions with or without preimplantation HLA typing.

RESULTS
A total of 1835 unaffected or HLA matched embryos free of 24-chromosome aneuploidy were detected, from which 616 were transferred in 533 cycles (1.15 embryos per transfer, on the average), resulting in

375 (70.5%) unaffected pregnancies, 347 deliveries (92.3%) and birth of 382 healthy or HLA matched children. This is significantly different from the outcome of PGD without concomitant 24-AT (70.5% vs. 49.9%), suggesting a significantly improved pregnancy rate in a combined 24-AT, despite transferring 1.15 vs. 1.79 embryos on the average in PGD cycles without 24-AT. In addition, a two-fold reduction of spontaneous abortion rate was observed in the outcome of these pregnancies: 29 (7.7%) spontaneous abortions in 376 pregnancies, compared to 211 (14.8%) in 1424 PGD pregnancies without 24-AT, despite comparable maternal age.

CONCLUSIONS
The results demonstrate significant improvement of reproductive outcome of PGD for SGD and HLA typing with introduction of concomitant 24-AT, including significant improvement of pregnancy rate from 49.9% to 70.5%, and reduction of spontaneous abortions rate from 14.8% to 7.7% .

Karyomapping: a versatile, high resolution tool for preimplantation genetics
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SNP genotyping and karyomapping is being used increasingly world-wide for linkage-based preimplantation genetic diagnosis (PGD) of single gene defects. The ability to genotype a universal set of hundreds of informative SNPs across each chromosome in a single, low cost test has significantly reduced the time and effort required to develop patient and disease specific tests.

The use of these markers for high resolution detection of chromosome abnormalities including aneuploidy and structural chromosome imbalance in translocation carriers is also increasing. Unlike quantitative methods of copy number analysis, including array comparative genomic hybridisation (array CGH) and next generation sequencing (NGS), karyomapping identifies the parental origin of these abnormalities.

This unique combination of features is providing new insights into the origins and mechanisms behind a range of chromosomal abnormalities in the preimplantation embryo.

Blastocentesis: innovation in embryo biopsy
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The process of cavitation and blastocoel formation in the human embryo normally happens 4 to 5 days after fertilization when the blastocyst stage initiates. The accumulation of the fluid inside the cavity and the continuous duplication of the surrounding cells lead to the expansion of the blastocyst and the concomitant thinning of the zona pellucida. A combination of repeated contractions/expansions, actin-based modifications and enzymatic digestion of TE cells seem then lead to the hatching process.

The blastocoelic fluid (BF) represents the natural medium for the ICM and develops into the yolk sac. The analysis of its composition has demonstrated the presence of proteins and metabolites in proportions that are similar to those found in blastocysts' cells. The presence of DNA has also been documented and this motivated the idea to evaluate whether it could be representative of the blastocyst genetic status.

According to prospective and observational studies, the biopsy of TE cells represents at the moment the ideal procedure providing the most reliable results for PGD. When testing for aneuploidy, the results obtained by TE biopsy give a significantly improved clinical outcome in comparison to conventional treatment. This means that TE cells are highly representative of the blastocyst chromosome condition, and for this reason, they were taken as a reference to evaluate the results of the DNA extracted from BF. The final goal was to estimate the degree of ploidy and chromosome concordance.

In all, we submitted 195 BFs to WGA and 173 amplified (89%). For 93 of them, a study of concordance could be performed as TE cells were also available. In the vast majority of blastocysts (80.6%), there was total ploidy concordance with all chromosomes having the same pattern both in BF and in TE cells. In 16.2% of cases, the ploidy concordance was partial as not all chromosomes had the same status in

BF and corresponding TE cells. In the remaining 3.2% of blastocysts, there was ploidy discordance with BF anticipating 2 false positives and 1 false negative compared to the corresponding TE cells. When evaluated per single chromosome, the grade of concordance was 98.4%.

DNA from BF could also detect the presence of segmental abnormalities in blastocysts generated by translocation carriers, as well as those generated de novo. This finding suggests the potential use of BF for PGD of translocations.

Finally, we observed cases in which the results from BF and corresponding TE cells were both not informative mainly due to failed or weak amplification, suggesting the presence of poor quality DNA in both compartments of the blastocyst irrespective of normal morphology.

The reduced invasiveness of BF sampling as well as the easiness of the technical procedure makes this approach advantageous for PGD.

PGD and Ovarian Reserve: Which Patients Benefit More?

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The number of embryos available for transfer is significantly lower in PGD than in regular IVF because of genetic selection. Thus, the clinical evaluation of a patient applying for PGD is important in order to maximize the possibility of transferring of a euploid embryo.

Over a three-year period, 665 PGD cycles were retrospectively analyzed and 1574 blastocysts were biopsied and diagnosed by aCGH. The probability of finding at least one euploid embryo (POFALOEE) per cycle and the rate of euploid embryos per diagnosed embryos (Euploidy Rate) were evaluated according to maternal age and ovarian reserve i.e. the number of cumulus-oocyte complexes (COC), MII oocytes retrieved and biopsied embryos.

According to our findings, regarding maternal age and POFALOEE, the decline in the POFALOEE per cycle was around 10% for each two-year age group from the age of 35, (35-37, 38-39). However, from the age of 40, for each two years the patient age increased (40-41, 42-43, 44-45), the POFALOEE per cycle decreased by 20% approximately.

The dataset was evaluated in four groups according to the number of COCs retrieved (1-4, 5-8, 9-11, ≥12). The ER increased by only 16% between the 1-4 COCs and the ≥12 COCs groups). However the POFALEE per cycle increased by nearly 3 fold from 29% to 75%.

When the COC groups were subcategorized into age groups <38 and ≥38, interestingly, the POFALOEE per cycle and the ER followed a similar pattern with perfectly parallel lines.

When looking at the effect of the number of blastocysts biopsied, we noted that it had a relatively low effect on the ER (27% and 41%, for 1 and >8 embryos, respectively), when compared to the POFALOEE, which increased from 27% to 100% for the same groups.

Also, diagnosed aneuploidies were categorized into three types; single chromosome, double chromosome and complex aneuploidies. When these groups were evaluated according to patients' age clusters, the percentage of single chromosome aneuploidies decreased from 56% to 31% for the patients under 35 and above 42 respectively, whereas complex aneuploidies increased from 21% to 39% in the same age categories. Interestingly, the percentage of aneuploidies affecting two chromosomes was found to be stable at between 23% and 33% for all age groups.

The best approach when counseling PGD cases for aneuploidy testing should be first to evaluate the ovarian reserve and to counsel the couple by giving an estimation of the possibility of finding at least one euploid embryo specific to maternal age and ovarian reserve.

Preimplantation Aneuploidy Testing: when it's necessary?
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Lifestyle and aneuploidy: Is there a correlation?
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Chromosome aneuploidy occurs frequently in humans, affecting up to 60-80% of all conceptions. The presence of chromosome aneuploidy has a tremendous impact on embryo development, and is the leading cause of pregnancy loss and developmental disabilities in humans. The etiology of aneuploidy in embryos is uniquely gender-specific, being predominantly maternal in origin (~>90%), with the exception of the sex chromosomes, for which there is a greater paternal contribution (50-100%).

Excluding the presence of a parental chromosomal aberration, advancing maternal age is the principal etiological factor associated with chromosome aneuploidy. There are several hypothesized biological explanations for these findings, however, it seems plausible that various lifestyle factors and/or environmental or occupational exposures could also contribute to the generation of aneuploidy. Studies investigating whether such factors are associated with perturbations in aneuploidy levels within gametes are slowly emerging but are notoriously difficult to design, interpret and compare. It should also be noted that data on associations between aneuploidy and lifestyle factors and/or environmental exposures are lacking in oocytes. This is primarily due to the difficulties in obtaining sufficient numbers of oocytes to study, in conjunction with the issue of teasing out potential age-effects on aneuploidy. Thus, the majority of such association studies have focused on spermatogenesis, investigating the association between sperm aneuploidy and various lifestyle factors (e.g., obesity; physical activity; smoking, alcohol, and caffeine consumption), and drug, environment or occupational exposures (e.g., chemotherapy; pesticides; air pollution; endocrine disruptors).

Although these studies are still in their infancy, they provide preliminary data to suggest that certain lifestyle factors and/or environmental exposures are associated with increases in sperm aneuploidy levels for specific chromosomes, when compared to unexposed controls. Furthermore, there is evidence to suggest that, at least for chemotherapy, these increased levels may be transient in nature, which has obvious clinical ramifications. Currently, these observations predominantly remain associations, with no proven cause and effect, and the molecular mechanism through which chromosomal nondisjunction occurs following exposure has yet to be elucidated. This is further complicated, given the striking differences between meiosis in males and females. Therefore, it seems likely that there will be gender and chromosome specific differences for aneuploidy susceptibility to lifestyle factors and environmental exposures.

The complexities of interpreting such studies due to differences in donor age; donor heterogeneity; dosing, timing and length of exposure will be discussed along with the clinical ramifications of the increased aneuploidy levels observed.

New perspectives on embryo biopsy: not how, but when and why.
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Preimplantation genetic diagnosis (PGD), as an effective treatment modality for reducing risks of children from inherited genetic disorders, such as monogenic diseases and unbalanced translocations, is indisputably well established. There is a growing tendency for biopsy at later stages of preimplantation development because more cells can be obtained for genetic analysis. Embryos after D5/6 biopsy may be cryopreserved for transfer at a non-stimulated cycle, which is believed to be beneficial for implantation.

For preimplantation genetic screening (PGS), however, controversy still exists in its effectiveness. With recently developed technology, whole chromosome copy numbers as well as gains and losses of small segments can be accurately detected by aCGH or SNP array or other technology. The use of next generation sequencing is dramatically increasing due to its power.

Currently various views can be found in the literature and at scientific conferences. These differ, from the view that PGS should be applied universally to all embryos, to the view that it should only be applied to specific indications. Although thousands of publications related to PGS over the last two decades can be found, RCTs for D3 biopsy and FISH- based PGS could not reach a clear conclusion. A recent meta-analysis, could only include 3 randomized controlled trials (RCTs) and 8 observational studies pertaining to the comparison of the latest PGS procedure (Dahdouh et al, 2015). This presentation will review literature as well as data from our own center, with over two decades of PGS experience, to discuss the biological, technical (embryology as well genetic testing), and medical issues relevant to this complex topic.

In summary, recent advancement in IVF, embryology and genetic testing has made PGS more accurate and efficient. PGS is, therefore, increasingly recognized as a useful technique to improve implantation rates when euploid embryos are transferred for all patient age groups. Yet, there is no clear evidence from publications that supports the notion that PGS will help to improve the live birth rate per single IVF attempt, particularly, for women of advanced maternal age. Like any medical treatment, applying a procedure to all patients may be inappropriate. In the long run, we will need to look for other technologies to improve IVF outcome, for example, use of stem cells to produce healthy oocytes will be the ultimate treatments for women of advanced ages.

Debate: Clinical results on day-3 biopsies

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Despite the controversy arosen regarding the usefulness of PGS, mainly criticizing day-3 biopsies, our own experience differs from these publications. We conducted two prospective, randomized trials using FISH for 9 chromosomes in AMA patients between 41 and 44 years old and RIF patients <40 years of age. We observed a significant increase in live birth rates in the PGS group compared to the blastocyst group for the AMA study (32.3% vs. 15.5%; p=0.0099), and a clear trend to better live birth rates for the RIF study (47.9% vs. 27.9%). We therefore concluded that PGS with classic FISH was beneficial for these two indications if proper blastomere biopsy procedures, good laboratory conditions and validated and robust genetic analysis were applied (Rubio et al., 2013). Despite these results, there was a clear need for a technique to analyze all chromosomes, while also producing reliable and faithful results in a short period of time.

With the application of CGH array, in cleavage stage biopsies, our clinical experience in 6,908 cycles shows the clinical benefits of embryo selection based on CGH array analysis in poor reproductive prognosis couples. Results are compared with 335 cycles with blastocyst biopsies performed in the same IVF settings. For population consistency, day-3 results will be compared with trophectoderm biopsies in three age groups: women < 38 years of age, between 38-42 years and between 43-46 years. False positive rates were similar for day-3 and blastocyst biopsy (Mir et al., 2015) and, low false negative rates were observed so far with cleavage stage biopsy, minimizing the impact of mosaicism in the accuracy of day-3 analysis.

Preliminary results of two RCTs comparing PGS with day-3 biopsies and blastocyst transfers without chromosomal analysis, performed in couples with AMA (38-41 years) and MF (≤2 million/mL) have revealed promising results. In the AMA study, 86 cycles in the conventional blastocyst transfer group and 75 in the PGS group. Ongoing pregnancy rate per transfer and per cycle were significantly increased in the PGS group (26.5 vs. 60.4; p=0.0001 and 25.6 vs. 42.7; p=0.0294, respectively). Miscarriage rates were dramatically higher in the blastocyst group that in the PGS group (43.6 vs. 3.3; p<0.0001) (Rubio, ASRM 2015).In the MF study, in the group of blastocyst transfer, 35 cycles were completed with 33 transfers and 15 ongoing pregnancies (45.4 ongoing pregnancy rate per transfer and 42.8 per cycle), whereas in the PGS group, 33 cycles were performed with 31 transfers and 22 ongoing pregnancies (71.0 ongoing pregnancy rate per transfer and 66.7 per cycle). One-side Chi-square test showed significant differences for ongoing pregnancy rates per transfer (p=0.0345) and per cycle

(p<0.0417). In the blastocyst transfer group, miscarriage rate was 28.6, whereas no miscarriage was observed in the CCS group (Rubio, ASRM 2014).

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Preimplantation Aneuploidy Testing: Blastocyst

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The choice of when to biopsy an embryo for genetic testing is complex. Factors include the putative impact on reproductive potential, the challenge of establishing efficient methods of extended culture, the ability to detect all origins of genetic abnormality, the accuracy of results obtained, the amount of time available for testing, and government restrictions.

At least 4 randomized controlled trials have demonstrated improved clinical outcomes with the use of comprehensive chromosome screening (CCS) on trophectoderm biopsies. In addition, blastocyst biopsy does not significantly impact the reproductive potential of the embryo, as demonstrated in a randomized blinded paired sibling embryo study. Many studies also indicate superior accuracy of genetic testing results when starting from multiple cells rather than single cells.

As technology for CCS has evolved, it has also become possible to improve sensitivity to detection of mosaicism within a trophectoderm biopsy, which may prove useful in enhancing the predictive value of aneuploidy screening.

In summary, trophectoderm biopsy for preimplantation aneuploidy testing is safer, more accurate, more sensitive, and more well established for improving clinical outcomes from IVF than any other stage of embryo biopsy.

Sperm contribution to aneuploidy

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Oocyte contribution to aneuploidy - SNP genotyping and MeioMapping of human oocytes: a powerful tool for analysis of patterns of recombination and chromosome segregation

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Human preimplantation embryos have an exceptionally high incidence of chromosome aneuploidy. These are predominantly of maternal origin and arise through errors in chromosome segregation in the two meiotic divisions (meiosis I and II) of oogenesis.

These errors increase with maternal age and contribute to the decline in a woman's fertility with advancing age as well as the associated increase in miscarriages and viable trisomic pregnancies.

SNP genotyping and maternal haplotyping (MeioMapping) of all three products of female meiosis, both polar bodies (PB) and the corresponding oocyte/embryo, allows simultaneous analysis of patterns of recombination and chromosome segregation. Analysis of oocyte-PB trios confirmed that premature sister chromatid separation was the most frequent cause of segregation errors in meiosis I but also identified a novel reverse segregation mechanism predisposing to errors in meiosis II.

The use of Meiomapping for diagnosis of female meiotic aneuploidies and the preclinical assessment of IVF protocols, including artificial oocyte activation, will be reviewed.

The developmental potential of mosaic embryos

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Introduction

Next generation sequencing (NGS) is increasingly being used for the purposes of preimplantation genetic screening (PGS). In addition to lowering PGS cost, NGS is both more powerful and more sensitive compared to other comprehensive chromosome screening methods, such as array CGH or real-time PCR. It is, therefore, capable of accurately identifying all types of chromosome abnormalities, including those of mosaic origin. Mosaicism is common during preimplantation development. The fate of mosaic embryos is unclear and consequently there is uncertainty about how to deal with such embryos clinically. We set out to assess the different types of chromosome errors as well as the rate of mosaicism in a group of blastocysts examined for PGS. Moreover, we attempted to determine the developmental potential of mosaic blastocysts.

Materials and Methods

We used NGS to examine the cytogenetic constitution of 848 blastocysts. These were generated by 138 couples (average female age of 38.7 years, range: 25-46 years). These couples were referred to Reprogenetics UK for PGS by 7 different IVF clinics. We also carried out a retrospective review of several hundred microarray-CGH results from blastocysts transferred to the uterus following PGS, and identified 44 with possible mosaicism. Biopsy samples from these embryos were re-assessed via NGS. The clinical outcomes obtained after the transfer of these 44 blastocysts were compared to those observed after the transfer of 51 blastocysts predicted to be uniformly euploid, derived from a well-matched contemporary control group.

Results

We identified chromosome abnormalities in a total of 73% of the TE samples. We also scored a significantly ($P<0.0001$) higher number of aneuploidies in blastocysts generated by reproductively older women. The total mosaicism rate was 49%, and was similar in blastocysts generated by younger and older women (48% vs. 45% respectively). Of all examined blastocysts, 20% consisted of mosaic errors only, while 28% had abnormalities affecting the entire TE sample (possible meiotic origin), and 29% had a combination of mosaic and meiotic anomalies. We did not see any influence of the PGS indication, or the biopsy day (day-5 vs day-6) on the mosaicism rate. We did, however, observe that some IVF clinics tended to generate more mosaic embryos, compared to others.

Of the re-analysed mosaic blastocysts, 62% did not implant, 12% miscarried and only 26% led to ongoing pregnancies. Comparison with the control group showed that both implantation (39%) and ongoing pregnancy rates (28.2%) were significantly reduced ($P=0.003$) when mosaic embryos were transferred. Blastocysts with mosaic whole chromosome aneuploidy failed to implant significantly more often than those with mosaic segmental abnormalities ($P=0.0038$).

Conclusions

NGS is capable of detecting mosaic abnormalities difficult or impossible to identify using other comprehensive chromosome screening methods. The total mosaicism rate was determined to be 49% and was independent of female age, PGS indication, or biopsy day. We observed, however, an effect of the referring IVF clinic on the number of mosaic embryos. Additionally, our results suggest that mosaic embryos have reduced developmental potential. If detected during IVF treatment, mosaic embryos should not necessarily be excluded, but should be given a lower priority for transfer than those that appear to be fully euploid, as the likelihood of producing a child is reduced.

Accepting mosaicism in PGS: a new paradigm shift
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Aneuploidies: the embryo's point of view.
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Human embryonic aneuploidy can be meiotic or mitotic in origin. The great majority of meiotic errors happen during oogenesis with premature division of sister chromatids being the prevalent form of aneuploidy-causing mechanism.

The most likely consequence of an aneuploid oocyte is the development of a uniformly aneuploid embryo, unless an event of aneuploidy rescue occurs. The first chance for the oocyte to correct single aneuploidy originated from the first meiotic division, is to compensate the error by a reciprocal error at meiosis II. The resulting zygote is most probably euploid and can develop regularly. More frequently, aneuploidy rescue ensues at the cleavage stage leading to a condition of mosaicism. Should this happen, there are two possible scenarios: 1) abnormal cells are relegated into the trophoblast, and the normal rescued cells become the fetus; and 2) the mosaic cell lines persist throughout the trophectoderm and inner cell mass. In either case, meiotic errors can be very harmful due to the early onset of the abnormality, for which the mosaic cell line has high probability to dominate.

Mitotic errors may also have extremely severe consequences depending on when they occur. It is well known that they are especially frequent during the first three divisions after fertilization, and decrease as embryos progress to the blastocyst stage, presumably due to arrest of abnormal cells or demise of the aneuploid embryo. The consequences can be the same described above, with aneuploid cell lines confined to the trophectoderm or affecting also the inner cell mass.

The embryo could react to the presence of an abnormal chromosome condition by tending to modify its cell cycle parameter timing, by generating micronuclei or by extruding cellular fragments. Whether the generation of fragments and micronuclei that may persist or become reabsorbed during interphase, are a potential means to correct aneuploidy is still a hypothesis. Nevertheless, the high frequency of aneuploidy and mosaicism in human embryos, a condition that seems to be typical also in vivo, poses the question of a possible evolutionary advantage of this condition. Maybe the embryo having a degree of aneuploidy that is compatible with development could have a stronger capacity to implant and to establish a dialogue with the endometrium to convince the mother to support its growth to term.

Aneuploidies: the uterus' point of view
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Human reproduction is characterized by a high rate of early pregnancy loss. This attrition is likely to derive from two features of human embryos; their intrinsic invasiveness and their high prevalence of chromosomal abnormalities. Genome wide screening of individual blastomeres taken from high-quality cleavage stage embryos IVF has shown around 70% to have complex chromosomal abnormalities, mostly arising from mitotic rather than meiotic non-disjunction. It is now clear that peri-implantation human embryos are intrinsically chromosomally diverse and predominantly mosaic, and therefore pose a challenge: how to facilitate implantation while simultaneously safeguarding the mother against prolonged investment in potentially developmentally abnormal embryos? In recent years, experimental evidence has indicated that spontaneous decidualization of the endometrium coupled to cyclic menstruation and regeneration may represent an elegant response to this challenge. Recently, additional functions have been uncovered, which indicate that the decidua is a key determinant of successful implantation, and is the active component of the maternal strategy to cope discerningly with genomically unstable embryos.

Evidence supporting the concept of active embryo selection at implantation in the human comes from in-vitro and in-vivo studies which showed the decidualized endometrium to respond more markedly to

the developmentally incompetent than the competent embryo. In-vivo studies in which human embryo conditioned medium was injected into mouse uteri confirmed these findings; resulting ina more profound dysregulation of gene expression when the media had been co-cultured with developmentally incompetent embryos. A dual phase response of the endometrium has been proposed be proposed, consisting of 'recognition' and 'selection'. The ability of the luminal epithilium to 'recognize' a viable embryo and modulate the decidual response it encounters on breaching the epithelium would aid subsequent nurturing and development in the post-implantation phase.

Recent studies have shown that rather than representing a passive matrix through which the embryo 'invades', active decidual cell migration and encapsulation of the embryo are also key elements of successful implantation. Thee propensity of decidual cells to migrate towards a conceptus was determined by embryo quality appears to be modulated by the viability of the embryo, being entirely inhibited in the presence of chromosomally abnormal tripronuclear (3PN) embryos.

It can be proposed that disruption of the selective decidual phenotype will result in either poorly viable embryo being allowed to implant, only to present later as a clinical miscarriage, or conversely, excessive 'selection'may result in embryos with minor chromosomal abnormalities which would not normally result in pregnancy failure or birth of baby with clinical traits,may present as recurrent implantation failure. The former concept is supported by evidence form clinical studies showing that women with recurrent pregnancy loss often report a shorter time to pregnancy than fertile controls. Whether recurrent implantation failure may represent the opposite disruption in decidual phenotype remain under investigation, but the implications of this work on current practice in PGS may be significant.
Given that the majority of human embryos can be shown to harbour a degree of aneuploidy, the question arises as to whether this may confer some advantage. Studies in single cell eukaryotes have shown that aneuploidy can confer a selective advantage under stressful environmental conditions, by resulting in over expression of efflux pump genes, suggesting that a degree of aneuploidy that is compatible with development may allow rapid evolution of advantageous phenotypic traits. In other words the genetic heterogeneity conferred by a high rate of moderate degree of aneuploidy may make the population adaptable to a wide range of environmental challenges. Indeed a high prevalence of aneuploidy has been shown to be present in healthy human liver. It can be further proposed that aneuploidy, a common feature of invasive malignant cells may confer a degree of invasiveness which may aid the breach of the epithelium. Clearly, should the extent of the chromosomal abnormality be incompatible with further normal development, the mother must have a means of preventing further investment in this.

Current approaches to in-vitro embryo selection are based on the premise that perfection is always best, particularly in relation to chromosomal constitution. Recent insights into the high prevalence of aneuploidy in human embryos, and the mechanisms by which the mother determines whether or not to permit establishment of pregnancy suggest that complete euploidy may not always be ideal. The current trend towards excluding all but the most perfect embryos from transfer after IVF may be ill advised.

From non-invasive PGD to NIPT: Analysis of the cumulus cells

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The oocyte is surrounded by several cell layers, cumulus cells samples (CCs), which are tightly connected to each other and to the oocyte through gap junctions such as Cx43 and Cx37, that facilitate the bi-directional traffic between the oocyte and CCs. The oocytes create and control their microenvironment by promoting differentiation of the CC phenotype through secretion of paracrine signaling factors, such as growth and differentiation factor 9 (GDF9) and bone morphogenic protein-15 (BMP15), which are members of the transforming growth factor- □ (TGF-□) family.

Cumulus cells play an essential role, particularly for normal oocyte growth, ovulation, fertilization and embryo development. Active components of the cumulus matrix come from several sources: they are synthesized directly by CCs under the control of endocrine and oocyte-derived factors, secreted by mural granulosa cells (GCs), or enter the follicle from blood plasma. In addition, CCs and oocytes have different metabolic needs. Oocytes themselves are unable to synthesize cholesterol and poorly metabolize glucose for energy production. Inversely, the CCs metabolize alternative substrates, such

as cholesterol and glucose, which are essential for the development and function of oocyte. The exact nature and diversity of oocyte–CC signaling molecules are complex and dynamic. Errors in the regulatory cumulus–oocyte complex may result in the production of oocytes unable to undergo embryo and pregnancy outcomes.

The study of the CCs gene expression profile offers the opportunity by a non-invasive method, to predict oocyte competence, because bi-directional communication between the CC and the oocyte are necessary for gradual acquisition of this competence. Using either RT–PCR or DNA microarrays, some studies have provided evidence for the genes expressed in CCs presenting potential biomarkers to predict embryo quality and pregnancy outcomes. The lecture provides an overview of the current knowledge about CCs as biomarkers for oocyte and embryo selection as an alternative of PGS under an IVF/ICSI program.

In addition, an enormous amount of knowledge about the human oocyte and CCs have been generated over the last years, due in part to the recent advances in gene expression technologies using microarray, CGH array and high-fidelity RNA amplification. Numerous small endogenous non-coding transcripts, termed microRNAs (miRNAs), have been found to execute key functions in silencing expression of specific target genes in plant, animal and human systems. Changes in miRNA expression profiles have been linked to pathologies such as cancer and infertility: female mice with global miRNA deficiency are sterile from several causes, including defects in oocyte function. In addition, the messenger RNA (mRNA) expression in mice and bovine during oogenesis shows that a large proportion of maternal genes are under the control of miRNAs. Thus, miRNA profiling offers an effective means of acquiring novel and valuable information regarding the regulation of transcripts involved in human reproduction.

The miRNAs study of oocyte-cumulus complex offers a promising opportunity, by a non-invasive method, to evaluate ovarian failure and pregnancy outcome. The lecture will demonstrate that the microarray approach is a very useful tool for the discovery of new candidate genes, and the genomic profiling of CCs can usefully serve as a high throughput and non-invasive means for the assessment of oocyte quality, embryo competence and pregnancy outcome.

DNA in Culture media

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Background

Cell-free DNA can be found in embryo culture media. An interesting option would be if this DNA could be used to characterise the quality and developmental potential of the embryos. The Cell-free DNA may be single or double stranded of various lengths and have genomic or mitochondrial origin. A complicating factor is that DNA in embryo culture media may come from various non-embryonic sources such as microbes or somatic cells in semen or vaginal fluid, viral or human DNA in the protein supplements used in culture media. (Kastrop, et al., 2007, Scalici, et al., 2014). Additionally, oocytes will have maternal DNA in cumulus cells and polar bodies and paternal DNA will be present from sperm cells. It is therefore important to select detection systems that are specific for the type of DNA one wants to study.

Analysis of cell free DNA s for embryo selection?

The presence of cell-free DNA of embryonic origin in the culture media may correlate to embryo developmental potential. This is particularly true for mitochondrial DNA. (Stigliani, et al., 2013, Stigliani, et al., 2014). A potential mechanism is shedding of membrane vesicles containing anything from intact chromosomes to relatively short DNA fragments. Blastomere necrosis would also release DNA to the media. Different culture media may induce a difference in embryo fragmentation judged by a traditional morphology based assessment of relative volume of fragments in the embryos (Cooke, et al., 2002). If this difference in fragmentation grade is paralleled by a difference in release of cell-free DNA to the culture media is unknown

An open question is the specificity and sensitivity predicting embryo developmental potential an analysis of cell-free DNA. No studies have yet been published comparing embryos selection based on analysis

of cell-free DNA compared to traditional methods based on morphology and growth kinetic and/or aneuploidy screening.

Analysis of cell free DNA for PGD?
Since embryos to some extent release chromosomal DNA to the culture media, one could at least theoretically imagine being able to do a specific disease specific genetic analysis of the cell-free DNA (Palini, et al., 2013). Not all embryos shed/release DNA, and it is reasonable to assume that it is random which part of chromosomal DNA that is released to the medium. It may perhaps be looked upon as a random partial embryo biopsy. Thus currently it does not seem likely that analysis of cell-free DNA in embryo culture media will prove as useful as the more traditional method for Preimplantation genetic diagnosis based on a trophectoderm biopsy.

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The impact of low fetal fraction on NIPT results

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Non-invasive prenatal testing (NIPT), based on the analysis of circulating fetal cell-free DNA (cfDNA) in maternal plasma, has become rapidly integrated into clinical practice for detection of common fetal chromosomal aneuploidies.

The fetal fraction (FF) is a key determinant of assay performance. The larger the FF, the better the ability to distinguish between euploid and aneuploid pregnancies, thereby the better the test performance. Instead, if the FF is too low, then a chromosomal abnormality could be masked by the overwhelming proportion of euploid maternal cfDNA, thereby increasing the risk of achieving false negative test results.

Clinical tests that rely on quantification of FF, require the amount of fetal cfDNA in maternal plasma to be above a minimum level for accurate aneuploidy assessment. A FF of ~4% or greater is the suggested minimum threshold currently employed by these NIPT methods for a reportable result. Below this value the cfDNA test is usually presented as a failure and no result is reported.

The 4% cut-off was defined using statistical modelling based on the requirement for sufficient sequencing read depth as a function of FF in order to detect fetal aneuploidies. The assumption was that at lower FF, the small differences in circulating cfDNA between trisomic and disomic pregnancies may not be detectable causing false negative results or leading to inaccurate or failed test results. However, no experimental data are available on the actual limit of detection (LOD) of these NIPT approaches (i.e. the lowest FF with a detectable aneuploidy), supporting the above hypothesis. As a consequence, the use of the above cut-off value could be not necessarily appropriate for all cfDNA testing methodologies.

We demonstrated that the minimum FF level required for accurate aneuploidy assessment should be related to the actual LOD of each specific NIPT approach used, and not necessarily fixed at 4% for all cfDNA testing methodologies. In fact, the NIPT protocol we used allowed the detection of chromosomal aneuploidies at FFs as low as 2%. This was demonstrated in the experimental model for the determination of the related LOD, as well as confirmed by the 23.8% of chromosomally abnormal pregnancies involving a FF<4% that were consistently detected. These high risk pregnancies would have not been identified if the 4% FF cut-off had been used.

A major advantage of using a FF cut-off adjusted to the LOD of the specific NIPT approach, is related to the potential of decreasing the incidence of test cancellations and, consequently, lowering the redraw rate. In this study, 8.5% of the samples involving a FF<4%, would have been reported as failed tests if using the suggested minimum threshold currently employed for a reportable result, compared with 2.2% of test failures that would have occurred if using the 2% cut-off related to the actual LOD of the approach. Although repeat sampling can be performed after a failed test result due to insufficient fetal fraction on the first draw, this adds additional time to the screening process and may delay the diagnosis of aneuploidy, thereby potentially limiting reproductive options, especially because only about 50–60% of repeat screens have been reported to provide a result.

In our study, a six-fold increased incidence of aneuploidy (6.9% vs. 1.1%, $p<0.001$) was observed in samples with FF<4% vs. samples with FF≥4%. The above data confirms the finding that a FF<4% is associated with a higher risk of aneuploidy and underscores the importance of testing samples with a very low amount of fetal cfDNA, using NIPT methods with a demonstrated accuracy at low FF.

In conclusion, this study underscores the importance of testing samples with low FF, which may reduce the incidence of test cancellations and shorten the time required for the diagnosis of aneuploidy by invasive prenatal testing. It may also be helpful in providing more truthful test performance calculations. The determination of the actual LOD is advisable for each specific NIPT methodology, rather than using a theoretically determined fixed FF cut-off for all cfDNA testing methodologies.

From non invasive PGD to NIPT: how to improve availability and affordability in the private and public sectors
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Developing a program culminating in a diagnostic test on which clinical management depends requires coordination between multiple units. This applies to preimplantation genetic diagnosis (aneuploidy testing; single gene diagnosis), traditional invasive procedures (amniocentesis, chorionic villus sampling), and most recently non-invasive prenatal screening using cell free DNA in maternal blood. Although physicians have for eons ordered tests and expect to act on results, genetic tests pose more challenges. The provider ordering a given test may or may not be aware of the appropriateness of the query, is typically less familiar with nuances of the indication (e.g., genetic heterogeneity and molecular heterogeneity for a given disorder). The technology is daunting to some, and once codified is quite likely to be altered frequently. Thus, coordination must exist between the genetic counseling unit, the clinicians responsible for requisite procedures, and the laboratory responsible for tasks under its aegis (embryo biopsy for PGD, culture, diagnosis).

At each step prowess is required. Some steps can be overseen readily, whereas others are heavily dependent on the performing individual having experience. Extrapolating from published information is not appropriate for counseling expectation in a unit just initiated. To facilitate consistency various processes exist. In some venues authoritative bodies exist, whereas in other oversight is said officially to be “voluntary”. In the US, different components of the process may be under different jurisdictions. For example, solitary (CLIA) laboratory ordinarily does not require FDA “approval” for its overall quality management and control, this being delegated instead to the “voluntary “ albeit

rigorous College of Pathology (CAP) inspection and approval. Yet assay components and devices ordinarily would be under FDA aegis.

Indications for a procedure or test also vary per venue/country. The authority may rest with government, or for rapidly evolving prenatal genetic diagnosis more often depends on society guidelines and practices. Determining whether a given diagnosis is desirable is in general less the issue in prenatal genetic diagnosis (PGD, traditional PND, and noninvasive NIPT) than the decision as to which method is preferable. In turn this must reflect experience, resources, and results of analytic validity, clinical validation, and comparative studies. The comparative study of choice is randomized clinical trials (RCT). However, pitfalls exist. The recommended Intent to Treat design may or may not be applicable when a surgical procedures (CVS, embryo biopsy) is part of one or more arms; the "learning curve" necessary for any surgical or laboratory procedure must have been passed if the trial is to be considered one on which recommendations can be made. Absent this, conclusions can be erroneous. The same principle applies for diagnostic accuracy. And, of course, clinical utility must be taken into account.

Once introduced, follow up is necessary to track performance and to recognize unexpected outcomes. It can be assume that any "test" newly introduced will be shown to have unexpected results. The team must be capable of handling unexpected outcomes (false positive or false negatives; procedure complications; unintentional ancillary findings). Perhaps of primary emphasis for the genetic community is that ability to shift indications, choice of method, and individual protocols must be considered the rule rather than the exception.

Evolution of PGD for translocations

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INTRODUCTION

PGD is the only hope for carries of balanced translocations, as their reproductive outcome is extremely poor, despite remote chance of having a normal offspring after numerous natural attempts. However, carriers experience repeated spontaneous abortions (SPA) with also a great risk of having an affected child.

MATERIAL AND METHODS

PGD was performed for couples with maternal and paternal balanced rearrangements. Biopsy procedures varied from polar bodies (PB) removal to blastomere (BL) or blastocyst biopsy, by mechanical or laser methods. Nuclear transfer or chemical conversion techniques were used to visualize second PB (PB2) and BL chromosomes. Biopsied materials were tested by FISH, microarray (aCGH), or next generation (NGS) technologies.

RESULTS

PGD for translocation was first introduced by PB1 testing, as it consists of metaphase chromosomes, analyzable by whole chromosome-specific fluorescence probes. But because of high frequency of chromatid exchanges during the first meiotic division, resulting genotype of oocyte cannot be established without PB2 testing, which in contrast never proceeds to metaphase, requiring especially designed conversion techniques for reprogramming PB2 nucleus by nuclear transfer technology. This was also applied to visualize BL nuclei, further improved by okadaic acid pretreatment, with success rate of 83%. Chemical conversion with caffeine simplified procedure, making it routine for "in house" PGD cases. Among other efficient methods were haplotyping by the use of a multiplex fluorescent PCR of highly polymorphic markers and aCGH or NGS, further improving the accuracy of PGD. Overall, the above methods were applied in 902 PGD cycles, including 227 by nuclear or chemical conversion, 90 using PB1 and PB2 testing, 368 by embryo biopsy and interphase FISH analysis, 217 by aCGH or NGS, the latter resulting in further improvement of pregnancy rates to 65%, with reduction SPA rates to 3.9%. The most recent approach involves distinguishing balanced from normal embryos by a specially designed NGS technology - mate pair sequencing (MPS), with a high depth mate pair sequencing to identify breakpoints regions, Sanger sequencing to define the exact breakpoints and design of primers for identifying normal and carrier embryos. The application of the above techniques resulted in significant improvement of pregnancy and over four-fold reduction of SPA rates.

CONCLUSIONS
PGD for translocations evolved from FISH to aCGH/ NGS, and high depth sequencing (MPS). Although no prior work-up may be required for performing a-CGH or NGS-based PGD, its accuracy is adversely affected even by slightest imprecision of the break points, which can now be identified by MPS, allowing also to distinguish normal from balanced embryos, to avoid postponing the problem to the next generation. Performing NGS also allows a concomitant 24-chromsome aneuploidy testing to further improve the pregnancy and reduce SPA rates.

Interchromosomal Effects: ICE, ICE… Maybe?

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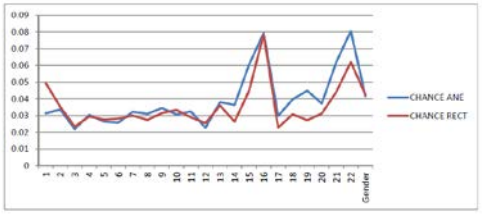
Interchromosomal effect (ICE) it is the disruption of meiotic behavior of chromosomes not involved in a structural rearrangement favoring the non-disjunction of such chromosomes and it was first postulated in 1963 by Lejeune. As such, patients with structural chromosome abnormalities will produce anenhanced rate of abnormal gametes not only due to the expected result of the segregation of the structurally abnormal chromosomes, but also for the postulated interchromosomal effect.

Since then, several studies have attempted to give an answer to Lejeune's postulate, from the beginning of the cytogenetics studies (karyotypes of newborns and prenatal cytogenetics), and later with theincorporation of new techniques. Fluorescence in situ Hybridization (FISH) was a new technique whichallowed for a series of studies in the sperm of translocation carriers and mostly supported the ICEtheory. Although these studies are rich in number of sperm analyzed, they are limited due to the smallnumber of chromosomes that can be analyzed at a time. These studies also contained few patients,revealing the behavior of a particular structural abnormality but not the behavior of the structuralabnormalities as a population. Currently Comprehensive Chromosome Screening (CCS) techniques areable to study all chromosomes, making them the most useful tools for ICE studies on embryos to-date.
Since the validation of aCGH for translocation carriers, a significant number of embryos fromtranslocation and inversion carriers have been analyzed by our group and several studies wereconducted which may help answer to the question "is there ICE?"
The first study compared diagnosis of embryos from patients who are carriers of a reciprocaltranslocation with embryos from patients who are carriers of an inversion. This study showed thatalthough both groups of patients have similar proportions of normal or balanced embryos, theproportion of embryos carrying aneuploidies not related to the chromosomes involved in the structuralabnormality are higher in the inversion group than in the translocation group, pointing to a heightenedICE in inversion carriers compared to translocation carriers.
The second study compared the sex of the carriers in reciprocal translocation cases. This studyconcluded that although both groups have also the same proportion of normal or balanced embryos,
the proportion of embryos carrying an aneuploidy not related to the translocation was higher in malecarriers.These two studies, though related to ICE, it doesn't address the core subject of this theory which is thedisruption of meiotic behavior of chromosomes not involved in a structural rearrangement.

To addressthis subject head on, we conducted a study which, first examines the meiotic behavior of chromosomesfrom cytogenetically normal IVF patients, and then compares those results with those patients that arecarriers of reciprocal translocations.

The following graph was obtained:



The results of this study reveal a close parallelism between both groups revealing that there is no ICE between both groups. Though these results seem to invalidate the presence of interchromosomal effect in reciprocal translocation carriers, still there is much to study in the subject matter. For instance which is the behavior in each age group (which will be discussed in this lecture), other types of structural abnormalities, sex of the carrier,…

MPS for translocations

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Preimplantation genetic diagnosis (PGD) for chromosomal rearrangements is widely used to avoid transferring embryos with genomic aberrations. Currently, genomic microarrays are predominantly used for the detection of unbalanced structural abnormalities and aneuploidies in embryos from parents at risk. We evaluated whether massive parallel sequencing (MPS) can be used in PGD for detecting chromosomal abnormalities on 47 day-5 embryo trophectoderm biopsies from 15 patients with a balanced structural rearrangement. Eight reciprocal translocations, 4 Robertsonian translocations, 2 inversions and one insertional translocation were to be diagnosed. As the samples consist only of 3 to 6 cells, whole genome amplification (WGA) is needed to amplify the available DNA before sequencing library preparation. Although all current WGA methods lead to amplification bias, SurePlex WGA is uniform enough for detection of copy number alterations (CNAs) at a 5 Mb resolution.
Low coverage MPS on a Nextseq 500 (Illumina) and Ion Proton (Life Technologies) instrument was performed in parallel for those 47 amplified samples. Data analysis was performed using the QDNAseq algorithm implemented in Vivar. In total, 5 normal and 42 abnormal embryos were analysed. All aberrations previously detected with arrayCGH could be readily detected in the MPS data and were correctly identified. The smallest detected abnormality was a 5 Mb deletion/duplication hence equaling or even exceeding the resolution of the routinely used microarrays.

Mitochondria: the engine of the oocyte

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Introduction:
Mitochondria are the „power house“ of most cells, where substrates as pyruvate or fatty acids (FA) are metabolised in the citric acid cycle or by fatty acid oxidation (FAO) in the matrix to produce $NADH/FADH_2$ that can drive the electron transport chain in the inner mitochondrial membrane and ROS and ATP production. Mitochondrial generation of ATP serves as major source of high energy substrates in most cell types and influences enzyme activities in metabolism, cytotoskeletal and cell cycle regulation, cell locomotion, redox regulation and overall cellular homeostasis during interphase and mitosis/meiosis. Mitochondria are involved in calcium storage and homeostasis and in steroidogenesis as well as induction or protection from apoptosis. Since mitochondria are maternally inherited, their numbers that do not increase during early stages of preimplantation embryogenesis and their differentiation and functional integrity are essential for embryo quality and developmental competence and for health of offspring.

Materials and Methods:
Many recent studies have analysed mitochondrial copy numbers (DNA) and mutations as well as morphology and distribution by sequencing technologies and fluorescence and electron microscopy in

oocytes, polar bodies and cumulus. Functionality has been assessed by analysis of inner mitochondrial membrane potential, redox potential, ATP production and calcium signalling in oocytes from healthy patients or with respect to maternal age, metabolic disease, IVM or cryopreservation in human oocytes or in experimental models e.g. mutant or chemically exposed oocytes.

Results and Conclusions
Mitochondrial integrity and mitochondrial stage-specific and spatio-temporal activity in oogenesis are essential for oocyte maturation and early embryogenesis. Suboptimal environment, disturbed bi-directional signalling between oocyte and cumulus as well as an unbalanced gene expression from the nuclear and mitochondrial genome, and maternal age and metabolic disease can induce mitochondrial dysfunction and mutation and ultimately effect oocyte quality and developmental potential.

Preferential segregation of Mitochondrial DNA mutation in human oocytes
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Several lines of evidence show that in Metazoa the variation within the germline of mitochondrial genomes is under purifying selection. The presence of this internal selection filter in the germline has importantconsequences for the evolutionary trajectory of mtDNA.
However the nature and localization of this internal filter are still unclear with several hypotheses proposed in the literature.

In this study, 60 mitochondrial genomes were sequenced from 17 sets of oocytes, first and second polar bodies, and peripheral blood, taken from 9 women between 38 and 43 years of age belonging to 6 different mitochondrial macro-haplogroups.

Whole Genome Amplification was performed only on the single cell samples and a Sanger sequencing was performed on amplicons.

The comparison of variant profiles between first and second polar body sequences showed no difference in substitution rates but displayed instead a sharp difference in pathogenicity scores of protein coding sequences using 3 different metrics (MutPred, Polyphen, SNP&GO). Second polar bodies, differently from first, did not show significant differences in pathogenic scores with blood and oocyte sequences.

This suggests that a filtering mechanism for disadvantegous variants operates during oocyte developmentbetween the expulsion of the first and second polar body.

Levels of Mitochondrial DNA and embryonic implantation potential
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Introduction
It is widely known that the transfer of morphologically good euploid embryos does not guarantee an ongoing pregnancy. mtDNA quantification has recently been proposed as a new biomarker of embryonic viability by our group and others. Specifically, we were the first to demonstrate a relationship between mtDNA copy number and embryonic implantation potential. Our results showed that euploid embryos with mtDNA above a certain threshold were incapable of implanting. Our current investigation had two aims: a retrospective analysis of the overall value of the established mtDNA threshold in previously completed clinical cases, and the first application of mtDNA quantification in a prospective blinded non-selection setting.

Materials and Methods
We initially retrospectively assessed the mtDNA of 572 euploid blastocysts, which were generated by 328 couples (average female age 34.95 ± 0.27 years) undergoing preimplantation genetic screening (PGS). Outcome data was collected from 6 different IVF centers for routine follow up.
We then proceeded to quantify the mtDNA of an additional 280 euploid blastocysts generated by 143 couples (average female age 37.24 years). Patients were referred for PGS by a single large IVF clinic.

The study took place in a non-selection blinded manner, i.e. mtDNA quantity was not known at the time of transfer (all were SETs). Clinical outcomes analysis followed to determine usefulness of mtDNA quantification in improving embryo selection.
PGS occurred via a next generation sequencing strategy (NGS). The same embryos were also tested using quantitative PCR, allowing highly accurate mtDNA quantification.

Results
14% (80/572) of the blastocysts assessed retrospectively had elevated mtDNA levels, and therefore lower implantation ability. To date 246 embryos have been replaced in SETs. mtDNA quantification identified 216 with normal/low mtDNA (153 implanted) and 30 with elevated levels (above the established threshold). Of the blastocysts with the elevated mtDNA levels one led to a successful pregnancy, with the remaining 29 not implanting. Therefore the negative predictive value of mtDNA quantification was calculated to be 96.7% (29/30).
A total of 111 blastocysts have been transferred so far without taking their mtDNA levels into consideration, i.e. in a blinded non-selection manner. All transfers involved a single euploid blastocyst of good morphology. Of these, 78 (70%) led to ongoing pregnancies, and had normal/low mtDNA levels. The remaining 33 (30%) blastocysts failed to implant. Among them there were 8 (24%) embryos with elevated levels of mtDNA. This meant that the ongoing pregnancy rate for morphologically good chromosomally normal blastocysts with normal/low levels of mtDNA was 76%, and 0% for the same type of embryos with elevated mtDNA levels. This difference was highly statistically significant (P<0.001).

Conclusions
This is the first blinded non-selection study to demonstrate that mtDNA quantification significantly improves ongoing pregnancy rates. 76% of embryos with normal/low mtDNA levels implanted vs. 70% for the cohort as a whole. This data confirms the usefulness of mtDNA copy number analysis as an additional biomarker of embryonic viability.

The predictive value of mitochondrial DNA
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The existence of a nuclear and mitochondrial genome originated from an endosymbiosis event between two prokaryotes, enabling the host to generate large amounts of intracellular ATP, at the price of increase in intracellular reactive oxygen species (ROS). The resulting high mutation rate in the mtDNA was balanced by endosymbiotic gene transfer (EGT) from the mtDNA to its nuclear counterpart and, in animals, by a high mtDNA copy number in combination with a genetic bottleneck and selection during transmission. We used the zebrafish model to characterize the mtDNA bottleneck mechanism further.

During oocyte maturation, the mtDNA content increased rapidly with high inter-individual and intra-individual variation. In zebrafish oocytes, the mtDNA copy number is higher than in mammalian oocytes, probably due to the metabolic demands of externally developing embryos. During development, the mtDNA copy number per cell decreases until the segmentation period. In PGCs from different embryonic stages we measured on average, ~170 mtDNA molecules at the bottom of the bottleneck, a value similar to the ~200 copies measured for the mouse germline. In corresponding non-PGCs, the bottleneck size was, on average, ~50 mtDNA molecules.

Our data indicated that considerable variation exists in mtDNA copy number among PGCs and non-PGCs of the same female at the bottom of the bottleneck, dictated by the highly variable mtDNA content in the oocyte. In patients, about 25% of the pathogenic mtDNA mutations are de novo and have a high mutation load. We determined de novo mutations and mutation load directly in the mtDNA of individual zebrafish oocytes using next-generation sequencing (NGS). Our data indicate that the de novo mutations most likely arise from errors of mtDNA replication occurring during oogenesis. Low mtDNA amounts at the bottom of the bottleneck create a larger risk for a de novo mutation to reach functional significance. If comparable in humans, this could explain the high frequency of de novo mtDNA disease.

The mtDNA is extensively replicated during oogenesis to achieve a high mtDNA copy number. This is essential for healthy reproduction, as it correlates with both the fertility and viability of oocytes and early

embryos. We demonstrate the existence of selection at OXPHOS and mitochondrial membrane potential (MMP) in human preimplantation embryos and calculated that, as in mice, about 50,000 mtDNA copies or functionally equivalents are required for normal oogenesis and early embryonic development. We investigated the relevant pathways associated with a decreased mtDNA copy number during early embryonic development in zebrafish by knocking-down the Tfam gene with morpholinos (MOs). Injected embryos had a 50-80% drop in the mtDNA level, causing an overall embryonic developmental delay, especially of the heart and the brain.

Using transcriptomics, we observed a decrease in expression of mtDNA-encoded proteins of the electron transport chain and various mitochondrial-related pathways, indicating a downregulation of energy metabolism and ATP production. Furthermore, probably as a feedback mechanism, we found a significant upregulation of the nuclear-encoded mitochondrial translation machinery. This imbalance between mtDNA replication and translation could contribute to the mitochondrial stress and pathology observed. The zebrafish model turns out to be an excellent model for in-depth characterization of the molecular mechanisms during mtDNA inheritance from PGCs to whole embryos and will most likely reveal options for intervention.

Mitochondrial replacement: from ART to Stem Cells

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Mitochondrial DNA (mtDNA) represents a small fraction of the total cell DNA content with 37 genes involved in mitochondrial function, including oxidative phosphorilation.

Mitochondrial disease arises when mutations occur in mtDNA and constitute an heterogenous group of disorders that can affect multiple organs. The percentage level of mutant mtDNA may vary among individuals within the same family and also among organs and tissues within the same individual (heteroplasmy).

Mitochondrial disease prevention can be performed through oocyte donation, preimplantation genetic diagnosis (PGD), mitocondrial transfer (MT) and genome editing (GE).PGD can reduce the risk of transmitting mtDNA disease and relies on the production of embryos with low levels of mtDNA mutation. PGD is not useful for homoplasmic women (100% of mtDNA mutated) or for heteroplasmic women who produce oocytes with high mutation loads (Treff, 2012).

MT techniques are useful for women with high mutation loads and include germinal vesicle/spindle transfer at the oocyte level or pronuclear transfer at the zygote stage.

Spindle transfer has been successfully achieved in primates and the reconstructed oocytes showed normal fertilisation, embryo development and production of healthy offspring (Tachibana, 2009). This technique has also been applied in human oocytes with normal embryo development and normal mitochondrial respiratory chain enzymatic activity and oxygen consumption in resulting stem and differentiated cells (Paull, 2013).

Pronuclear transfer has proven to be compatible with normal development in the mouse model while preventing mtDNA transmission. Preliminary results have been published in 3 PN human zygotes (Craven,2010)

Selective elimination of mitochondrial mutations in the germline has been reported by GE. MtDNA heteroplasmy was induced through the elimination of mutated mtDNA in the mouse model and human mutated mtDNA levels responsible for Leber's neuropathy were reduced in artificial mammalian oocytes (Reddy,2015).

Co-evolution of the mitochondrial and maternal nuclear genomes might result in adverse effects arising from the creation of a new combination of mtDNA and nuclear DNA.
SCNT derived mouse ESCs trigger an immune rejection in spite of their matching with the entire nuclear genome of the recipient mouse strain (Deuse,2015).

Genetically and functionally corrected pluripotent stem cells from mtDNA disease patients have been generated. Clones with exclusively wild type mtDNA due to spontaneous segregation of heteroplasmic mtDNA have been recovered (Ma,2015)

MT has been proposed as a mean to ameliorate the effects of female age on fertility. Cytoplasmic transfer from oocytes from young donors to advanced aged patients was reported in the 1990's and banned by the FDA due to safety concerns. Isolation, transfer and injection of mitochondria from oogonial stem cells has also been recently proposed for oocyte quality improvement with controversial results.

In the UK, the House of Lords has recently approved the use of MT to prevent mitocondrial disease while in the USA, the FDA allows the use of this technique for clinical investigations under certain conditions.

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Can Success Rate in Human ART Be 95%

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Personalized or precision medicine approaches to medicine are evolving strongly with the increasing availability and sophistication of genomic, metabolic, cellular and related information. These data sets continue to be applied to reproductive performance and the algorithms improved despite some setbacks in improving IVF success rates. The important parameters appear to be; optimized and automated culture conditions that enables data to be recovered on the developmental changes and timing of cell cleavage and embryo morphometry; mitochondrial vitality which is closely associated with embryo metabolism; euploidy and DNA rearrangements, deletions and mutations in developmental and disease

related genes; and the evolution of a viable transcriptome necessary to ensure full embryonic developmental potential.

Both chromosomal and mitochondrial copy number can be measured in embryonic cells, together with the detection of small pathogenic copy number variants within the genome. Variant gene dysfunction prediction due to point mutations can also be applied to rank embryonic viability and normality of the conceptus. The quantum of information can be used to accurately predict the developmental competence and normality of preimplantation embryos. Consequently in patients that have difficulty in conception the batching and vitrification of embryos from several cycles of IVF treatment enables the selection of single very high implant potential embryo for transfer. The limitation to the use of these technologies is the increasing cost that may decrease, as the volume of testing and analyses increases.

Free Communications

ORAL PRESENTATIONS MONDAY 9th MAY 2016

Chromosomal analysis on spermatozoa from infertile couples.

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Introduction. The application of multicolor fluorescence in situ hybridization (FISH) was proposed during the last decade, and enabled comprehensive studies on numerical and structural chromosomal abnormalities in human spermatozoa. The reliability and feasibility of this technique for sperm analysis has included the numerical study of chromosomes as a routine test for the screening of infertile couples, even in cases of severe oligospermia including testicular and epididymal samples.

Materials and methods. Between April 2003 and December 2015, 1462 sperm samples from infertile patients underwent a FISH test. Aneuploidy for nine chromosomes (XY, 13, 15, 16, 17, 18, 21, 22) were tested in 1391 samples from couples with at least 3 previous IVF failures and/or miscarriages: 313 were normospermic (N), 706 were moderate oligoasthenoteratospermic (m-OAT), 346 were severe OAT (s-OAT), 15 samples had been retrieved by TESE (NOA) and 11 by MESA. In addition, 71 samples from carriers of translocations (10 Robertsonian and 61 reciprocal) and were tested to evaluate the reproductive risk by detecting the proportion of spermatozoa with an unbalanced rearrangement. For the evaluation of the results, a statistical analysis based on chi-square test for rare frequencies by applying the binomial distribution of Poissons was applied. The results were interpreted by assigning clinical relevance of the detected aneuploidy at P values lower than 0.001.

Results. In the cases tested for aneuploidy, the incidence of samples with an increased frequency of abnormalities for the 9 analyzed chromosomes was 0.32% in N samples, 10.1% in m-OAT, 67.6% in s-OAT 80% in TESE and 45.5% in MESA samples (all P<0.001 vs. N).
The proportion of aneuploid spermatozoa in the different sperm categories compared to the total number of diagnosed cells was 1.3% in N samples (range 0.1-6.3%), 1.9% in m-OAT (range 0.4-16.6%), 4.4% in s-OAT (range 0.5-50%), 5.2% in TESE (1.5-33.3%) and 2.4% in MESA (range 1.5-24.2%).
The analysis of aneuploidy for the tested chromosomes, showed different frequency of variation with the highest values for the gonosomes followed by chromosome 21, 13, 22, 17 and 15. In most cases, aneuploidy events involved between 2 and 6 chromosomes.
In the 71 translocation carriers, the proportion of unbalanced chromosomes was 15.8% for Robertsonian translocations (range 4.3-51%) and 61.9% for reciprocal translocations (range 10-87.4%). In 42 of these samples, aneuploidy was also evaluated and revealed a frequency that was similar in both translocation categories (5.2±3.3% in Robertsonian, range 1.3-9.3%; and 4.9±3.4% in reciprocal translocations, range 1-15.5%).

Conclusions. The analysis of 9 chromosomes in sperm samples from infertile patients revealed that a male factor condition is characterized by a significantly higher incidence of aneuploidy compared to normospermic samples. The general proportion of aneuploid spermatozoa is not especially high when considering the mean value, but the wide range observed suggests the importance of this test according to the specific couple's reproductive history. Some chromosomes are especially involved in these abnormalities, including those, which are compatible with embryo implantation.
If this predisposition to generate aneuploid gametes in couples carrying a male factor of infertility could have implications in the clinical outcome of their pregnancies, is still to be determined. Nevertheless, preliminary data on the infants born after ICSI have raised concern. It is possible indeed that the

introduction of ICSI, that completely bypasses any mechanism of natural sperm selection, could lead to recalculate the contribution of the male gamete to the generation of aneuploid concepti.
For translocation carriers, the FISH test provides useful information to predict the couple's reproductive risk. Due to the high incidence of unbalanced gametes generated in translocation carriers, the number of embryos destined to chromosomal analysis is crucial to increase the chances of performing embryo transfer.

Reproducible segmental organization of telomeres and centromeres in sperm: Implications for early embryonic development

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Introduction: Genomes are organized non-randomly in the 3D space of nuclei, with potential functional implications beginning to emerge. Similarly chromosomes and telomeres in sperm cells have a unique spatial arrangement that is hypothesized to be critical for the ordered exodus of the paternal genome immediately following fertilization. The current "hairpin-loop" model of organization in sperm describes telomeres localizing to the extreme periphery and centromeres aggregating in the center of the nucleus to form a chromocenter. This study evaluated the spatial arrangement (radially and longitudinally) of centromeres and telomeres and its reproducibility in multiple subjects using 3D and 2D approaches.

Materials and Methods: Ten normozoospermic subjects were recruited, and this study was approved by the local IRB. FISH was employed to assess the topology of all telomeres; 3D-radial (n=300), 3D and 2D-longitudinal (n=300 and n=964) and all centromeres; 3D-radial (n=300), 2D longitudinal (n=964). Assessment of the 3D organization was achieved using wide-field microscopy and model rendering that allowed measurement of distances from the geometrical center of telomeres and centromeres to the closest nuclear edge. Organization in 2D was assessed using standard fluorescence microscopy and a custom plugin for Image J. Statistical analysis was performed using the chi-squared goodness-of-fit test to determine nonrandom organization of areas of interest ($p<0.05$).

Results: The results showed reproducible non-random distribution for telomeres and centromeres with both 3D and 2D approaches ($p=<0.05$). Telomeres demonstrated the following 3D radial localization in the nucleus: 43% peripheral, 43% intermediate, and 14% interior. In terms of longitudinal organization, both 3D and 2D approaches revealed a remarkably similar telomere distribution with approximately 25%, 50%, and 25% being found in the tail, mid, and head region of sperm cells, respectively. Centromeres demonstrated the following 3D radial distribution in the nucleus: 49% peripheral, 44% intermediate, and 7% interior. In terms of longitudinal organization (2D), the distribution of centromeres in the tail, mid and head regions was 21%, 54%, and 25%, respectively. Telomeres and centromeres were observed to form an average of 20 and 7 clusters, respectively, throughout the sperm nucleus.

Conclusions: This was the first study that simultaneously radially and longitudinally mapped telomeres and centromeres in more than one individual using 3D and 2D approaches. The results demonstrate that not all telomeres are in the nuclear periphery as previously hypothesized. Furthermore, we showed the presence of multiple centromere clusters (i.e. chromocenters) that were not restricted to being interiorly localized. These findings suggest the sperm nucleus is more segmentally organized. This segmental organization is likely an essential pre-requisite for early embryonic development and serves several critical functions including: i) to efficiently compact the paternal DNA and to protect against DNA damage; ii) to effectively silence gene expression, but allow rapid reactivation post-fertilization; and iii) to deliver the paternal genome in a sequential order to the maternal oocyte for chromatin remodeling and pronucleus formation. The more segmental distribution of telomeres and centromeres described in this study more readily accommodates and facilitates the sequential exodus of paternal chromosomes following fertilization than the current proposed "hairpin-loop" model.

Accurate Recombination Risk Prediction For Preimplantation Genetic Diagnosis (PGD) Of Monogenic Disorders

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Introduction: Genotyping of a disease causing mutation during PCR based PGD can be compromised by amplification failure. To minimize such a risk, Short Tandem Repeat (STR-) markers in close proximity of the mutation are simultaneously analyzed, called linkage analysis. The basepair distance of the STR-markers to the mutation is a linearly dependent predictor of the recombination risk: STRs, which are 1 Megabase away from each other have an average recombination probability (RP) of 1% or 1 Centimorgan approximately. This rule of thumb between the genetic unit Centimorgan and the basepair distance is imprecise. Available high resolution SNP datasets from the HapMap-project can be used to render more precisely recombination risks during PGD. Retrospectively, SNP- and basepair distance- based recombination risk predictions were compared to actual recombination events occurred at a PGD of the dominant inherited disease tuberous sclerosis.

Method: Recombination rates and genetic distance values between a tuberous sclerosis causing deletion and neighboring STR-markers were extracted from the latest HapMap datasets. The number of recombination events, predicted by the 1 Centimorgan = 1 Megabase equation and the SNP based HapMap data were compared in terms of concordance to actual recombination events during PGD cycles for the same patient family.

Results: The HapMap based recombination probability between the TSC2 proximal and distal STR-markers showed several recombination hotspots, which exhibit a local 10- to 55 times increase of the RP. In general, a tendency towards a higher RP of distal STR-markers was predicted. This was confirmed during actual PGD cycles for tuberous sclerosis: recombination events occurred mainly between two distal markers, which had the highest SNP based RP of all markers used.

Conclusion: PGD requires comprehensive validation including ADO-rate, total amplification failure rate and RP determination of used STR-markers. High resolution SNP datasets allow a more realistic RP prediction than the widely used 1 Centimorgan = 1 Megabase equation by giving additional information of localized recombination hotspots. High recombination probabilities between STR-markers can also implicate, that the haplotypes of the parents and/or their children might be recombinant themselves. Therefore, haplotype confirmation by two meiotic products is highly recommended.

Whole Genome Sequencing of a Single Human Cell by Linear Amplification via Transposon Insertion (LIANTI)

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Introduction: We have developed a new whole genome amplification (WGA) method for a single human cell called LInear Amplification via Transposon Insertion (LIANTI), which has superseded existing WGA methods in all aspects. LIANTI involves random insertion of transposon sequences into the genomic DNA, creating genomic DNA fragments that are linearly amplified prior to library preparation for next generation sequencing.

Material and Methods: Picograms of genomic DNA from a single human cell are linearly amplified via random insertion of transposon sequences into the genomic DNA (LIANTI), followed by standard DNA library preparation with NEB kit (New England Biolabs, MA, USA). We obtained a final product of hundreds of nanograms that is then sequenced by Illumina Hiseq platform (Illumina, CA, USA).

Results:LIANTI offers many advantages compared to currently existing methods for human cell whole genome amplification, including highest genome coverage, lowest false positive and false negative rates for detecting single nucleotide variation (SNV), lowest chimera formation rate, and the highest accuracy and spatial resolution for detecting copy number variation (CNV).

Conclusions:LIANTI makes it possible for the first time to detect micro CNVs in a single human cell, which has immediate applications to *in vitro* fertilization and prenatal diagnosis. The methodology and applications will be discussed.

ORAL PRESENTATIONS TUESDAY 10th MAY 2016

Impact of mosaicism detection on PGS clinical outcome

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Introduction: Preimplantation genetic screening (PGS) enables to select euploid embryos in order to increase implantation rate and reduce miscarriage rate after embryo transfer. In comparison to previously used array technology, aneuploidy screening for all chromosomes by next-generation sequencing (NGS) provides higher dynamic range for detection of copy number changes. PGS is currently performed on trophectoderm samples from blastocysts. The trophectoderm biopsy contains typically 5 – 10 cells and therefore it may consist of euploid and aneuploid cell lines which persist in embryo as a result of genetic instability in early mitotic divisions. Implantation potential of mosaic embryos is potentially different from those embryos containing only euploid cells. Therefore, identification of mosaic embryos could influence PGS clinical outcome.

Material and methods: All samples were originally amplified by Picoplex WGA Kit (Rubicon Genomics) and tested for chromosomal aneuploidies by arrayCGH using 24sureV3 microarrays (Illumina). The same amplified DNA samples were subsequently reanalyzed by NGS using VeriSeq PGS Kit and MiSeq (Illumina). Reanalysis of mosaicism was performed in the group of 50 livebirths and in the control group of 50 non-implanted embryos covering the same time period in our clinic. Additionally, VeriSeq reanalysis was carried out in the group of 21 miscarriages after PGS from 2013 to 2015 and in the group of 8 embryos which were already reported as mosaic after arrayCGH and transferred to patients after genetic counselling. No ongoing pregnancy – 4 miscarriages and 4 non-implanted – was recorded in this group.

Results: In order to evaluate clinical impact of mosaicism detection, retrospective analysis of 100 randomized samples was performed covering the group of 50 livebirths and the control group of 50 non-implanted embryos. Chromosomal copy number changes in mosaic were scored for all chromosomes including segmental changes. After de-randomization of reanalyzed samples, 8 mosaics were reported in the group of 50 livebirths compared to 17 mosaics reported in the group of 50 non-implanted embryos (16% vs. 34%, P = 0.038). To further analyze impact of different types of mosaic findings on the clinical outcome, 9 mosaic embryos (43%) from the group of 21 miscarriages after PGS and 8 embryos transferred as a reported mosaics were compared in terms of specific mosaic type to mosaic embryos from the groups of livebirths and non-implanted. Differences in the type of mosaic findings were observed in the studied groups.

Conclusions: Randomized retrospective analysis of mosaicism by NGS revealed that mosaic embryos are two times more frequent in the control group of non-implanted embryos proving that mosaicism is a real biological phenomenon rather than an artefact linked to DNA amplification or sequencing data processing. Implantation potential of mosaic embryos appears to be half-reduced compared to euploid embryos. Though mosaic embryo can result in a live birth, mosaics were reported in 42% embryo biopsies in the miscarriage group which probably reflects increased miscarriage rate for mosaic embryos. Our data also supports the hypothesis that implantation potential depends on type of mosaic finding in the embryo such as whole chromosomal versus segmental or number and extent of mosaic changes.

Improved detection of chromosomal mosaicism by Next Generation Sequencing (NGS)-based preimplantation genetic screening

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Introduction: Aneuploidy screening of embryos at blastocyst stage can be jeopardized by the presence of chromosomal mosaicism, a phenomenon characterized as a mixture of diploid and aneuploid cell lines in the same embryo. Conventional methods have precluded accurate detection of mosaicism after preimplantation genetic screening of embryos preventing, in some case, transfer of the only viable mosaic embryos (false positive), or transferring undetected mosaic embryos (false negative). Here we applied next generation sequencing (NGS) technologies-based preimplatation genetic screening (PGS) to investigate the potential of this methodology to improve the detection of embryos with chromosomal

mosaicism over conventional methods, such as array comparative genomic hybridization (array-CGH) technique.

Material & methods: This study was organized into three steps. The first involved mixing experiments with different ratios of euploid and aneuploid single cells, to mimic chromosomal mosaicism. The aim was to determine the accuracy and the minimum ratio of aneuploid to euploid cells that is needed to detect a copy number variation by NGS and array-CGH technologies. The second step was a retrospective assessment of 2399 whole genome amplification (WGA) products, selected from previously performed clinical PGS cycles analysed with array-CGH. The third consisted in a prospective trial involving a blind parallel evaluation of 1860 blastocysts, with both NGS and array-CGH techniques.

Results: Reconstitution experiments results demonstrated that NGS accurately identified chromosomal mosaicism at 10%, and showed higher specificity throughout each mixture compared to array-CGH. Comparison between NGS and array-CGH results showed concordant results in all 2466 embryos but revealed that 188/1798 (10.5%) of array-CGH-diagnosed aneuploid embryos were indeed mosaic embryos (Table). Following transfer of 43 mosaic embryos in 42 women, 17 clinical pregnancies ensued, of which one resulted in the birth of twin and 13 resulted in the birth of a singleton infant, confirmed to have a normal karyotype.

Conclusions: This study demonstrates that the increased dynamic range and the higher sensitivity of quantification of NGS technique allows the detection of mosaic embryos undetectable with array-CGH. Consequently, NGS may represent a more robust method for aneuploidy screening of embryos at blastocyst stage with the potential to lower the risk of false negative and false positive results.

Mosaic embryos, detected by NGS, are mostly classified as euploid by aCGH

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Introduction: Array Comparative Genomic Hybridization (aCGH) has been the gold standard for Comprehensive Chromosome Screening (CCS). Most recently, Next Generation Sequencing (NGS) has been implemented for CCS due to its increased resolution and the ability to detect mosaicism and most polyploidy. For example, data presented recently showed 5% of embryos were mosaic by aCGH but 20-30% by NGS. In this study, we reanalyzed four groups of NGS trophectoderm biopsies classified as mosaic, euploid, triploid, or aneuploidy, by aCGH, to determine how NGS mosaics are classified by aCGH and if the excess of mosaics observed by NGS come mostly from euploid embryos (potentially false negatives) or from aneuploid ones (potentially false positives).

Materials and Methods: 70 SurePlex whole genome amplification products of trophectoderm biopsies from the same IVF center were processed first using Illumina VeriSeq PGS assay with the MiSeq sequencing platform for NGS. Results were interpreted through BlueFuse Multi analysis software and diagnosed as euploid, triploid, aneuploid, or mosaic for NGS. Next, the same 70 SurePlex whole genome amplification products of trophectoderm biopsies were processed via Illumina's 24Sure aCGH protocol and analyzed via BlueFuse Software into euploid, aneuploid, or mosaic (aCGH cannot detect polyploidy).

Results: All 20 euploid NGS embryos after reanalysis were confirmed euploid by aCGH. 19/20 aneuploid NGS embryos were concordant with the one exception being a partial monosomy 7 on aCGH whereas NGS diagnosed it as a full Monosomy 7. However, 16/20 mosaic trophectoderm biopsies were diagnosed as euploid on aCGH and the remaining 4/20 were classified as aneuploid (table 1). None of the embryos was classified as mosaic by aCGH. 6/10 triploids were classified as euploid on aCGH and the others as aneuploid for gonosomes.

Table 1:

	aCGH Results		
NGS	Euploid	Abnormal	Concordance
20 Euploid	20	0	100%
20 Aneuploid	0	20	100%
20 Mosaic	16	4	20%
10 Triploid	6	4	N/A

Conclusions:
This study shows that aCGH main limitation is its inability to detect mosaicism and triploidy in trophectoderm biopsies. aCGH classify triploids euploid in about 40% of the occasions. Whereas polyploid embryos are a small fraction of the embryo population, mosaic embryos are quite more abundant. Thus the detection of mosaic and polyploid embryos by NGS should reduce further the risk of miscarriage and improve implantation rates, since mosaics by NGS have been shown to implant less and miscarry more and polyploid embryos are also at risk of miscarry or produce a hydatidiform mola. Some of these mosaics do implant and go to term, so mosaic embryos may be consider for transfer if no euploid embryos are available.

Mosaicism rates in embryos resulting in live birth or miscarriage

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Introduction: Next generation sequencing (NGS) is used to screen human blastocysts undergoing preimplantation genetic diagnosis (PGD) for comprehensive chromosome screening (CCS). This high resolution technique allows for the identification of mosaic samples. Unlike NGS, array comparative genomic hybridization (aCGH) cannot detect mosaicism. Therefore, NGS provides additional information not detected by aCGH which may improve embryonic selection compared to aCGH technology. This study attempts to determine the frequency of mosaic embryos diagnosed as euploid by aCGH that result in live birth or miscarriage.

Materials and Methods: For this retrospective study, biopsied samples from patients who had experienced miscarriages following biochemical or clinical pregnancies from transfer of blastocysts originally diagnosed as euploid via aCGH technology (Illumina, USA) were reanalyzed using VeriSeq NGS (Illumina, USA) and results were compared. Aliquots of the same amplified product from a single biopsy per embryo were utilized for the two tests.

Results: A total of 68 blastocyst samples originally tested using aCGH and diagnosed as euploid were reanalyzed. Of the biochemical pregnancies, 72.7% (32/44) samples had euploid results via NGS and aCGH, 25% (11/44) samples had mosaic results via NGS, while 1 was found to be triploid (69, XXY) via NGS. Of the pregnancies resulting in spontaneous abortion (SAB), 66.7% (16/24) of the samples had euploid results as diagnosed through NGS and aCGH, while 33.3% (8/24) were mosaic via NGS. In contrast, of the viable pregnancies, only 9% (6/67) of the samples were mosaic via NGS.

Conclusions: NGS provides additional information that can significantly improve embryonic selection by detecting mosaic embryos that may result in SAB or biochemical pregnancies followed by miscarriage. Additionally, unlike aCGH, NGS technology can clearly identify 69, XXY embryos. NGS provides a more comprehensive look into the chromosomal complement of preimplantation embryos, substantially enhancing embryonic selection and leading to higher chances of achieving a healthy pregnancy. Selection against mosaic embryos when other fully euploid ones are available for transfer may increase further ongoing pregnancy rates. In the case of no euploid embryos available, mosaic embryos could be considered for replacement but may more likely result in SAB or biochemical pregnancies followed by miscarriage.

Keywords: next generation sequencing, comprehensive chromosome screening, aCGH, mosaic

Mosaicism of single segmental chromosomal changes in trophectoderm biopsy

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Introduction: Chromosome aneuploidy observed in human embryos is a major cause for implantation failure and miscarriage and can affect the health of the offspring. Both, whole chromosomal changes and subchromosomal segmental changes are observed. Although the high postzygotic chromosomal instability associated with cleavage stage is in blastocysts substantially lowered, a certain proportion of mosaic findings still persists. In case of mosaicism the capability of the embryo to corrected or "outcompete" aneuploidy cells was discussed. Recently, successful implantation and birth of healthy children after transfers of diploid/aneuploid mosaic blastocysts were reported.
This study is aimed to assess the level of mosaicism of single segmental chromosomal changes in trophectoderm cells (TE).

Material & methods: Aneuploidy screening was performed in 1049 TE samples of 182 infertile couples during years 2014-2015. The biopsied samples underwent whole genome amplification (SurePlex (Illumina), PicoPlex (Rubicon Genomics)) and were tested by aCGH (24sure V3/+ (Illumina)). The evaluation of the results was performed in SW BlueFuse Multi (Illumina). Thereof, 80 TE samples obtained from 80 embryos with a proven single segmental change (defined as segmental changes

larger than 20Mbp) were assessed for mosaicism. Level of mosaicism was determined according to the log_2 ratio of changes compared with X-separation value (level A: 50-60% mosaicism with trisomy or monosomy of the given chromosomal segment, level B: 60-80% mosaicism with trisomy or monosomy of the segment, level C: (non-mosaic trisomy or monosomy of the segment), level D (mosaicism of trisomy/tetrasomy or nullisomy/monosomy of the segment), level E (non-mosaic tetrasomy or nullisomy of the segment).

Results: In 8% (80/1049) of analysed samples a single segmental change was found. Thereof, in 45/80 samples (56%) a single segmental loss (sL) was observed. 27/45 (60%) samples with sL were classified as diploid/monosomy mosaics (level A+B), 2/45 (4%) as monosomy/nullisomy mosaics (level D), while only 16/45 (36%) were assessed as non-mosaic finding (level C+E).
In 35/80 samples (44%) a single segmental gain (sG) was observed. Out of these embryos 12/35 (34%) samples with sG were classified as diploid/trisomy mosaics (level A+B), 6/35 (17%) as trisomy/tetrasomy mosaics (level D), while 17/35 (49%) were assessed as non-mosaic finding (level C+E). Lower frequency of mosaics in embryos with segmental gains might be partly explained by lower sensitivity of gains recognition by aCGH.

Conclusions: In total, 49% (39/80) of all blastocysts with diagnosed single segmental changes were found to be mosaic with concurrent presence of normal diploid cell line. Embryos with single segmental chromosomal changes proven in diploid/aneuploid mosaics have been discussed as "2nd chance embryos" when no euploid embryo is available for transfer. Further 10% (8/80) of the embryos were diagnosed with aneuploid/aneuploid mosaicism. In contrast, 41% of the analysed TE-cells showed no mosaicism in sG or sL. Following studies are necessary to analyse whether non-mosaic status in these embryos is proven for the whole embryo, including the inner cell mass.

Combined time-lapse imaging and Preimplantation Genetic Screening (PGS): A valuable strategy for embryo selection

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INTRODUCTION: Previous studies have confirmed that preimplantation genetic screening (PGS) of human blastocysts significantly improves pregnancy rates per cycle diminishing the likelihood of miscarriage. Unfortunately,there is still a considerable number of euploid embryos that fail to implant. New embryo selection markers have been developed (mtDNA content, metabolomics or morphokinetic assessment). Time-lapse imaging (TLI) has emerged as an interesting non-invasive tool for embryo selection. No conclusive data about the utility of this technology for chromosome abnormalities determination is available; in the present study we wonder if combined TLI using Eeva Test and next generation sequencing PGS embryo selection may be a valuable strategy to identify those embryos with the best chances of success.

MATERIAL & METHODS: This unicentric and retrospective study included 159 PGS-cycles from IVF and egg-donation patients seeking ART treatment at our centre between September 2013 and December 2015. The control group (*PGS-only)* comprised 70 cycles in which embryos had been selected for transfer following euploidy criteria only. The study group (*PGS+Eeva*) comprised 89 cycles in which embryo selection for transfer was based on combined PGS and Eeva predictions. All embryos were cultured, biopsied and vitrified at the blastocyst stage. Genetic analyses were performed by Next-Generation Sequencing (NGS). Differed single euploid blastocyst transfers under HRT were performed in all cases. Within the *PGS-only* group (control), the best morphological euploid embryo available was transferred. Within the *PGS+Eeva* group, the euploid blastocyst with the highest Eeva-prediction available was prioritized for transfer. Biochemical pregnancy rates of study groups were statistically compared.

RESULTS: Clinical characteristics were comparable between groups; no significant differences in terms of MII, fertilized eggs, blastocyst rate, high quality blastocyst rate and euploidy rate were found. However, when comparing pregnancy rates, a significantly higher ($p<0.05$) positive pregnancy rate was found in the *PGS+Eeva* group cases where euploid embryos with *high* implantation potential as predicted by Eeva were transferred [73.4% (47/64)] compared to the *PGS-only* group [52.9% (37/70)]. No such differences were found when pregnancy rates from transfers of euploid embryos classified as *medium* or *low* by Eeva were compared to controls.

Similar results were obtained when IVF and egg donation cycles were independently analysed. A significantly higher pregnancy rate was achieved in transfers were *high* Eeva prediction in addition to PGS were used for embryo selection [(69.2% vs 48% in IVF cycles ($p<0.05$) and 71.9% vs 54.1% in egg-donation cycles ($p<0.05$)]. These results show that Eeva Test provides valuable information for euploid embryo selection significantly increasing the chances of pregnancy in cycles where chromosomally normal embryos predicted by Eeva as *high* are prioritized for transfer.

CONCLUSIONS: Eeva Test provides valuable information for embryo selection increasing the chances of pregnancy by accurately predicting the implantation potential of euploid embryos. To our knowledge, this is the largest dataset of single euploid embryo transfers selected by a TLI system. This analysis reveals the ability of early cleavage TLI parameters to predict embryo implantation potential and suggests that its combined use with PGS of embryos would significantly improve ART outcomes.

Single cell testing versus amniocentesis/NIPT: Ultra-high resolution preimplantation screening for microduplication/microdeletion syndromes

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Background: Invasive prenatal testing and recent advances in noninvasive prenatal testing (NIPT) have afforded pregnant couples the ability to test for some pathogenic microdeletions during pregnancy. These structural chromosomal abnormalities include 22q11.2 deletion (DiGeorge syndrome), 1p36 deletion, Cri-du-chat, Prader-Willi, and Angelman syndrome deletions which, altogether, appear in 1:1000 childbirths. Unlike Down syndrome, the risk for the aforementioned disorders is independent of maternal age such that prenatal testing of pregnant women of all ages is recommended. Nonetheless, due to the small size of the deletions (ranging from 1Mb-3Mb long), preimplantation screening for high-risk *de-novo* microdeletions remains too technically challenging for large-scale application. Therefore, we aimed to assess whether moderately low coverage next generation sequencing can be utilized to screen clinically relevant microdeletion/microduplication syndromes in preimplantation embryos.

Materials and Methods: Structural chromosomal aberrations, resulting from parental balanced translocations, were tested in 96 different blastomere/blastocyst biopsies by array comparative genomic hybridization (aCGH) on the Illumina/BlueGnome platform at the Shaare Zedek Medical Center (SZMC) in Jerusalem. Residual whole genome amplified DNA from the same aCGH workflow was also subjected to moderately low coverage whole genome shotgun sequencing on a NextSeq 500 (Illumina) instrument. All resultant reads were aligned to the human reference genome (hg19) and custom in-house software was established for the determination of ultra-high resolution chromosomal copy number across the entire human genome. This same software was applied for the detection of pathogenic microdeletions in 12 polar body samples from the Hou et al. (2013) deep sequencing data set.

Results: Deep sequencing identified all 84 unbalanced and 12 balanced/euploid embryo samples that were detected by aCGH (100% of the cases). Notably, even copy number variation (CNV) gains/losses (unbalanced translocations) as small as 4Mb-7Mb long were easily detected by deep sequencing in 16 of the 84 unbalanced embryo biopsies. Therefore, to further assess the sensitivity of our CNV detection, slightly higher coverage sequencing data from the Hou et al. study was scrutinized for smaller copy number gains and losses. Strikingly, this analysis identified multiple structural abnormalities with pathological implications. In addition to large structural abnormalities, CNVs as small as 0.3Mb and 0.5Mb were detected with high confidence. The 0.3Mb microdeletion included the *PARK2* gene whose loss-of-function is associated with juvenile Parkinson disease; the 0.5Mb deletion included the *NPC2* gene whose deletion is associated with Niemann-Pick Disease, Type C.

Conclusions: We have expanded the scope of preimplantation genetic screening (PGS) to include genetic disorders that are currently screened only by amniocentesis or NIPT. Indeed, our moderate low coverage deep sequencing workflow offers the requisite high resolution CNV detection which is crucial for preimplantation diagnosis of common microdeletion disorders such as DiGeorge, Cri-du-chat, Prader-Willi, and Angelman syndromes. This methodology is also applicable for any couple performing PGS or PGD for a familial balanced translocation. Moreover, our method is rapid (24 hours from biopsy

to test result), robust (accommodating tens of samples per batch), and highly cost-effective with respect to aCGH.

Reference list: Hou Y, Fan W, Yan L, Li R, Lian Y, Huang J, Li J, Xu L, Tang F, Xie XS, Qiao J. (2013)

PGD for variants of unknown significance (VUS) ; perform or not to perform ?

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Introduction: Whole Exom Sequencing (WES) has become an effective tool for delineating the mutations to enable PGD application for families without previous diagnosis of their affected child. A family was referred to our clinic who had two deceased children with phenotypes resembling a complex metabolic disorder. Due to lack of clinical diagnosis WES was performed and two disorders (Menkes, Joubert Syndrome) were highlighted in results.

Menkes syndrome is a disorder that affects copper levels in the body. Joubert syndrome is a disorder that affects many parts of the body. The signs and symptoms of this condition vary among affected individuals. Combination of these disorders may lead to an unexpected phenotype making diagnosis quite difficult for clinicians who face cases with overlappping phetoypes of multiple disorders.

Material & methods: The couple referred to our center for genetic counseling. Family history reported 2 deceased male siblings with similar phenotype . Whole exome sequencing (WES) was performed for the healthy son and parents. After couple's consent, PGD was designed specificly for three variants of unknown significance.

Results: WES analysis performed on a healty child and parents revealed three VUS . The WES results of the trio analysis, concluded that several disorders might partially account for the deceased children phenotypes including X-linked Menkes disease and autosomal recessive ciliopathies. In the light of this assumption, PGD application was designed for these variants. We have performed two IVF/PGD cycles for this family. In their first attempt only one embryo was appropriate for transfer . Due to failure in achieving succesful pregnancy, second IVF/PGD cycle was performed. Eight embryos were evaluated and two normal embryos were transferred. The rest of the embryos were vitrified for future considerations.

Conclusions:WES results reveal vast amount of data and it is not always straight forward to filter out the mutation responsible for the disorder. Unusal phenotypes also turns analysis stage into a difficult one. In cases where a published mutation can not be delineated variants of unknown significance (VUS) comes into the scenario. It is not always easy to report VUS and it is even harder to interperete them If you are planning to perform PGD for the familiy. The families need need to be informed about the risk of PGD applications based on VUS elimination may not related with the phenotype of the affected child.

WES results revealed three VUS in our case with each disorder having an overlapping phenotype with the index cases. We have decided to try and exclude all three VUS with the PGD since none of them could be excluded due to complexity of the pheotype. Frequent use of WES for families demanding PGD may change our approach towards PGD cases. We may have to consider performing new techniques like karyomapping enabling us to investigate multiple target regions but the more we try to exclude the less likely we will find an appropriate embryo for transfer. In these cases the families should be informed of this possibility before consenting to PGD applications.

POSTERS

Application of Next Generation Sequencing on Preimplantation Genetic Screening

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Introduction Largely because of efforts required to complete the Human Genome Project, DNA sequencing has undergone a steady transformation with still-ongoing developments of high-throughput sequencing machines for which the cost per reaction is falling drastically. Similarly, the fast-changing landscape of reproductive technologies has been improved by genetic approaches. Preimplantation genetic diagnosis and screening were established more than two decades ago for selecting genetically normal embryos to avoid inherited diseases and to give the highest potential to achieve stable pregnancies.

Study design: cross-sectional parallel evaluation, with both NGS and array-CGH techniques, of 38 blastocysts obtained from clinical PGS cycles.

Participants/materials, setting, methods: 38 PGD cycles were carried out on blastomeres after cleavage-stage. The samples were analyzed using both comparative genomic hybridization and semiconductor high-throughput sequencing (Ion torrent). Whole genome amplification (WGA) was carried out by PCR-based method for 28 samples and by MDA for 10 samples.

Main results: Comparative genomic hybridization showed that 25 samples (65.7%) had normal karyotype, 13 samples (34.3%) had different aneuploidies. Among 13 embryos with aneuploidies 4 have multiple chromosomal rearrangements. In 36 samples results of NGS were identical with aCGH, and only in 2 cases (5,2%) the aCGH results were different. This findings are due to the more strict criteria of data interpretation by aCGH in comparison with NGS. We are going to continue to work on the development of method and analysis of results of aCGH and NGS. An examples of distribution of signals received from the same sample by using two methods are shown on the pictures 1.
NGS method allows the detection of whole-chromosome aneuploidies in trophectoderm biopsies with the same efficiency as aCGH. Despite comparable results were obtained by using both amplification methods, MDA amplification gives sufficiently less noise in the final data.

Conclusion: NGS techniques are being validated and all the studies carried out conclude that it is a reliable technique that will most likely become the widely used technique for PGS in the near future.

Trial registration number:MD-6043.2015.7

Pictures 1. An examples of distribution of signals received from the same sample by using two methods.

Morphokinetics and multinucleation patterns in aneuploid and euploid human embryos

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Introduction: Prediction of good quality embryos in IVF cycles is still a challenge. Pre-implantation chromosomal instability is a complex, understudied process of aneuploidy generation affecting 50-80% of cleavage stage embryos. Current data suggest abnormal meiotic chromosome segregation in oocytes as a primary mechanism of reproductive failure, but abnormal nuclear formation during early mitotic cell divisions also plays a significant role. Multinucleation (MN) is a common nuclear abnormality observed in early human embryos and is associated with diminished potential of embryo development. Previous studies have shown that although MN is highly associated with aneuploidy, there is a proportion of MN 2-cell stage embryos, that are not aneuploid suggesting a "self-correction" event.

Time-lapse morphokinetics imaging coupled with advanced genetic technologies, provide early insight into the biology of embryo development.

Aim: To evaluate and describe the pattern of morphokinetics and MN in euploid and aneuploid embryos analyzed by aCGH–PGS.

Material and methods: Patients (n=113) undergoing IVF treatments and PGS screening were included. Trophectoderm biopsies were performed on 607 embryos at blastocyte stage (day 5-6). Comprehensive chromosome screening was carried out using aCGH, 24Sure-kit (Illumina Inc. USA) and data was analyzed with BlueFuseMulti™ software (Illumina Inc. USA).
Development and multinucleation status of embryos were annotated by EmbryoViewerTM image-software (Unisense; Fertilitech), using EMBRYOSCOPETM. Recorded morphokinetics parameters were: time of pronuclear fading (tPNf), cleavage time to 2-, 3-, 4-, and 5-cell stage, beginning of blastulation and formation of full blastocyte.

Results: From 607 blastocystes analyzed by aCGH, 49.8% were euploid, 33.8% aneuploid and 13.6% carried segmental aneuploidy. All 24 chromosomes contributed to blastocyte aneuploidy.
MN at 2-cell stage was present in 40.8% of euploid, 46.7% of aneuploid and 36.1% of segmental aneuploid embryos. There was no significant difference found between euploid and aneuploid embryos (p=0.5). Assessment of the MN level during the transition from 2- to 4-cell stage showed reduction of MN from 43.2% to 15% in the total embryo population. Monosomies were more likely to show MN than trisomies (59% vs 41%, p=0.02) with preference of chr 2, 9, 15 and 16. When non-MN aneuploid embryos were analyzed there was no difference between the frequency of monosomies and trisomies. Aneuploidies of chromosomes 21 and 22 were associated with significantly lower number of MN events ($p<0.04$ and $p<0.03$, respectively). Neither chromosomal aneuploidy was associated with significantly higher number of MN events.
Aneuploid embryos showed longer time of each cleavage event than euploid embryos ($p<0.001$, all stages). Euploid embryos with MN had longer time of each cleavage event from tPNf to t5 compared with euploid embryos without MN ($p<0.0001$). Aneuploid embryos didn't exhibit such difference.

Conclusions: Ploidy status of day-5 embryos is not associated with multinucleation at early cleavage stages. Observed high multinucleation rate reduces with transition to 4 cell stage. Aneuploid (regardless of multinucleation) and euploid embryos with multinucleation need longer time for each cleavage stage, supporting the concept of self-correction in the early stages of embryo development. Observed multinucleation pattern with specific chromosome aneuploidy needs to be further elucidated.

Is morphology embryonic on day 3 and 5 related to aneuploidy rate?
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IntroductionEmbryo morphology is checked every day in order to select the embryo with the highest implantation potential; however it is not always enough to achieve a healthy pregnancy (Fragouli et al., 2010; Magli et al., 2007; Munne et al., 2009). Different types of aneuploidy may cause miscarriages or implantation failure in spite of transfer a perfect morphology embryo. Preimplantation genetic diagnosis (PGD) allows us to select an euploid embryo. The great inconvenient is that it is an expensive technique that increases the price of the treatment and not all patients can do it. Some studies show that most of euploid embryos reach the blastocyst stage , while this is the case in only a minority of chromosomally abnormal embryos (Sandalinas et al., 2001), and other authors observed there are many aneuploidy blastocysts with the greatest capacity to produce clinical pregnancies , such as trisomy 21 (Fragouli et al.,2014). The aim of this study was to find a good morphology parameter that can decide if the embryo is aneuploidy or euploid and be used in cases where PGD is not performed.

Material & Methods: A retrospective and observational study including 1616 cycles from our CCS program of PGD conducted from August 2012 to February 2016. Over the study period, 7281 embryos were biopsied on day 3 of development and analysed through array-comparative genome hybridization (CGH). The mean age of our female population was 40 years old and the indications included were advance maternal age (75%), recurrent miscarriages (11%), implantation failure (5%), male factor (4%), inversions and translocations (3%) and others indications (2%). We study if there is a relation between

the morphology on day 3 and day 5 with the euploid embryos. Chromosomally normal embryos were transferred on day 5.
Statistical analysis was performed using the Anova test, Chi-squareand Logistic Regression to compare all the variables.

Results: Of the 7281 embryos analyzed from the PGS program, we found significant differences for all day 3 morphologies variables: number of cells on day 3 ($p < 0.001$), symmetry on day 3 ($p < 0.001$) and low fragmentation on day 3 ($p=0.362$). Nevertheless on day 5 we can not predict the chromosomal status only with the score morphology because of the best quality embryos were aneuploidy in a 53.6% versus euploid embryos, 46.4%.

Conclusion: The results of this study suggest that there are not morphological parameters at day 5 that help us to predict the chromosomal status of the embryo. So it is always to recommend performing a biopsy in order to confirm chromosomal normality.

What the patient really wants to know?
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What the patient really wants to know?
As a former patient and the chair of CHEN – patient Fertility Association I would like to challenge the view that patients must choose and decide about their medical procedure in details.
We have to keep in mind that most of the patients are not part of the medical team and have no medical education. They want to be parents. Some of them want to understand the procedure but not more than that.
The questions should be:
Should the doctor update the patients with all the medical details of the treatments and what is the line that make it too much for the patients to bear? How we will make the medical decisions and did we become a cover-up nation that informing the patients about all the difficulties and procedures?
Speaking about IVF does the patient need to know the chemical components of the medication she has to take? Does she need to know the procedure of PGD? Or it is OK for her to understand why PGD is needed in general for her problem. Does the patient need to know how PDG is being done in details?
What is the line between knowledge and fear?
Is it ethical to speak about highly expensive option when you know that the patient cannot pay for it?
I know the questions but not know all the answers. We learned that not all of them positive.

A retrospective case series of clinical application of karyomapping for couples requiring preimplantation genetic diagnosis
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Introduction: Preimplantation genetic diagnosis (PGD) is an early form of prenatal diagnosis. Although generally successful, traditional PGD strategies based upon the polymerase chain reaction (PCR) are associated with several technical and logistical limitations. These include allele dropout, long work-up times and failure to detect lethal aneuploidies. In contrast, karyomapping represents a more generic approach. The method utilises microarray technology to determine the genotype of >300,000 single nucleotide polymorphisms (SNP). The genetic status of individual embryos can be determined by analysis of linked SNP alleles inherited along with the mutant gene. This study was carried out to determine whether karyomapping can be applied in a clinical setting to couples requiring PGD for single gene disorders and/or chromosomal rearrangements.

Methods: This is a retrospective case series of PGD cycles from May 2014 to December 2015. Karyomapping was considered feasible if the DNA from a relative of known genetic status or from an affected foetus was available.

Couples self-referred or were referred from regional genetic centres. Couples were counselled and standard IVF protocols were applied. Initially, couples had day-three embryo biopsy and embryo

transfer of a suitable blastocyst. This strategy was changed in May 2014 to blastocyst biopsy, vitrification and subsequent frozen embryo replacement.
Karyomapping was carried out by Reprogenetics, UK, essentially as described by Natesan et al. (2014). Patients were advised to do urinary pregnancy test 16 days after embryo transfer.

Results: Karyomapping was applicable in 90 couples during this period of time. 76/90 (84%) couples had PGD by karyomapping. The remaining 14 couples (16%) are awaiting treatment at the time of writing.
85/90 (94%) and 3/90 (3%) couples were referred for autosomal single gene disorder and X-linked disease, respectively. The remaining 2/90 (2%) were referred for chromosomal rearrangements. Additional, PCR or array comparative genomic hybridization was carried out in order to increase diagnostic accuracy in 5/90 (6%) and 1/90 cases, respectively. A total of 85 cycles of embryo biopsies were performed. Day-3 embryo and blastocyst biopsies were performed in 16/85 (19%, 95% CI 12-28) and 69/85 (81%, 95% CI 72-88) cycles, respectively.
A total of 72 embryo transfers were performed. 60/72 (83%, 95% CI 73-90%) and 12/72 (17%, 95% CI 10-27) were frozen and fresh embryo transfers, respectively. From 63 embryo transfers with a known pregnancy outcome, 42 (67%, 95% CI 54-77) had a positive pregnancy test. The ongoing pregnancy rate is 37/63 (59%, 95% CI 46-70). 2/63 (3%, 95% CI 1-11) and 3/63 (5%, 95% CI 2-13) embryo transfers resulted in biochemical pregnancies and first trimester miscarriages, respectively. 21/63 (33%%, 95% CI 23-46) had a negative pregnancy test. In 9/72 (13%, 95% CI 7-22) embryo transfers, the outcome is pending.

Conclusions: Karyomapping is reliable, accurate and efficient for couples requiring PGD for single gene disorders and/or chromosomal rearrangements. Its utilisation of a generic protocol dramatically accelerates test work-up compared to conventional PCR, helping to expedite the start of treatment. Additionally, it provides aneuploidy screening, minimising risks of miscarriage and implantation failure.

Reference:
Natesan et al. 2014 *Genet Med*doi: 10.1038/gim.2014.45

Preimplantation Genetic Diagnosis associated to Duchenne Muscular Dystrophy
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Introduction: Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disease (Xp21.2) which occurs due to changes in the gene encoding dystrophin. The deletions and duplications of one or more exons of the gene are responsible for 65% of pathogenic mutations, and the remaining cases are attributed to point mutations. Most of the mutations cause progressive and irreversible degeneration of muscles. The first symptom, usually weakness in the pelvic girdle muscles, arises in the first five years of life and disease leads to death around the 2nd decade from heart or respiratory compromise.It affects 1:3,000 boys.

Material and Methods: EPR, 38y, symptomatic patient heterozygous for a 2 to 47 exon mutation in *DMD* gene and GTS, 39y, sought genetic counseling service of the Institute Ideia Fertil, Human Reproduction and Genetics Center of Faculdade de Medicina do ABC/Brazil in February 2014 to know about the Preimplantation Genetic Diagnosis (PGD) process. They had a 6 year old son who died in 2012 of the disease complications. The couple undergone four cycles of ICSI using 200 UI FSHr protocol for controlled ovarian hyperstimulation. Eight D5/D6 embryos biopsies were analyzed by PCR for specific mutation analysis, followed by CGH array for aneuploidy analysis.

Results: PGD disclosed that two embryos had inherited the maternal *DMD* mutation, one embryo had a chromosomal alteration [47,XY,del(8)(q24.11-qter),+18] and five embryos (3 males and two females) were normal. One blastocyst was transferred and resulted in a successful pregnancy. The child was a female, born after 38 gestational weeks by cesarean section, with 2970 g of weight and 43 cm height, uneventfully. The other embryos remain vitrified.

Conclusions: Embryo analysis using the associated techniques ofPCR and CGH array showed to be safe for embryo selection in cases of X-linked disorders, as Duchenne Muscular Dystrophy.

Oocyte accumulation by vitrification increases the number of viable euploid embryos. PGD-A using NGS

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Introduction: Preimplantation genetic diagnosis for aneuploidy (PGD-A) refers on chromosomalprofiling of embryosprior to implantation with the aim to transfer *in uterus* only euploid embryos.
Oocyte vitrification preserves oocyte integrity by exposure to very low temperatures thus arresting biological activity until warming for clinical use. Fertilization and pregnancy rates after oocyte vitrification are similar to results from fresh oocytes in ICSI treatments. In the present study, we considered the hypothesis to accumulated vitrified oocytes in a view to increase the number of blastocyst to diagnose for patients candidates for PGD-A to increase the number of euploid blastocyst to transfer.

Material and methods: Fifty-one patients with a mean age of age 36.8 years and a mean basal FSH of 6.8 IU/l underwent PGD-A for repeated implantation failure or recurrent miscarriage. They were proposed to undergo several cycles of ovarian stimulation. In the first cycles, 468 metaphase II oocytes were vitrified and consequently accumulated. In the last cycle, the freshly 342 produced matured oocytes and the previously accumulated ones were micro-injected with the same partner's semen sample. PGD-A was performed on 101 blastocysts from vitrified/warmed oocytes and 100 blastocyst from fresh oocytes. The chromosomal analysis of biopsied trophectoderm cells was done using next generation sequencing strategy.

Results: The aneuploidy rates were comparable between blastocyst obtained from vitrified/warmed oocytes (57.4%) and those obtained from fresh oocytes (64.0%, p>0.05). A mean of 10.2% (35/342) euploid blastocyst per micro-injected fresh oocyte and 9.2% (43/468) euploid blastocyst per micro-injected vitrified/warmed oocytes were obtained ($p<0.05$).

Conclusion:Oocyte vitrification and warming do not generate aneuploidy in blastocyst. Euploid blastocysts generated from vitrified oocytes have an implantation potential comparable to euploid blastocyst from fresh oocytes. Oocyte accumulation by vitrification is a valid strategy to increase the number of viable euploid embryos to transfer after PGD-A and the chances to obtain a pregnancy.

Fragile X retraction on the CGG repeats length was observed on cleavage stage embryos

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Introduction: Fragile X syndrome is a common form of X-linked mental retardation caused by expansion of an unstable CGG trinucleotide repeat in the 5'UTR of the *FMR1* gene; unaffected or normal alleles (<45 CGG), intermediate (45-54 CGG), premutation 55-200 CGG) and full mutation (>200 CGG). Female premutation carriers are at risk of having offsprings with further CGG expansion, which may result in a full mutation. Preimplantation genetic diagnosis (PGD) on Fragile X syndrome has been successfully performed. We reported here a case of PGD on Fragile X syndrome by linkage analysis and sex determination by amplification of *sry* and amelogenin.

Material & methods:A premutation carrier of 97+/-3 CGG repeats underwent IVF-PGD. One blastomere was removed from each embryo on day 3, followed by whole genome amplification (WGA). PGD was performed by linkage analysis with 9 microsatellite markers flanking *FMR1* gene, together with sex determination by amplification of *sry* and amelogenin. CGG repeat length was determined retrospectively on archived WGA products of blastomere biopsy by AmplideX FMR1 PCR kit (Asuragen).

Results: Blastomere biopsy was performed on 12 embryos, 9 embryos with conclusive results (2 normal, 7 carriers), 2 embryos with inconclusive result due to suspected sex chromosome aneuploidies and there was no result on 1 embryo due to failure in whole genome amplification. One normal female blastocyst was replaced on day 5 (3BB) and one good quality blastocyst was vitrified on day 5. She got pregnant and prenatal testing showed that the fetus had a normal karyotype of 46,XX with no expansion

on *FMR1* gene. Unfortunately she miscarried at 19 weeks of gestation. CGG repeats length was determined retrospectively on 11 WGA samples, 3 samples showed retraction in the CGG repeats number of maternal allele from 97+/-3 CGG to 53 CGG, 36 CGG and 63 CGG respectively. Previous test on WGA product of single premutation carrier lymphocyte show concordant result on CGG repeats number determination, indicating that CGG repeats number remains unchanged after WGA. This is the first report on the retraction of CGG repeat on cleavage stage embryos in PGD.

Conclusions: Our result showed that retraction in the repeat length was possible when premutation is inherited to the next generation. Further research is needed to confirm whether this retraction is mosaic in cleavage stage embryos. As premature ovarian insufficiency is common in fragile X permutation carriers, one may consider to counsel the patient to replace the embryo with retracted CGG repeats, in case no normal embryo is available after PGD.

Importance of genetic diagnosis preimplantation: case report of heterozygous couple for cystic fibrosis

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Introduction: PancreaticCystic fibrosis, or as commonly designated cystic fibrosis is a genetic disease caused by mutations on *CFTR* gene. The disease affects about 70,000 people worldwide and presents an autosomal recessive inheritance. Manifestations relate not only to the disruption of exocrine function of the pancreas but also to intestinal glands (meconium ileus), biliary tree (biliary cirrhosis), bronchial glands (chronic bronchopulmonary infection with emphysema), and sweat glands (high sweat electrolyte with depletion in a hot environment). Infertility occurs in males and females. In Brazil less than half of patients performed genetic analysis, and heterozygous incidence is not well known. However, the growing case of records in the last years demonstrates increased chances of heterozygous weddings. Although heterozygous patients usually have no symptoms they have a 25% risk of having children with cystic fibrosis. In this work we report a non-consanguineous heterozygous young couple (CFTR p.Ile507del) that already had lost two children with Cystic Fibrosis after ileostomy, one with 6 months old and the other with 18 days old. They electively decided for an ART in order to perform embryo selection.

Material & methods: She underwent standard assisted reproduction procedures and had 12 days of ovarian stimulation with recombinant human FSH (rhFSH). As a result, 12 oocytes were retrieved, 9 MII. Mature oocytes were submitted to ICSI. Embryo biopsy of 6 embryos occurred on the 5th and 6th day post fertilization (blastocyst stage). Polymerase chain reaction (PCR) was performed to screen embryo DNA for *CFTR* mutation.

Results: After PCR the following result was obtained: 1 normal homozygous embryo; 4 heterozygous and one embryo got no PCR result. The couple decided not screen for chromosomal aberration. Endometrial preparation for transfer was difficult and she went to 3 preparation cycles. The normal embryo was transferred but resulted in no gestation. New endometrial preparation will be performed for one heterozygous embryo transference.

Conclusions: With the evolution of assisted reproduction techniques, especially the PGD techniques, couples or individuals with a history of genetic disorders can be really benefited. The use of PGD was efficient for cystic fibrosis detection in embryos and the positive experience serves as an incentive for more frequent use of PGD technique in cases of familial genetic diseases screening, as it prevents great suffering to the pair.

Keywords: Preimplantation Genetic Diagnosis, cystic fibrosis, rare disease embryo selection.

The transition from blastomere to trophectoderm biopsy. Comparing two PGS strategies.

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Introduction: Until recently, most biopsy procedures for Preimplantation Genetic Diagnosis (PGD) were performed on day 3 embryos through the analysis of a single cell. Nowadays, improvements in ART laboratories have made trophectoderm (TE) biopsy a real option to implement in PGD treatments. TE biopsy has been presented as the alternative to overcome the limitations derived from single cell analysis. Having more initial DNA for the study as a result of having more than one cell (3 to 8 cells) should improve the reliability of results, and increase the percentage of embryos with conclusive diagnosis after biopsy. Moreover, TE biopsy has been suggested to be less harmful as a smaller proportion of the embryo is biopsied when compared to cleavage stage biopsy and TE cells are not be part of the developing embryo itself. Taking all this into consideration an improvement in clinical outcomes should be expected.
The aim of this study was to compare the results obtained in our PGS programme by two different approaches: blastomere biopsy and TE biopsy.

Materials and methods: PGS cycles with own gametes performed in a private IVF centre in Barcelona between January 2014 and December 2015 were included. Single blastomere biopsy was performed on day 3 embryos. For trophectoderm biopsy zona opening was performed on day 3 embryos and those developing to blastocyst stage between day 5 and day 7 were biopsied and vitrified post-biopsy. In both cases analysis was performed using array-CGH. Fresh euploid blastocyst transfer was carried out for embryos biopsied on day 3 while a frozen-thawed euploid embryo transfer policy was followed for embryos biopsied at the blastocyst stage.
The results and clinical outcomes were compared between blastomere biopsy and TE biopsy group.

Results: A total of 183 cycles were included in blastomere biopsy group and 173 in trophectoderm biopsy group. Mean maternal age (38.6±3.6 vs 38.2±4.0), number of MII oocytes (13.1±4.5 vs 13.7±5.5) and fertilisation rates (72.4% vs 73.1%) showed no significant differences between groups. The mean number of embryos biopsied was higher in blastomere biopsy group (8.3±3.4 vs 4.9±3.0). A higher percentage of embryos with conclusive results after biopsy was observed in TE biopsy group (89.2% vs 95.9%). The aneuploidy rate was higher in blastomere biopsy group (82.1% vs 63.5%) as well as its complexity (51% vs 13.6% of complex aneuploidies). The mean number of euploid embryos transferred was lower in TE biopsy group (1.4±0.5 vs 1.2±0.4). Clinical pregnancy rates per transfer (56.1% vs 60.2%), implantation rates (50.3% vs 56.8%), and miscarriage rates (10.0% vs 10.4%) were similar between groups.

Conclusions: Although the trophectoderm biopsy strategy with frozen-thawed embryo transfer offers multiple advantages including lower cost and better laboratory organisation, PGS with blastomere biopsy should still be considered as a valid alternative for centres that cannotassume the transition to TE biopsy.

Quantification of mitochondrial DNA from blastocysts as a strategy to improve identification of viable embryos

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Introduction: Mitochondrial DNA (mtDNA) is a high copy-number, maternally inherited genome that codes for a small number of essential proteins involved in cellular metabolism and the generation of energy. mtDNA content in oocytes has been positively correlates with both fertilization and embryo viability, suggesting that it could be used as a potential biomarker. However, little is known about the contribution of changes in the number of mtDNA genomes (i.e. the 'mtDNA copy number') in a blastocyst, to human embryos developmental potential. Here, we survey mtDNA copy number variation across chromosomally distinct human embryos and embryos with different clinical outcomes after transfer into maternal utero.

Material & methods: This study investigated the mtDNA, and nuclear DNA copy number variation in 231 blastocysts obtained from previously performed preimplantation genetic screening (PGS) cycles. These included 102 euploid embryos, 72 aneuploid, 26 mosaic and 31 degenerated embryos. mtDNA copy number of euploid and mosaic embryos was correlated with clinical outcomes (implantation, miscarriage, and live birth) obtained after embryo transfer. All embryos were cultured to blastocyst stage. Mitochondrial and chromosomal DNA copy number variation were examined simultaneously with next generation sequencing (NGS) methodology. Analysis was accomplished with BlueFuse Multi software. mtDNA copy numbers was based on the observed ratios of sequence coverages between mtDNA and nuclear DNA.

Results: The analysis of the relative amount of mitochondrial DNA revealed that degenerated embryos had the highest average value of mtDNA/nDNA; the aneuploid embryos had an mtDNA average amount 4 times lower than the degenerate did and 2 times higher than that of the euploid embryos; the mosaic embryos had similar values to those of euploid embryos. Assessment of clinical outcomes after transfer of 62 euploid and 19 mosaic embryos to the uterus revealed that blastocysts that successfully implanted and resulted in baby born, contained significantly ($p<0.001$) lower mtDNA quantities compared with those that failed to implant or resulted in biochemical pregnancies.

Conclusions: The results of this study suggest that increased mtDNA may be related to reduced viability and embryo degeneration. The correlation between mtDNA copy number and clinical outcomes obtained after transfer of euploid or mosaic embryos strongly suggest that mtDNA quantification has the potential to provide clinically relevant information additional to that provided by aneuploidy testing, representing a valuable biomarker of embryo viability.

Single-cell uniform genomic amplification (SUGA): a new approach for comprehensive PGD

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Introduction: Whole genome amplification (WGA) is a standard method used to generate sufficient embryonic DNA from biopsy samples for downstream PGD. In general, current commercial WGA kits use PCR or MDA amplification methods can provide a suitable DNA template for PCR, array or NGS based genetic analyses. However, genome coverage is biased and ADO rates are variable across the genome, prohibiting high-resolution genetic analysis of embryos. The aim of the study was to design and validate a novel WGA method that provides more extensive and uniform genome coverage for comprehensive chromosomal and single gene analysis.

Methods: The new method developed called Single-cell Uniform Genomic Amplification (SUGA) was based on proprietary technology for random amplification of the genome. The performance of SUGA for chromosomal analysis was evaluated by NGS using single cell and five-cell samples with known chromosomal abnormalities. Single cell and 5-cell ADO rates were assessed by comparing the call rate of genome-wide heterozygous loci on SNP arrays against the corresponding genomic DNA sample.

Results: Reliable amplification was achieved for the majority of single cells tested, with a success rate of 95%. By density plots of the mapped sequencing reads, consistent uniform coverage of the genome was demonstrated across all 24 chromosomes. Chromosomal analysis of SUGA products from single cell samples correctly identified known aneuploidies as well as segmental imbalances as small as 2-3 Mb with high sensitivity, specificity and reproducibility. In addition, expected deletion and duplication intervals were precisely defined. SNP array analysis of single cell and five cell samples demonstrated a call rate for genome-wide heterozygous loci of > 80% and > 95%, respectively. Further, the intra-and intragenic SNP patterns of genes commonly tested in single gene PGD cases derived from single cell and 5-cell samples were highly concordant with the known genomic DNA SNP pattern, with only occasional ADO when the starting template was five cells.

Conclusions: At the single and five-cell level, mimicking a blastomere or blastocyst biopsy sample, SUGA provided uniform and representative genome-wide coverage and ADO rates were low. Based on initial validation data, SUGA has the potential to improve the resolution and accuracy of all available molecular assays currently used for PGD of chromosomal and single gene diseases.

Next generation sequencing for the detection of reciprocal translocations

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Introduction: Translocation carriers are at risk of producing unbalanced gametes. This can result in an increased risk of infertility, miscarriage or the birth of chromosomally unbalanced offspring. Preimplantation Genetic Diagnosis (PGD) provides the opportunity to distinguish between normal/balanced and unbalanced embryos and can increase the chance of a successful ongoing pregnancy. The rapid progression of new technologies such as Next Generation Sequencing (NGS) for comprehensive chromosome screening has contributed significantly to providing faster and more accurate PGD diagnoses.

The aim of this study was to perform a proof-of-principle study to determine the reliability and accuracy of NGS when used for PGD in embryos from reciprocal translocation carriers.

Methods/Materials: Embryos were biopsied at the blastocyst stage on day 5 or 6 of development and vitrified post-biopsy pending PGD results. Biopsy samples were amplified using SurePlex whole genome amplification (Illumina, USA) and subjected to array Comparative Genomic Hybridisation (aCGH) using the 24Sure+ platform (Illumina). Following diagnosis, embryos diagnosed as unbalanced were re-assessed using the 'Veriseq' NGS comprehensive chromosome screening platform (Illumina) to determine concordance of results. Data obtained was analyzed using Bluefuse Multi analysis software (Illumina).

Results: 65 known unbalanced embryos from 26 reciprocal translocation couples were re-examined using NGS technology. All 65 embryos gave a conclusive NGS result. In 65/65 (100%) cases, the PGD result obtained using NGS was concordant with the initial result obtained using aCGH. A total of 130 distinct chromosome breakpoints were examined, 128 (98.4%) of which were clearly detected using NGS. The remaining two chromosome breakpoints were not detected using either aCGH or NGS technology. Of note, 33/128 (25.7%) of the segmental aneuploidies detected were below the 20Mb recommended detection capacity for NGS, with the smallest segment being just 3.5Mb (4 bins). 20 out of the 22 autosomal chromosomes were represented in this data set (there were no patients with translocations involving chromosomes 8 or 19). Adjacent-I, Adjacent-II and 3:1 segregation patterns were all observed.

Conclusion: This data suggests that NGS can be used as a reliable and accurate method for the detection of unbalanced embryos from reciprocal translocation carriers, providing a more efficient and cost effective alternative to aCGH. Each translocation should be assessed on a case-by-case basis prior to proceeding with PGD.

Trophectoderm biopsy and transfer in a frozen embryo replacement cycle in preimplantation genetic diagnosis cycles

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Introduction: Traditionally, embryo biopsies for PGD cycles have been performed at cleavage stage with fresh transfer of a suitable blastocyst. ESHRE PGD Consortium collection I-XII data showed 92% and 0.7% of embryo biopsies were at cleavage and blastocyst stage, respectively. Blastocyst biopsy compared with cleavage stage biopsy has been reported to have higher rate of genotyping, a lower rate of amplification failure, less allele drop out and fewer embryos to biopsy. It is also associated with higher implantation and pregnancy rates than cleavage stage biopsy. Furthermore, FER can prevent late onset ovarian hyperstimulation syndrome (OHSS), is associated with higher ongoing and clinical pregnancy rates and improved perinatal outcomes.

The study was carried out to determine pregnancy outcome of trophectoderm biopsy and transfer in a subsequent frozen embryo replacement (FER) cycle in couples undergoing preimplantation genetic diagnosis (PGD).

Methods: Couples were either referred from a regional genetic centre, or self-referred. Couples were counselled and standard IVF protocols were applied. Embryos were cultured to blastocyst and biopsied.

The cells were sent to the reference laboratory for genotyping. Standard procedures for thaw of embryos were performed and blastocysts transferred in a subsequent medicated FER cycle. Patients were advised to do their urinary pregnancy test 16 days later.

Results: This is a retrospective case series of PGD cycles performed from July 2014 to December 2015. 85 cycles of trophoectoderm biopsy and vitrification were performed. 72/85 (85%) and 13/85 (15%) cycles were for single gene disorders and chromosomal rearrangements, respectively. A total of 398 blastocyts were biopsied. 182/398 (48%) blastocysts were suitable for transfer. One blastocyst required repeat thaw and biopsy, due to initial amplification failure, which successfully resulted in a suitable embryo to transfer. None of the blastocysts failed to survive the thaw process on the day of FER. All couples had elective single embryo transfer.
Of 54 PGD cycles for single gene disorder with known pregnancy outcome, 42 (78%, 95% CI 65-87) FER cycles resulted in a positive pregnancy test. The ongoing pregnancy rate is 38/54 (70%, 95% CI 57-81). 2/54 (4%, 95% CI 1-13) FER cycles were biochemical pregnancies and 2/54 (4%, 95% CI 1-13) resulted in first trimester miscarriage. 12/54 (22%, 95% CI 13-35) FER cycles had a negative pregnancy test.
Of 12 PGD cycle for chromosomal rearrangements with known pregnancy outcome, 9 (75%, 95% CI 47-91) FER cycles resulted in a positive pregnancy test. The ongoing pregnancy rate is 7/12 (58%, 95% CI 32-81). 2/12 (17%, 95% CI 5-45) such cycles resulted in first trimester miscarriage.

Conclusions: Trophectoderm biopsy, vitrification and FER can be successfully applied to PGD cycles for single gene disorders and chromosomal rearrangements. This approach increases the rate of genotyping and number of suitable embryos to transfer. Moreover, implantation and pregnancy rates exceed those following cleavage stage biopsy. However, the results from this data set are retrospective and non-randomized.

Application of NGS for aneuploidy screening in embryos after IVF

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Introduction: Today the majority of modern clinics worldwide consider that PGS (preimplantation genetic screening) became an essential part of IVF (in vitro fertilization) program. The possibility of embryo analysis outside the mother organism made IVF with PGS an attractive procedure for fertile couples with high risk of child with genetic disorders birth. Many articles prove that including of PGS in IVF program provides a several fold increase in efficacy of ART (artificial reproductive technologies). It is important to note that efficacy rate depends on quantity of analyzed chromosomes and method of analysis.

Materials and methods:"Mother and child" group of companies (29 clinics) began to use NGS (next generation sequencing) for PGS from January 2016. The study included 69 women from 22 to 48 years old, mean 40±6,05 years. 168 embryos were analyzed.

Results: Different pathological abnormalities were found in 105 of 168 embryos (62,5%). In 54 of 105 cases (51,4%) there was one abnormal chromosome (monosomy, trisomy, mosaic, deletion, duplication) per embryo. Two abnormal chromosomes per embryo and combined pathology (including multiple aneuploidy) were observed in 23 (21,9%) and 28 (26,7%) of 105 cases. Pathology of chromosomes 21 and 22 was seen more often. In 24 of 168 cases (14,8%) the 21 chromosome pathology has been detected; and in 23 of 168 cases (14,2%) the abnormal 22 chromosome has been seen. The most common form of aneuploidy of chromosome 21 was trisomy. Monosomy was the most common form of aneuploidy of chromosome 22.

Conclusion: Results of this study again prove that ART with PGS allows choosing embryos with normal karyotype what results in pregnancies with healthy babies birth.

The analysis of relationship between chromosomal mosaicism in trophectoderm and abnormal sperm morphology rates

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Introduction. It is well known thatsperm morphology link to a higher incidence of embryo aneuploidy. But less is said of spermatozoal morphological characteristics' impact on the occurrence of chromosomal mosaicism in the blastocyst despite the fact that the violation of the centrosomal structure leads to the appearance of mosaicism and is most likely of paternal origin.

Material and Methods. From March 2014 to January 2016, the trophectoderm of 154 blastocysts **gotten from** 59 fresh and frozen IVF+PGS cycles were analyzed in IGR Medical centre. Prior to ICSI, patient's semenal parameters were evaluated in accordance with the WHO laboratory manual forexamination and processing of human semen 2010 namely; concentration, motility and proportion of morphologically abnormal sperm. On the post-fertilization day five, blastocyst biopsy was performed, trophectoderm samples were fixed and diagnosed using FISH on chromosomes 13, 16, 18, 21, 22, X, Y. Statistical analysis had been carried out using Shapiro-Wilk test for normality and Spearman correlation.

Results. The study was conducted based on the patient's sperm characteristics such as concentration (the range 2 - 450 mln/ml), motility (the range 3 – 80%), the number of morphologically abnormal spermatozoa (the range 32 - 90%) and estimation of their effect to the embryos' chromosomal set such as euploid (60 embryos or 39,0%), aneuploid (47 embryos or 30.5%) and mosaic (47 embryos or 30.5%). A positive correlation between the percentage of morphologically abnormal sperm and the proportion of mosaic blastocyst ($r = 0,77$, $p < 0,001$) was found. An inverse relationship was shown between the percentage of morphologically abnormal sperm and the proportion of euploid blastocysts ($r = - 0,60$, $p < 0,001$).That may be associated with violation of the division spindle structures formation from morphologically abnormal spermatozoa after fertilization of the oocyte. There was a trend relationship between the amount of motile spermatozoa and proportion of euploid blastocysts ($r = - 0,22$, $p < 0.09$). The analysis of other sperm parameters of patients and their embryonic parameters detected no correlation between signs. In the same time analysis revealed that there was a statistically significant positive relationship between sperm concentration and the amount of motile spermatozoa ($r= 0,33$, $p< 0,01$). This indicates a pleiotropic effect of the factors determining semenal parameters and as a consequence the embryonic parameters.

Conclusions. The greatest influence on the embryos' chromosomal set was shown by qualitative semenal parameters. Therefore, evaluation of the ejaculate parameters is important not only for the clinical management of patients, but also to predict the genetic and morphological quality of the embryos on ART programs.

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Combined PGD/PGS using whole genome sequencing in a model of blastomere and trophectoderm biopsy

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Introduction: Whole genome sequencing (WGS) has the potential to provide combined preimplantation genetic diagnosis and screening (PGD/PGS). In addition, WGS can diagnose de novo mutations which would be undetectable by other means of PGD. The limited genomic material available and requirement for whole genome amplification (WGA) has led to concern regarding allele drop out in genes of interest and the introduction of false positive genotype calls in de novo mutation analysis. This study involved

WGS of single cells and multiple cells from cell lines with known abnormalities as a model of blastomere and trophectoderm biopsy.

Material and Methods: Two cell lines were obtained from Coriell Cell Repository (euploid *BRCA1* positive cell line (GM14090B) and *BRCA1* negative, aneuploid cell line 48,XY,+16,+21 (GM03576)). Genomic DNA was extracted and single cell and multiple cell samples were manually sorted from each cell line. Cell samples underwent WGA using Reproductive Health Science's (RHS) DOP-PCR as per manufacturer's instructions (EmbryoCellect^{TM}). Purified WGA products and 300ng of unamplified genomic DNA were sequenced on the Illumina X Ten using the standard Nextera workflow. The reads were aligned to the reference human genome using a custom bioinformatics workflow through DNA Nexus. *BRCA1* was analysed for intronic and exonic variants when compared to the reference sequence using the Integrated Genomics Viewer (IGV, Broad Institute) and their presence or absence confirmed in the WGA products. Exonic regions of *BRCA1* were analysed to determine the incidence of false positive variants introduced during the WGA, library preparation and sequencing protocols in the amplified cellular and genomic DNA samples.

Results: Sequencing generated an average 116260 Mbp at median coverage 22.07x. Aneuploidy was readily detectable using a depth of coverage approach. Analysis of *BRCA1* in genomic DNA samples identified 167 sequence variants in the *BRCA1*-positive cell line and 9 sequence variants in the *BRCA1*-negative cell line. Single cell sample reads covered 36/167 (21.5%) and 1/9 (11.1%) of these variants, whereas multiple cell samples covered 111/167 (66.5%) and 3/9 (33.3%) of the same variants. Average coverage of the variants identified was 28.6x and 19x for single cell sample variants and 12.4x and 9.7x for multiple cell sample variants. The pathogenic variant (2bp deletion 185delAG in exon 3) was directly sequenced in the 5 cell *BRCA1*-positive sample (5/11 reads, confirming that the deletion was heterozygous) but was not sequenced in the single cell sample. However, analysis of sequence variants in close proximity of the 2bp deletion was possible. Analysis of *BRCA1* exonic regions identified an average of 2.75 false positive genotype calls per sample.

Conclusions: WGS has the potential to provide combined PGD/PGS by direct sequence analysis and using surrounding informative markers. Direct detection of a pathogenic mutation was demonstrated in the *BRCA1*-positive multiple cell sample. False positive variants may limit the use of WGS for detection of de novo mutations. Use of parental genomic data may ensure that false positives do not preclude embryo transfer.

Mitochondria quantification in human IVF embryos using Next-generation sequencing

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Introduction: The successful development of the human embryo depends on numerous factors. Correct cell divisions, low level of blastomere fragmentation and the absence of aneuploidy are the main prerequisites for embryo implantation and viable foetus formation. Nonetheless, the quality of the oocyte's cytoplasm and energy levels in preimplantation embryos seems to be important especially in the first week of embryo development. With respect to the high energy demands of developing embryos, the role of mitochondria as ATP synthesis factories is essential. Thus quantification of mitochondria based on the amount of mitochondrial DNA (mtDNA) in developing embryos might be of significance in human IVF programme.

Material & methods: We have quantified mtDNA in 175 blastocysts obtained from couples undergoing PGS programme in our centre for assisted reproduction. Trophectoderm biopsy underwent Whole Genome Amplification (PicoPlex, Rubicon Genomics). Subsequently, the amplified DNA was diluted, quantified and dual index sequencing libraries were prepared using VeriSeq PGS Kit (Illumina). The final purified library was sequenced on the MiSeq sequencing system (Illumina). A quantification of mtDNA using NGS involved a determination of fraction of mtDNA sequence reads related to the total number of reads (analysed by BAMStats and BlueFuse Multi, Illumina). An unpaired two-tailed *t*-test was used to statistically analyse the data.

Results: First, we quantified mtDNA in 46 euploid (mean age 34.8 ± 3.8, range 27-41) and 44 aneuploid (mean age 35.3 ± 4.1, range 27-42) blastocysts obtained from 43 couples. We observed a significant increase in mtDNA in a group of aneuploid embryos (P = 0.007). The embryos with more than two

chromosome abnormalities clearly showed much higher mtDNA levels. Further, we analysed mtDNA in 42 blastocysts (mean age 32.5 ± 4.0, range 21-39) producing clinical pregnancies compared to 34 blastocysts (mean age 32.1 ± 4.2, range 24-38) failing to give pregnancy. Both groups were highly comparable; the difference was without statistical significance (P = 0.4). Finally, we measured mtDNA content in 9 euploid blastocysts (mean age 35.6, range 30-40) which gave clinical pregnancy but the embryo was miscarried later. The quantity of mtDNA was lower compared to the control group of viable pregnancies, however, it was inconclusive due to a low number of samples in the tested group.

Conclusions: Our findings are concordant with several studies (Fragouli *et al.* 2015; Diez-Juan *et al.* 2015) showing increased mtDNA levels in aneuploid embryos. This indicates that aneuploid embryos display higher energy demands to cope with their aneuploid status. In contrast, we did not observe statistical significance between embryos producing viable pregnancies vs. embryos failing to achieve this. However, in our study the average age of women was considerably lower.

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Mechanical vs. laser-assisted trophectodem biopsy, depending on blastocyst stage

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INTRODUCTION: Laser assisted trophectoderm biopsy (TEB) aimed at obtaining specimen for PGD may appear quite traumatic procedure. The major potential adverse effect includes the possibility of damaging an excessive number of trophectoderm (TE) cells. In addition, the obtained biopsied material, and the resulting blastocyst edge become sticky, leading to an increased chance of a loss during the sample transfer into the testing tube. Accordingly, the resulting nuclei fragments of the damaged TE cells may interfere with correct interpretation of embryo genetic status, and may contribute to a fraction of mosaicism reported in preimplantation 24-aneuploidy testing (24-AT) performed on blastocysd biopsy samples, which currently represents the major problem of 24-AT at the present time. So the objective of this report is to investigate the usefulness of alternative non-laser assisted techniques of TEB.

MATERIALS AND METHODS: The force of surface tension on the boundary of culture media of biopsy drop and mineral oil was used as substitution to laser pulse, and was applied only on grade 2 to 2-3 blastocysts, as the connection (tight junction) between TE cells of early blastocyst graded 2 and 2-3 is not as strong as cell connection in more advanced blastocyst graded 3-4, 4 and higher. The grade 2 and 2-3 blastocyst are placed into 5 mkl equilibrated culture media drops covered by 2ml of equilibrated mineral oil, held by holding pipette on the left side, while TEB pipette (ID 25 mkm) on the right side is used to suck into 3-5 cells of the extruded TE. Embryo is then moved on to the right edge of the biopsy drop with the biopsy pipette, moving slowly toward the inside of the oil environment, making cytoplasm bridge between blastocyst and sample thinner, until finally removed and placed into a separate 5 mkl culture media drop in the same dish. The usefulness of the method was evaluated by amplification efficiency of the biopsied samples and results of 24-AT.

RESULTS:A total of 55 early day 5 mechanical blastocyst biopsies were performed, with the results of amplification efficiency and PGD for single gene disorders and 24-chromosome aneuploidy testing compared to the those results of 87 cases of the regular laser-assisted Day 5 – Day 6 biopsies. Significant differences were observed in the amplification efficiency, from 84% to 96.4% (73 of 87 in regular, to 53 of 55 in early day 5 biopsies), with the trend of the euploidy rate increase from 41.1% to 43,4% (30 of 73 in regular, to 23 of 53 in early day 5), but requiring more data for conclusions, as well as the need for further follow up data to analyze the possible impact on the mosaicism rates.

CONCLUSION: The described biopsy procedure resulted in obtaining a non-sticky TE samples with no evidence of damaged nuclei, avoiding number of the damaged blastocyst TE cells, and decreasing percent of non-amplified or debatable samples, which may related to the prevalence of mosaicism.

Combined PGD and PGS: Enrichment of PGD genes during whole genome amplification

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Introduction: Microarray-based comparative genomic hybridisation (aCGH) presents an opportunity to determine the genetic composition of uncharacterised human genomes. The resolution and specific characteristics of the microarray dictate the genetic information that can be derived. Some arrays are able to detect copy number variations, microdeletions and microduplications. In the preimplantation setting, the disadvantage in using high resolution arrays is that they frequently detect genetic alterations of unknown clinical consequence. Reproductive Health Science Ltd (RHS) has developed a proprietary low resolution array specifically for the detection of whole chromosomal aneuploidy. In parallel with the array, RHS has developed a single cell whole genome amplification (WGA) method to provide sufficient DNA for analysis. The ability to screen PGD embryos for aneuploidy is technically challenging as the method used to amplify limited starting material is usually optimised to the downstream platform. However, by multiplexing WGA primers and gene specific PCR primers, a method has been developed for aneuploidy detection by aCGH and also for single gene disorder diagnosis and evaluation using higher resolution molecular tests. The aim of this study was to determine the suitability of PGD for monogenic disorders during aCGH screening for aneuploidy utilizing synchronous whole genome amplification and gene specific amplification by PCR.

Material and methods: Single cells sorted from aneuploid cell lines (Coriell Institute for Medical Research, USA) were subjected to cell lysis and WGA with the inclusion of sequence specific PCR primers in the WGA PCR reaction. Single cell controls were amplified in the absence of additional sequence specific PCR primers. To determine the chromosomal complement of the test cell, an aliquot of the WGA DNA was labelled with Cy3-like fluorescent dye, then mixed with WGA amplified male reference gDNA that had been labelled with a Cy5-like fluorescent dye, and competitively hybridized to the RHS microarray. The arrays were scanned using a GenePix 4000B microarray reader (Axon Instruments, USA) and analyzed using GenePix 6.0 software (Axon Instruments). The ratio of test to reference dye intensity after normalization was used to determine the ploidy status of each chromosome. Enrichment of the specific target sequence during the WGA was determined using semi-quantitative gene specific PCRs and agarose gel electrophoresis.

Results: Correct aneuploidy diagnoses were achieved from single cells carrying either trisomy 15 and 21. In addition, correct diagnoses from cell lines with abnormal sex chromosome numbers such as XXY were obtained. Semi-quantitative gene specific PCR and agarose gel electrophoresis indicated enrichment of the target sequence in comparison to the control single cell WGA DNA.

Conclusions: The RHS arrays have the capacity to reliably and accurately detect whole chromosomal abnormalities of clinical significance in single cells. Combined PGS and PGD provides an attractive new technology for improving PGD outcomes for patients following enrichment of single and/or multiple gene sequences for further evaluation.

Analysis of PGS results based on day of biopsy and stage of blastulation

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Introduction: Previous authors have compared euploidy rates and outcomes between day 5 (D5) and day 6 (D6) blastocysts. Some studies have shown that D6 embryos have a lower incidence of euploidy, although euploid D6 embryos give positive outcomes when used in treatment. Others concluded that there was no difference in euploidy rates or outcomes between D5 and D6. The time of the start of blastulation has previously been shown to be correlated with ploidy. This study investigates whether ploidy is correlated with blastocyst expansion.

Materials and Methods: Embryos for biopsy and PGS were scored for expansion according to the Gardner Embryo Grading scheme, stages 1 – 6 with increasing expansion through to hatched blastocysts.
PGS testing was carried out on 164 biopsied trophectoderm cells of embryos from 46 patients (average age 38.9yrs). The biopsied cells from April 2011 – June 2015 were tested using arrayCGH (aCGH; 23 treatments, 76 embryos; 24Sure, Genesis Genetics) and from June – December 2015 using NGS (27

treatments, 88 embryos; NGS testing). Biopsies of 110 embryos occurred on D5 and 54 on D6. Embryos tested on D5 using aCGH were biopsied with a view to fresh D6 embryo transfer (ET) of 1 or 2 euploid embryos. Embryos that had not reached expansion stage ≥3 on D5, but did so on D6 were biopsied and frozen pending results of aCGH testing. Embryos with no results (NR) from D5 biopsy were re-biopsied and frozen on D6 if they had developed to a suitable grade for freezing. All embryos for NGS testing were biopsied and frozen pending results.

Results: Comparing screening results of embryos biopsied on D5 (n=110: 23.6% euploid (E), 66.4% aneuploidy (A), 10.0% NR) versus D6 (n=54; 29.6% E, 63% A, 5.6% NR) the proportion of euploid embryos did not differ significantly (P=0.49; Two-tailed Fisher's exact test)
When results are compared by expansion stage, embryos biopsied on D5 at expansion stages <3 were less likely to be euploid (n=16; 0% E, 93.3% A, 6.7% NR) than stages ≥ 3 (n=93: 29% E, 62.4% A, 11.8%NR; P=0.007). On D6 all embryos at stages 1 – 4 were aneuploid, any euploids were stage 5 or 6 (P=0.001).

Conclusion: Embryos cultured to D6 for biopsy and freezing for PGS are worth utilizing only if they are fully expanded or hatching /hatched (Stage 5 – 6). Also, if PGS wasn't being considered the stage of embryos on D6 would be a strong guide to avoiding transfer of aneuploid embryos.
All Embryos were laser breached in advance and so the hatching status is more advanced than in non-breached embryos.
There are limitations in considering a blastocyst as 'D5' or 'D6' and static assessment of morphology or expansion stage. Time lapse imaging allows more frequent assessments and its use with PGS may improve biopsy efficiency and improved comparisons of precise developmental stage at biopsy. Further data analysis of an increased data will be done and could include looking into differences in the complexity of aneuploidies.

The effect of cell number on day 3 on embryo development after two cell-biopsy

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Introduction: In the preimplantation genetic diagnosis (PGD) program, the question has arisen, how the cell number on day 3 influences the further development of an embryo to the blastocyst stage after two cell-biopsy not to compromise the outcome in terms of pregnancy.

Material & methods: From Februar 2015 to March 2016 we performed 29 IVF-PGD cycles in 25 women, aged 31,7 years,with 133 cleavage-stage embryos with 5 to 11 cells and no or low fragmentation, which underwent the biopsy of two blastomeres on day 3. For the study, these embryos were devided into 2 groups according to the number of cells before the biopsy on Day 3: *Group 1*-embryos with 5 or 6 cells (22 embryos) and *Group 2*-embryos with 7 to 11 cells (111 embryos). The biopsy of two blastomers was performed in each embryo by a laser system (Saturn 5^{TM}, Research Instruments) to perform genetic analysis. After biopsy the embryos were cultured to the blastocyst stage in sequential media and on day 5 they were classified as blastocysts, morulae or arrested embryos according to their developmental potential. Both groups of embryos were compared in terms of embryo devleopment to the blastocyste stage (blastocyst rate) or developmental arrest using Chi-Square test. The statistical significance was set at $P < 0.05$.

Results: After biopsy, a significantly lower proportion of embryos developed to the blastocyst stage and a higher proportion of embryos was developmentally arrested in *Group 1* embryos than in *Group 2* embryos: 13,6% (3/22) and 55% (12/22) vs. 51,3% (57/111) and 18% (20/111) ($P < 0.05$) although the groups did not differ in terms of female age.

Conclusions: The genetic laboratory usually prefers to diagnose two blastomeres from an embryo to provide a more relevant diagnosis. But from the embryonal point of view, it is preferable to isolate only one blastomere from low, i.e. 5-6-cell, cleavage-stage embryo not to compromise the embryo development and pregnancy.

Preimplantation Genetic Diagnosis for Single Gene Disorders and Aneuploidy Screening: Karyomapping and Next Generation Sequencing

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Introduction: Patients undergoing preimplantation genetic diagnosis (PGD) for single gene disorders (SGD) are often of young age and also, do not face any infertility issues. Nonetheless, as has been shown previously by different studies a considerable amount of embryos derived from patients of younger ages are still found to be aneuploid. Adoption of a strategy that will allow highly accurate embryo testing for PGD of SGD in combination with comprehensive chromosome screening without changing usual embryology practices (i.e. performance of a single embryo biopsy for PGD), will be highly beneficial for these patients. Next generation sequencing (NGS), was recently introduced into preimplantation genetic diagnosis for aneuploidy screening [preimplantation genetics screening (PGS)]. NGS offers advantages over other methodologies with one of the most important ones being detection of abnormalities present only in some cells of the trophectoderm biopsied (i.e. mosaicism).

Materials and Methods: PGD for SGD in combination with PGS was carried out for 55 separate clinical cases (average maternal age: 34.9±0.6). A single trophectoderm biopsy was carried out on 322 blastocyst embryos. Each sample was whole genome amplified and aliquots from each amplified product were used to perform Karyomapping (Illumina, USA) for PGD of SGD and NGS for PGS. The VeriSeq PGS assay and a MiSeq desktop sequencer (Illumina) were used for NGS. The BlueFuse Multi analysis software (Illumina) was utilized for interpretation of results.

Results: 55.6% of the embryos were found to be available for transfer after PGD-SGD while, 45.8% of the embryos were found to be euploid via NGS. 23.9% of the overall embryos assessed were seen to carry 1 or 2 aneuploid chromosomes, with no other abnormalities detected. 13.6% of the overall embryos assessed were seen to carry 1 or 2 mosaic aneuploid chromosomes, with no other abnormalities detected. 3.7% of the embryos had one mosaic aneuploid chromosome and one aneuploid chromosome. 3.7% of the embryos were determined to be complex mosaic (≥3 mosaic aneuploid chromosomes) and 9% were complex abnormal (≥3 aneuploid chromosomes or ≥3 abnormalities involving full and mosaic gains and losses). 29 of the embryos found to be chromosomally abnormal carried segmental abnormalities ranging from 7.8 to 136 megabases in size. A triploid (69,XXY) embryo was also identified. A total of 86 (26.7%) embryos were found to be at low risk for the single gene disorder assessed and aneuploidy and could therefore be considered for transfer.

Conclusions: The successful clinical application of PGD for SGD in combination with NGS for PGS using a single trophectoderm biopsy is clearly documented in this study. Whole chromosome aneuploidies and segmental abnormalities as small as 7.8Mb were readily detected. Mosaic abnormalities were also identified in some embryos. Importantly, 17.3% of embryos assessed in this study presented no chromosomal abnormalities other than those of mosaic nature. The combination of powerful and highly accurate methodologies such as Karyomapping for SGD and NGS for PGS, is expected to benefit patients undergoing *in vitro* fertilization (IVF)/PGD and can enhance their chances for a successful cycle leading to a healthy pregnancy.

To PGD or not to PGD: The importance of the pre-case workup

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Introduction: A couple came to our genetic couselling practise requesting PGD for generalized arterial calcification of infancy (GACI) syndrome. The male partner is infertile due to severe oligozoospermia. The couple had recently lost their ICSI-conceived daughter at the age of 3,5 weeks. The phenotype of the deceased child was consistent with GACI syndrome. However, the prenatal ultrasound had also revealed multiple dysmorphic features. Prenatal chromosome analysis showed a normal female karyotype 46,XX. Sequencing analysis of the *EPNN1* gene responsible for GACI syndrome had revealed an apparent homozygosity for a disease causing point mutation as well as several SNPs in the *EPNN1* gene. In segregation analysis the mother had been shown to be a heterozygous carrier of the mutation whereas no mutation was revealed in the sample of the father. This had lead to the assumption of a large deletion in the father.

Material and Methods: We used a standard array-CGH (CytoChip ISCA 4x180k, Illumina, San Diego, USA) for the detection of genomic anomalies in the DNA extracted from blood samples. The data was analysed using the software BlueFuse Multi v4.3 (Illumina). Further analysis was performed on the CytoSNP-850k (Illumina, San Diego, USA) and analysed with the software Genome Studio (Illumina).

Results: In order to find the second causative mutation resulting in GACI syndrome, we analysed the sample of the father on a standard oligo-based aCGH. Against the original hypothesis, no relevant genomic anomalies were detected. However in the sample of the deceased child the analysis revealed two large deletions; 2,9 Mb in chromosome 4q13.2q13.3 and 14,2 Mb in chromosome 6q22.31q23.2 comprising the *EPNN1* gene. We utilized a high-resolution SNP array to further delineate the deletions and to determine their parental origin. The analysis indicated the loss of paternal allele in both deletions and confirmed the paternity. A DNA fragmentation analysis could not be performed as the ejaculate contained no sperm.

Conclusions: Our findings underline the importance of a proper pre-case workup before starting a PGD procedure. No standard PGD can be offered to the couple as the incidence of the GACI syndrome in the family can be assumed to be either a pure strike of chance, due to germ line mosaicism in the father or attributable to some other, unknown factors. The detected deletions are responsible for the complex phenotype of the deceased child, while hemizygosity for the *ENPP1* mutation resulted in GACI syndrome. Unfortunately, no second tissue was available to further evaluate the possible mosaicism in the father. He shows a significantly defective spermatogenesis, which together with the detection of relatively large deletions in paternally inherited chromosomes rises the question of DNA quality in the sperm. The reproductive options of the couple must be considered carefully: whether the DNA quality in sperm is compromised thus rendering a predisposition for further chromosomally abnormal offspring cannot be reliably estimated. Instead of PGD, a PGS with the highest possible resolution appears to be the best alternative.

Maternal age does not affect clinical outcomes of vitrified-warmed euploid blastocysts: an Asian Study

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Introduction: Harton et. al. 2013 reported no statistical difference in implantation rates for women from different age groups (up to 42 years old) when euploid blastocysts were transferred after preimplantation genetic screening. This retrospective study analyses the clinical pregnancy and implantation rates for vitrified-warmed euploid blastocysts in an Asian population.

Materials & Methods: This is a retrospective analysis of outcomes for 154 Asian women undergoing preimplantation genetic screening, who had their euploid blastocysts vitrified and warmed using the Cryotec Method in Alpha Fertility Centre from July 2013 to December 2015. Non-Asian women were excluded in this study. The patients were divided into 5 age groups, age <35, 35-37, 38-39 40-41 and 41-46. The number of cycles in each age group was 95, 26, 19, 12 and 2 respectively. Three to 5 trophectoderm cells were biopsied from each blastocyst on Day 5 or 6 and proceeded with PGS by microarray CGH or next generation sequencing (NGS). All biopsied blastocysts were of at least average grade (Gardner et. al., 1999). The biopsied blastocysts were vitrified shortly after biopsy using the Cryotec Method.

Results: The mean age for age group <35, 35-37, 38-39 40-41 and >41 were 28.6, 36.0, 38.4, 40.5 and 42.0 respectively. The mean number of blastocysts transferred were 1.4, 1.2, 1.3, 1.1 and 1.0 respectively. All 206 euploid blastocysts were warmed and survived with morphologically intact inner cell mass and trophectoderm cells (100% post-thaw survival rate), enabling transfer in all 154 cases.Clinical pregnancy rates were 63.2%, 61.5%, 63.2%, 58.3% and 50.0% respectively. The on-going pregnancy/ delivery rates were 60.0%, 57.7%, 47.4%, 58.3% and 50.0% respectively. Implantation rates were 54.8%, 56.3%, 58.3%, 61.5% and 50.0% respectively. There was no statistical significance in clinical pregnancy rates, implantation rates or on-going pregnancy rates between all age groups. Of the 96 on-going or delivered pregnancies, 77 were singleton (80.2%) and 19 were twins (19.8%). There are no higher order pregnancies.

Conclusion: This study shows that advanced maternal age does not affect the pregnancy and implantation rates in Asian women when vitrified-warmed euploid blastocysts are transferred. This study is in keeping with the studies done on predominantly non-Asian populations (e.g. Harton et. al., 2013).

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Comparison of ongoing pregnancy rate after blastomere biopsy in stimulated cycles and frozenthawed embryo cycles

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Background: Blastomere biopsy followed by fresh embryo transfer after preimplantation genetic diagnosis (PGD) was the standard treatment logistic. However, under many circumstances, fresh embryo transfer may not be feasible, for example, the risk of ovarian hyperstimulation syndrome. Some concerns may be arisen due to the possible adverse effect of vitrification after biopsy in these blastocysts. Some evidence showed comparable pregnancy rate after trophectoderm biopsy with preimplantation genetic screening (PGS) with fresh embryo transfer or frozen-thawed embryo transfer subsequently (1). Evidence of vitrification, followed by warming and re-vitrification after trophectoderm biopsy, did not adversely affect the implantation rate and pregnancy rate (2). We would like to look at the effect of blastomere biopsy and vitrification.

Methods: A retrospective analysis on the pregnancy rate in fresh cycles and frozen-thawed embryo transfer cycles (FET) after PGD.

Results: All PGD cycles in 2014-2015 were retrieved from our database. Cycles with fresh embryo transfer were recruited. Blastocyst transfer (BT) cycles without PGD were used as control. There were 68 PGD fresh cycles and 86 BT cycles, while 86 PGD FET and 227 BT FET cycles were retrieved. In stimulated cycles, although the women's age was significantly lower in the PGD group (34 [32-35.8] vs 35 [32-37] years, p 0.023), ongoing pregnancy rate (OPR) after blastomere biopsy on day 3 was lower than control group (35.3% vs 50%, p 0.068), but not reaching statistical significance, with similar number of embryo transferred. In FET cycles, OPR after PGD was comparable with control BT cycles (41.9% vs 44.1%, p 0.727). The women's age was significantly younger in the PGD group (33.5 vs 35 years, p 0.013) and the number of blastocyst transferred was significantly higher in the control group. A comparison of the PGD fresh cycles with PGD FET cycles showed the OPR was comparable (35.3% vs 41.9%, p 0.407), with a comparable miscarriage rate (13.3% vs 21.7%, p 0.355).

Conclusion: Vitrification after blastomere biopsy did not jeopardise the ongoing pregnancy rate and OPR was comparable in stimulated PGD cycles and FET PGD cycles. As the OPR in FET cycle after biopsy and PGD did not differ from control FET BT cycles, biopsy followed by vitrification probably did not adversely affect the pregnancy rate. The lower OPR in fresh PGD cycles may be related to the poorer endometrial receptivity after higher ovarian stimulation in fresh PGD cycles compared with control cycles. Further larger prospective study should be performed to confirm these findings.

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Ongoing pregnancy following simultaneous alpha thalassemia SEA PGD & PGS from a single biopsy using NGS

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Introduction

Alpha-thalassemia is a common Mendelian inherited autosomal recessive blood disorder particularly in Southeast Asia. Transmission of the mutated alpha gene to the next generation can be prevented by PGD. This case report describes our first case of PGD & PGS using whole genome amplification (WGA) product from a single biopsy on a single NGS platform in Alpha Fertility Centre (AFC), resulting in an unaffected ongoing pregnancy.

Materials and Methods

The couple, aged 28 years (female) and 30 years (male), both alpha-thalassemia heterozygous carriers (SEA deletion) underwent IVF treatment with PGD & PGS in September 2015. This is AFC's first attempt performing PGD & PGS simultaneously on a single biopsy using a single NGS platform. Following oocyte retrieval, 10 oocytes were injected with motile spermatozoa using PIEZO-ICSI (Primetech, Japan) and were cultured sequentially. Seven blastocysts with at least average grade (Gardner, 1999) were biopsied and the biopsied samples underwent WGA using the multiple displacement amplification approach. Each of the WGA product from respective blastocysts were split into 2 aliquots. One aliquot was used to construct a library using a customised thalassemia Ampliseq panel for PGD; another aliquot was used for PGS library construction. The libraries from Ampliseq panel and PGS were pooled and sequenced together using NGS according to manufacturer's specifications (Thermo Fisher, USA).

Results

Results were obtained for all seven samples. Of the diagnosed samples, 2 were homozygous for the alpha-thalassemia SEA deletion. One had chromosomal aneuploidies and the other one was euploid. The remaining 5 blastocysts showed no SEA deletion in the alpha-globin gene. Of these, 4 resulted in chromosomal aneuploidies. Only one blastocyst was found to be euploid with no SEA deletion. The blastocyst was vitrified and thawed for frozen embryo transfer and resulted in a singleton pregnancy. At the time of writing, the patient is in her 22nd week of pregnancy.

Conclusions

AFC commenced alpha thalassemia PGD with PGS from a single biopsy using a single NGS platform in September 2015. PGD for genetic disease diagnosis & PGS for comprehensive chromosomal screening enables two tests to be performed on a single platform from a single biopsy using NGS (Ion Torrent^{TM}). This eliminates the need to biopsy the same blastocyst twice, avoiding potential damage to the blastocyst and allows the selection of euploid blastocyst without alpha-thalassemia mutation for transfer.

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Euploidy predictability by blastocyst morphology and early-time lapse parameters in Egg donation embryos

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INTRODUCTION: The ability to select the embryo with the highest potential is essential to maximize IVF success rates. Classical assessment of embryo morphology provides a weak indication of IVF outcome. Several studies have confirmed that preimplantation genetic screening (PGS) is a valuable tool to select those embryos with the best chances of developing into a healthy baby. PGS is, however, still an invasive technique not available in every clinic due to legal or social reasons. Time-lapse imaging (TLI) has emerged as an interesting non-invasive selection tool. Several groups have explored the relationship between embryo morphology and chromosome imbalances and the link between morphokinetics and aneuploidy. To our knowledge, no study exploring the association between these parameters has been conducted in embryos from an egg donation program. We wonder if the previously

described association between morphology and aneuploidy is still present in embryos derived from good quality oocytes and also aim to evaluate the ability of TLI for chromosome abnormalities determination.

MATERIAL & METHODS: This unicentric and retrospective study evaluated 429 embryos from IVF and Egg-donation PGS cycles from our centre between September 2013 and December 2015. The relationship between embryo ploidy, morphokinetics and morphology was explored. All embryos were cultured into the TLI Eeva system®, biopsied, and vitrified at the blastocyst stage. Genetic analyses were performed in a reference laboratory by next-generation sequencing. Eeva test provided 3 morphokinetic categories related to embryo competence (*High*, *Medium* and *Low*) depending on 2 early cleavage parameters: P2 (time from 2 to 3 cells) and P3 (time from 3 to 4 cells). Blastocyst morphology was evaluated using Gardner classification.

RESULTS: No statistical relationship was found between morphokinetics and chromosome status. No significant differences were detected when comparing euploidy rates within the 3 Eeva-classified categories, neither in the Egg-Donation Program embryos (52.3% for High, 54.5% for Medium and 56% for Low) nor in the embryos from the IVF program (37% for High, 48.1% for Medium and 37.1% for Low).
In contrast, a statistically significant association was found between embryo morphology and chromosome status. A significantly higher euploidy rate ($p<0.03$) was detected in good morphology embryos (57.4%) from our Egg donation-PGS program as compared to fair (36.4%) and poor (42.9%) quality blastocysts. This association, although statistically significant, could not be used as a predictive value since a considerable proportion of good morphology blastocysts were found to be aneuploid (42.6%). A similar tendency was observed in the embryos from the own eggs IVF PGS Program although no statistical significant differences were detected.

CONCLUSIONS: Our results reveal, on one hand, that Eeva morphokinetics parameters are unable to provide valuable information for the identification of euploid embryos. On the other hand, our data confirm an association between good morphology and euploidy in blastocysts derived from donor eggs. Still a considerable proportion of good quality blastocysts are aneuploid, suggesting that PGS might be a suitable strategy in egg donation programs to avoid the transfer and storage of good morphology aneuploid embryos

Live twin birth following transfer of a 2PN and a 0PN embryo after PGD-MaCGH

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Introduction: Normal fertilisation is routinely assessed 18-20 hours after oocyte insemination. The appearance of 2 pronuclei (2PN) indicates that normal fertilisation has taken place and they enter syngamy 20-22 hours post-insemination leading to subsequent cleavage. 2PN can be detected as early as 5 hours post-insemination - their absence does not necessarily indicate fertilisation failure. A study found that 20% of 0PN oocytes went on to cleave and it was suggested that the PN in these embryos may have been missed due to syngamy before fertilisation check or that PN only formed after fertilisation check (Feenan & Herbert 2006). We earlier reported the birth of a healthy baby resulting from a 0PN embryo following 5-probe FISH analysis and embryo transfer. The baby was confirmed chromosomally normal through amniocentesis (Khoo et al, 2007). We had also reported that 0PN-derived embryos have a euploidy rate similar to embryos resulting from 2PN (Lee et al, 2013). This case report describes a successful birth of twins following the transfer of one euploid-2PN embryo and one euploid-0PN embryo after microarray comparative genomic hybridisation (MaCGH) analysis.

Material and Methods:A 44 year old patient who previously had a trisomy 18 baby which died after birth underwent a donor-IVF cycle with preimplantation genetic screening in September 2014. Twenty-three donor's (aged 22) oocytes were retrieved, of which 15 were inseminated. Fertilisation check was conducted at 20 hours post-insemination. From these, 7 were normally fertilised exhibiting 2PN, one 3PN and seven 0PNs. Cleaved embryos with at least average grade (Gardner's grading, 1999) were biopsied and biopsied cells were analysed using MaCGH (Illumina, UK). Three OPN oocytes went on to cleave normally producing good graded embryos. There were 9 embryos for biopsy on Day 3: 6 from 2PN and 3 from 0PN. The patient had fresh embryo transfer on Day 4.

Results: Euploidy was confirmed in 3 embryos while the remaining 6 embryos displayed various chromosomal anomalies. Of the 3 euploid embryos, 2 were derived from 2PN and 1 derived from 0PN. The patient being 44 years old only wanted 1 final attempt at pregnancy and insisted on having 2 embryos for transfer. Two good graded euploid embryos (one 2PN and one 0PN) were transferred. The reason the euploid-0PN embryo was chosen for transfer rather than the other euploid-2PN embryo was because the 0PN embryo was of better quality. Both embryos implanted. An uneventful delivery at 36 weeks by caesarean section resulted in the birth of 2 normal healthy babies, weighing 2.8kg and 2.5kg respectively.

Conclusions: This case report indicates that 0PN zygotes that go on to cleave may have been normally fertilised and these embryos can be chromosomally normal and result in live birth. These embryos should not be discarded but instead be treated as if they were 2PN embryos and be considered for transfer.

Age of vitrified-warmed euploid blastocysts at the time of transfer vs their clinical outcomes

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Introduction: As a result of advances in vitrification and warming technology for blastocysts, vitrified-warmed blastocysts yield good post-thaw survival rates, and achieve high clinical pregnancy and implantation rates. Since initiating the use of the Cryotec Vitrification and Warming Method in July 2013, our centre has gradually switched from fresh transfer to elective frozen embryo transfer for IVF cases undergoing preimplantation genetic screening cases. In this retrospective study, we assessed the correlation between age of vitrified-warmed euploid blastocysts at the time of transfer and their clinical pregnancy and implantation rates.

Materials & Methods: One hundred and thirty-three (133) women had 173 vitrified-warmed euploid blastocysts transferred from July 2013 to December 2015 in Alpha Fertility Centre. These 133 cases were divided into 4 blastocyst age groups at the time of embryo transfer, age <120, 120-130, 131-140 and >140 hours. The age of blastocysts at the time of transfer refers to the duration from insemination to embryo transfer. The number of cycles in each group was 42, 14, 8 and 69 respectively. Three to 5 trophectoderm cells were biopsied from each blastocyst on Day 5 or 6. All biopsied blastocysts were of at least average grade (Gardner et. al., 1999). Samples and reference DNAs were amplified, labelled and hybridized according to manufacturer's (Illumina / Life Technologies) specifications. Biopsied blastocysts were frozen shortly after biopsy using Cryotec Method (Cryotech, Japan). When PGS results showed at least 1 euploid blastocysts available, the patient will be prepared for FET.

Results: The mean age for blastocyst age group <120, 120-130, 131-140 and >140 hours were 32.4, 29.4, 32.9, and 32.9 respectively. The mean number of blastocysts transferred were 1.3, 1.4, 1.1, and 1.3 respectively.All 173 euploid blastocysts survived with morphologically intact inner cell mass and trophectoderm cells (100% post-thaw survival rate), enabling transfer in all 133 cases. Clinical pregnancy rates were 78.6%, 64.3%, 25.0% and 62.3% respectively. Implantation rates were 75.5%, 50.0%, 33.3% and 56.0% respectively. There was statistical significance in clinical pregnancy rates between blastocyst age <120 and 120-130 hours post-insemination. There were statistical significance in implantation rates for blastocyst age <120 compared to group 120-130, 131-140 and >140. The singleton vs twin pregnancy rates for each group was 78.8% vs 21.2%, 88.9% vs 11.1%, 100% vs 0% and 81.4% vs 18.6% respectively. There were no higher order pregnancies.

Conclusion: Blastocysts aged less than 120 hours (from insemination to the time of embryo transfer procedure) demonstrated superior clinical pregnancy and implantation rates. When more than one euploid blastocysts are available, it is recommended to select blastocyst with an age of <120 hours.

Preimplantation Genetic Diagnosis in combination with human leukocyte antigen typing: 8 years of experience

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INTRODUCTION: Preimplantation genetic diagnosis (PGD) in combination with human leukocyte antigen (HLA) typing is offered for treatment of affected siblings requiring HLA identical stem cell transplantation.
Here we present the results of our 8 years of experience (2008-2016) on this technique in the context of a public hospital.

MATERIAL & METHODS: 34 PGD cycles were performed for 10 different couples, all of them parents of children needed of HLA identical stem cell transplantation. These children were affected of acquired haematological diseases, such us adquired severe bone marrow aplasia and a *de novo* mutation related to diamond- blackfand anemia or were carriers of single gene disorders, such as talasemia major, shwachman-diamond syndrome, adenosine deaminase deficiency and X linked chronic granulomatous disease.
Embryos were biopsied on day 3 via laser technique, followed by cell lysis. A one-step multiplex single-cell fluorescent PCR was used for the simultaneous amplification of informative markers linked to the specific mutation (STRs) and a panel of markers at the HLA locus, using QIAGEN® Multiplex PCR kit (QIAGEN, USA). PCR products were analyzed on an ABI3730 automated sequencer (Applied Biosystem, USA). Multiple displacement amplification (MDA) was also used in 15 cycles since 2011 in order to ensure the quantity of DNA was enough to perform the analyses.

RESULTS: In 34 cycles, 264 embryos were obtained, 228 of them were biopsied and 215 (94.29%) had a successful genetic result. 108 of those diagnosed embryos were unaffected (50.23%) and 12 of them (11.1%) were HLA compatible and suitable for transfer.
12 embryos of 4 couples were transferred in 10 cycles (2 of them were cryotransfers cycles), yielding to 3 pregnacies and 2 births of unaffected and HLA compatible babies.
The results of the PGD were confirmed in the newborns and cord blood haematopoietic stem cells were obtained and frozen for subsequent transplantation.
The affected siblings have already received their identical HLA stem cells and are currently healthy.

CONCLUSION: Preimplantation genetic diagnosis in combination with human leukocyte antigen offers to families not only the possibility of having unaffected children, but also a new therapeutic options for a diseased sibling who need haematopoietic stem cell transplantation as the unique option of curative treatment.

Validation of detecting monogenetic diseases and aneuploidy simultaneously by next generation sequencing with linkage analysis

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Key Words
PGD, MALBAC, NGS, linkage analysis

Instruction: Here we report three supportive cases by screening embryos with our well-established and published method, named “mutated allele revealed by sequencing with aneuploidy and linkage analyses” (MARSALA), which combines multiple annealing and looping based amplification cycles (MALBACTM) and Next Generation Sequencing with linkage analysis. The MARSALA strategy is able to detect aneuploidy and targeted gene mutations simultaneously in preimplantation genetic diagnosis (PGD) process during an in vitro fertilization (IVF) cycle. The three cases demonstrated here justified the effectiveness of MARSALA that may provide a precision and low cost method in PGD procedure.

Material & methods:

Blastocyst Biopsy

We collected a few TE cells from each hatching blastocyst on day 5 or day 6. All embryos were obtained from patients who chose to be subjected to an IVF procedure and voluntarily gave their consent for providing the samples for this study.

Single cell whole genome amplification (WGA) with MALBAC

The WGA process was performed with the standard protocol provided by the commercial kit, MALBAC^{TM} Single Cell WGA Kit (Cat. #: YK001A/B, Yikon Genomics). For blood samples, we extracted the gDNA by using the QIAamp DNA Blood Mini Kit (QIAGEN) and amplified 1 ng gDNA using the MALBAC^{TM} Single Cell WGA Kit (Cat. #: YK001A/B, Yikon Genomics).

MARSALA method

Eight nano-grams of MALBAC products were used as the template for PCR reactions targeting disease-cause mutation sites. The PCR products was then added into the corresponding MALBAC products at 1-5%. The mixture was used to construct a sequencing library using the NEBNext Ultra DNA library Prep kit (New England Biolabs). The sequencing was done on the Illumina HiSeq 2500 platform with a low depth of 0.1x genome coverage. Following this procedure, targeted point mutations and aneuploidy can be detected simultaneously.

Linkage Analysis with MARSALA

We sequence the genome of the parents and relatives carrying the monogenic disease allele with the depth of 2x genome coverage. Then, from the sequencing results of each embryo, the SNP readouts (heterozygous or homozygous) adjacent to the disease-cause mutation sites allowed the identification of the disease-carrying allele in the embryo.

Results

Case1

Fig. 1.MARSALA analyses of blastocyst sequencing for case 1 in which both the husband and wife were carrying SLC26A4 mutations that cause autosomal recessive deaf.

Case 2

Fig. 2.MARSALA analyses of blastocyst sequencing for case 2 in wife was affected by a PKD1 mutation that causes autosomal dominant polycystic kidney.

Case 3

Fig. 3.MARSALA analyses of blastocyst sequencing for case 2 in wife was affected by the X chromosomal gene F8 that cause hemophilia A.

Conclusion: We demonstrated that our streamlined MASALA method can simultaneously screen aneuploidy and monogenic diseases, accurately and cost-efficiently, combining the MALBAC^{TM} technology and NGS platform.

Treatment outcomes using embryos undergoing two rounds of biopsy, vitrification and thawing

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We have previously presented data at this conference evidencing the efficacy of performing trophectoderm biopsy and vitrification following the return of no result from blastomere biopsy. Subsequent thaw and transfer of these embryos resulted in a clinical pregnancy rate of 33% per cycle and 42% per transfer for PGD patients and 67% per cycle and transfer for PGS patients.

Current best practise for PGD and PGS is now widely considered to be trophectoderm biopsy and vitrification pending the return of results. Unaffected or euploid embryos can be thawed for use in a frozen embryo transfer. This raises issues with respect to embryos returning no result as it necessitates the embryos to be thawed, rebiopsied and then re-vitrified. While the no result rate is lower in trophectoderm biopsy than blastomere biopsy, it still occurs. We also recommend rebiopsy for any embryo where contamination has been noted in the corresponding media wash blank.

Since we have begun performing trophectoderm biopsy and vitrification across our organisation, we have undertaken a second round of biopsy and vitrification for 13 PGD embryos and 8 PGS embryos. Results were obtained for all but one of the rebiopsies. 4/13 PGD embryos were suitable for patient use

which has resulted in 1 ongoing pregnancy (EDD July 2016), 1 clinical miscarriage and 2 negative tests. 3/8 PGS embryos were suitable for patient use but none of these patients have used the embryos as yet.

The number of embryos used at our clinics remains too small at present to draw definite conclusions about the efficacy and safety of this approach. However, we are encouraged by the ongoing pregnancy and continue to counsel patients that retesting is possible but that data remains limited and the repeat procedures may reduce the implantation potential of embryos.

Identification of triploid and tetraploid embryos using next generation sequencing

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In an IVF treatment, the first aim in the laboratory is to successfully fertilise the oocytes. This can be achieved via standard IVF or ICSI and is confirmed by the embryologist by the presence of the male and female pronuclei 16-18hours post insemination. As well as oocytes failing to fertilise, abnormal fertilisations may be identified, where an abnormal number of pronuclei are observed, resulting in haploid or polyploid embryos. While these embryos will not form a viable pregnancy, they may form blastocysts, implant, and potentially form a full or partial molar pregnancy.

The majority of haploid and polyploid embryos are excluded from treatment by the embryologist performing the fertilisation check and noting an abnormal number of pronuclei. However, pronuclear development is dynamic and appearance can be asynchronous. Thus embryos scored as normally fertilised by the embryologist may later display additional pronuclei and in fact be polyploid embryos.

The nature of array based comparative genomic hybridisation as a technology meant it was unable to consistently or accurately identify polyploid embryos. However, quantification of read counts in NGS results in an improved dynamic range and clearer changes. This allows us to more accurately examine X and Y values and identify triploid XXY samples (X=1.67, Y=0.3) and tetraploid XXXY samples (X=1.5, Y=0.5). In some cases where such results have been obtained we have been able to access timelapse imaging from IVF clinics – in one case the development of a third pronuclei after the initial fertilisation check was not noted, in another a micro-pronuclei was not scored. The majority of cases were not cultured in timelapse incubation.

NGS still does not allow for the identification of 69 XXX or 92 XXXX samples. However, we have observed trophectoderm samples from good quality blastocysts that have given chaotic mosaic results with good quality metrics. It is possible that these are triploid XXX or tetraploid XXXX samples with chromosomal gains or losses. It is conceivable that this interferes with the software's ability to plot where the midline of 2 copies lies. Thus, for such samples we hope to be able to obtain parental DNA and use SNP arrays to examine this phenomenon further.

First trimester QUAD screening for prenatal aneuploidy, preeclampsia risk assessment and NIPT indication

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Objective: The aim of this pilot study is to compare combined dual first trimester screening with first trimester QUAD (1T QUAD) strategy using additional biomarkers: PLGF, AFP, NT, including ductus venosus flow, uterine artery pulsatility, mean arterial pressure (MAP) and required clinical data for early preeclampsia (PE) screening, prevention and aneuploidy detection and exactness of its risk ascertainment for NIPT indication.

Methods: Dual screening was performed on Kryptor (Brahms) with software Lifecycle 3.2 (Perkin Elmer), the QUAD on Delfia Xpress using Lifecyle 4.0 and Preeclampsia Predictor (PerkinElmer). First trimester screening was supplemented by IInd trimester dual screening (Kryptor) and consequently by triple test (Delfia Xpress). Aneuploidy risk was ascertained in parallel in 408 first trimester samples within weeeks 8 to 13+6, 38 frozen sera for retrospective comparison of 38 T21 cases, disclosed within years 2008-2015 for QUAD reinvestigation. PE screening success was validated in 14 PE cases selected according international diagnostic criteria and in 23 cases of gestational hypertension (GH).

Results: QUAD increased reliability of aneuploidy risk ascertainment. Risk of T21 <1:50000 was in 56.3% comparing dual 2.10%. In 36/38 T21 cases DR was 94.73% with 1/38 (2.63%) intermediate risk indicating triple test/NIPT that allows final DR 97.40%, close to presumed 98% DR limit. Cut-off level 1:300 was used for final risk and „biochemistry only" to avoid false negativity due to US errors. PLGF, PAPP, and fβhCG provided PE DR 57.89%. Combination with clinical risks enabled in 13/14 cases (92.85%) detection of all PE types. Normal levels of QUAD biomarkers in all 23 women with GH were disclosed.
Exactness of aneuploidy risk detection was significantly higher also in negative screening risk results with the shift to the lower risk categories for optimal and NIPT indications to risk lower than 1:2500.

Conclusions:
This pilot study confirmed the possibility of presumed 98% DR limit for QUAD as the best in present time combined Ist trimester screening strategy that thanks to combination with NIPT or triple test can assure the most reliable prenatal screening not only of aneuploidies, but also in combination with exact clinical risk and biophysical markers evaluation by specific software the early prevention and treatment of PE, other severe complications as of IUGR, premature delivery or abortion. Normal levels of biochemical markers in first trimester might contribute to discrimination of severe GH and PE. QUAD provides also optimal risk calculations for NIPT indication in the lowest aneuploidy risk categories.

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Preimplantation genetic diagnosis for diastrophic dysplasia: a case report
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Introduction: Diastrophic dysplasia (DTD) is caused by homozygous or compound heterozygous mutation in the SLC26A2 gene on chromosome 5q32. Prenatal diagnosis is mainly performed by ultrasound in the second trimester. We report our case of preimplantation genetic diagnosis (PGD) for DTD. To develop PGD protocol we looked through publications and did not find any description PGD for DTD. Later on a PGD for DTD case was published [Mattos et al., 2014].

Material & methods: A couple (33 year-old man and 38 year-old woman) from Blagoveshchensk city addressed to Krasnoyarsk Center Reproductive Medicine (KCRM) for PGD. They had had four terminations of pregnancy because of skeletal dysplasia of fetuses. DTD was confirmed for the last fetus by molecular analysis: R178X and C653S mutations of SLC26A2 gene were revealed.
PGD setup was started in May 2014. We got blood samples of couple, their parents and DNA of fetus. Direct and indirect analysis of mutation for PGD was developed in KCRM and tested on single lymphocytes of the patient and her husband. Ovarian stimulation was carried out in Blagoveshchensk. IVF-ICSI, biopsy (day 3), PGD and embryo transfer (day 5) were performed in KCRM in September 2014.

Results: It took us two months to create and test single cell DTD protocol. We used nested PCR, restriction analysis for mutations and STR marker analysis. We investigated 11 polymorphic markers, 8 of them were informative. CSF1PO locus commonly used for paternity analysis, was useful for DTD analysis. During IVF-ICSI cycle 11 oocytes were retrieved and 9 embryos were biopsied and tested for mutation and DNA markers. Two embryos were compounds for mutation; one had no result (failed amplification). We recommended six embryos for transfer. Two embryos (normal and carrier) were transferred and two cryopreseved. The result was twin pregnancy. Boy and girl were born in May 2015. They are healthy and we performed molecular confirmation for both at the age of 10 month.

Conclusions: Molecular analysis by preimplantation genetic diagnosis (PGD) may be a good solution for DTD high risk couples.

Genome-wide haplotyping of preimplantation embryos in the clinic: principles guiding embryo selection in Leuven

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Introduction: Recently developed methods in preimplantation genetic diagnosis give a general overview of the embryonal genome and enable the simultaneous detection of multiple variants, mutations and imbalances genome-wide. Therefore, their introduction in diagnostic settings raises novel ethical questions that need to be addressed regarding the selection criteria and procedures.

Material and methods: Here we present the principles guiding embryo selection and prioritization that are applied at our center according to the chromosomal content and mutational load of the embryos, in an attempt to introduce for the first time a series of guidelines for genome-wide preimplantation genetic diagnosis. Additionally, we discuss the results of the clinical implementation of our novel method, single-cell haplotyping and imputation of linked disease variants (siCHILD).

Results: From June 2014 until March 2016, 366 embryos from 60 couples had been tested in 102 cycles, leading to 16 clinical pregnancies (35.5% clinical pregnancy rate per cycle for which embryos were available for transfer) and the birth of 7 healthy babies so far. Forty different indications, 36 for monogenic disorders, 3 chromosomal aberrations and 1 case of combined monogenic disorder with chromosomal aberration have been included.

Conclusions: Our embryo selection principles are based not only on technical and biological, but also on ethical criteria and have a profound impact on the organization of PGD operations and on the information that is transferred amongst the genetic unit, the fertility clinic and the patients. Those principles are also important for the organization of pre- and post-counselling and influence the way of interpreting and reporting preimplantation genotyping results. As novel genome-wide approaches for embryo selection are revolutionizing the field of reproductive genetics, such guidelines are becoming of outmost importance for good clinical practice in IVF and genetic centers.

Different strategies to improve the number of embryos to analyze in Preimplantation Genetic Screening

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Introduction: It is well known that all women not respond equally to the same stimulation protocol. The efficiency of the preimplantation genetic screening (PGS) cycle could be improved by accumulating oocytes, day-2 embryos or day-3 embryos in several cycles when patients have poor response.
The objective of this study was to assess the results obtained in couples from our PGS program depending on different cycle strategies compared with fresh cycle results.

Material & methods: Retrospective study involving a total of 1,474 cycles (January 2011 – December 2015) from our PGS program. Group A (control group): 617 fresh cycles with 3,110 analyzed embryos, Group B: 78 oocyte vitrification cycles with 351 analyzed embryos, Group C: 162 cycles with day-2 embryos vitrified with 955 analyzed embryos, Group D: 454 cycles with day-3 embryos vitrified with 2,673 analyzed embryos and Group E: 163 cycles with blastocyst biopsied with 758 analyzed blastocysts. Oocytes, embryos and blastocysts were vitrified using the Cryotop® method. In groups A, B, C and D, biopsies were performed on day-3 and embryos were cultured until day-5, when euploid embryos were transferred or vitrified (surplus euploid blastocysts). In group E, biopsies were performed on day-5 or day-6 and one hour after biopsy the blastocysts were vitrified. Euploid blastocysts were

transferred in a next cryotransfer. Microarray comparative genomic hybridization (array CGH) technology (© BlueGenome) was used to analyze aneuploidy for 24-chromosomes in all groups. Statistical comparisons were performed using Fisher's exact test and t-Student test ($p<0.05$).

Results: Comparisons between group A and other groups showed similar mean age except group B (39.1 *vs.* 40.6, $p<0.0001$). We obtained statistically higher mean number of MII oocytes in groups C (9.3 *vs.* 11.4, $p<001$) and E (9.3 *vs.* 13.3, $p<0.0001$). Similar results were obtained in terms of informative embryos in all groups. Percentage of abnormal embryos was similar when we compared groups with day-3 biopsy. However, statistical differences were found when we compared control group (day-3 biopsy) vs. blastocyst biopsy (79.6 vs. 56.8, $p<0.0001$). Pregnancy rates were similar in day-3 biopsy groups (A: 53.3%, B: 48.6%; C: 55.1%; D: 52.2%). Although pregnancy rate was higher in group E (62.2%), this difference did not reach statistical significance. Implantation rates were similar in all groups with day-3 biopsy but we obtained differences statistically significant when we compared group A and E (48.9 *vs.* 59.3, $p<0.05$).

Conclusions: In this study, biopsy on day-3 gives similar results with different strategies to improve number of embryos to biopsy. Statistically lower percentage of abnormal blastocysts was obtained with blastocyst biopsy, probably because abnormal embryos arrested during their development. Higher implantation rate was obtained when blastocyst biopsy was performed.

A Case Report: Pre-implantation Genetic Diagnosis Patient with Sex Chromosome Anomalies

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Aim: Robertsonian translocation 13/14 is the most common chromosome rearrangement in humans. It increases the risk of infertility, spontaneous abortions or chromosomally unbalanced offspring. Pre-implantation genetic diagnosis (PGD) is the only solution to help this group of patients to select normal embryos to achieve a normal pregnancy.

Method: This is a case report of a 38 year-old woman with regular menstrual cycle who suffered from primary infertility for 11 years. She underwent her first IVF cycle in 2014 but did not conceive. She was pregnant subsequently from the frozen embryo cycle after a day 5 blastocyst was transferred, but had a miscarriage at 12 weeks of gestation. A chromosomal analysis of the product of conception showed a trisomy 13 fetus. Her husband was found to have a Robertsonian translocation between chromosomes 13 and 14. A PGD program witha CGH-based PGD analysis was recommended to the couple. The PGD cycle was carried out in 2015. 6 oocytes were collected, out of which 5 were viable for intra-cytoplasmic injection (ICSI) and 3 were fertilized normally. A biopsy of three Day 3 embryos was carried out.

Result: Two embryos were chromosomally balanced and suitable for transfer, while the third embryo was chromosomally unbalanced and not suitable for transfer. Embryo transfer of the two chromosomally normal embryos was done on Day 5.
A post-transfer blood test was done on Day 16, elevated levels of beta-HCG shown positive. It was followed-up by an ultra-sound scan at 6 weeks, showing a single viable fetal pole of about 5-6 weeks gestation. The pregnancy is still on-going with a gestation of 37/52.

Conclusion: PGD is strongly recommended for these group of patients to select chromosomally normal embryos for transfer.

First live birth following in vitro fertilization combined with Karyomapping in Hungary

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Introduction: Preimplantation genetic diagnosis (PGD) for single gene defects obtained from embryos at different stages is a well established method in assisted reproductive technologies (ART). Lately, the simultaneous analysis of the number of chromosomes besides the monogenetic diseases became a

reasonable demand of embryos produces in in vitro fertilization (IVF). Karyomapping is a genome wide parental haplotyping using a high density single nucleotide polymorphism (SNP) array that allows the diagnosis of any single gene defects and provides information of meiotic trisomies and monosomies.

Methods: A couple with an affected child with primary congenital glaucoma (PCG) attended at our clinic. They were both previously dignosed as heterozygous carriers of PCG. Following genetic consultation, IVF combined with PGD using Karyomapping was recommended. Blood samples were obtained from both parents and affected child prior to the IVF cycle for reference for Karyomapping. Twenty year old female patient received ovarian stimulation with antagonist protocol. Partner was a 24 year old male with normal semen parameters.

Results: Thirty six hours before oocyte collection an hCG trigger was administered and six oocyte-cumulus-complex was retrieved. Intracytoplasmic sperm injection was used as insemination method on the three mature oocytes. At day 1, one zygote showed the signs of normal fertilization and was cultured for five days. Trophectoderm biopsy was carried out 120 hours post-fertilization The obtained sample was sent to Karyomapping analysis and the embryo was vitrified. Result showed that the embryo is a heterozygous carrier for PCG. In the following cycle the embryo was thawed and transferred. A positive hCG result was obtained two weeks after embryo transfer and a single heart beat was detected at week six. A 2970 g healthy girl was delivered by ceasarian section at week 39.

Conclusions: Here we report the first live birth following IVF combined with PGD using Karyomapping in Hungary. Previously, it has been shown that genome wide karyomapping is able to accurately detect single gene disorders from a limited amount of samples. It can also provide information about meiotic aneuploidies that responsible about two third of all aneuploidies. Furthermore, it can be used without a significant preclinical workup.

Whole Exom Sequencing Enables Preimplantation Genetic Diagnosis For Couples With Undiagnosed Disorders

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Introduction: Initial step of preimplantation genetic diagnosis for single gene disorders is the investigation of a pathogenic mutation associated with the clinically diagnosed disorder in parents.
In some cases clinical diagnosis of the baby with disease symptoms is elusive. Another scenario is the failure in detecting a pathogenic mutation associated with diagnosed disease. These situations prevent the possibility of parents to benefit from prenatal or preimplantation genetic diagnosis.
WES has been applied in different areas of research and diagnostics esspecially for families who had babies witohut any molecular diagnosis or in cases with heterogenous disorders (Rabbani et al 2014). This conditions include complex neurological disorders.

Band like calcification with simplified gyration and polymicrogyria (BLC-PMG) is a rare autosomal-recessive neurological disorder and known to result from mutations in the Occludin(OCLN) gene.
Intracranial calcification (ICC) and polymicrogyria (PMG) can be seen in a large and heterogeneous group of neurological disorders with diverse etiologies so differential diagnosis is complicated. Due to rareness of disease and complexity of differential diagnosis for ICC and polymicrogyria, WES was the best appropriate method for those patients.

Material & methods: The consanguineous couple referred to our center for genetic counseling of their two affected daughters. First patient was 5,5 years old and the second patient was 4 years old. Epileptic seizures, mental retardation, muscle weakness, delayed motor milestones, delayed speech, development regression, dysmorphic findings, growth retardation, microcephaly, muscle weakness were noted in both girls. Cranial MRI findings of second child were microcephaly, bifrontal ploymicrogyria, ventricular dilatation, generalized calsifications, calsifications at thalamus, dental nuclues and pons. Whole exome sequencing (WES) was performed for patients and parents. After couple's consent, PGD was designed specificly for detected mutation and flanking informative markers

to exclude allele drop out and false positive /negative results. Multiplex Nested PCR followed by RFLP or Minisequencing to gether with STR analysis was performed.

Results: WES performed on both siblings and parents revealed homozygous previously unreported variant c.173_194del (p.Trp58Phefs*10)in OCLN gene. Both parents are heterozygous carrier of the detected variant. In the light of this results PGD was suggested to this family and they have consented to PGD application.

PGD results revealed absence of mutation in 2 embryos (designated as normal) . Two embryos were heterozygous and 2 embryos were homozygous for c.173_194deletion.
According to PGD results 2 embryos were normal, 2 and 2 were found to be homozygously mutant. After transfer of normal embryo to mother succesfull pregnancy was observed.

Conclusions:WES identified the novel OCLN gene mutation in a family. Due to rareness of disease and complexity of differential diagnosis for ICC and polymicrogyria, WES was the best appropriate method for those patients. Our case presentation emphasizes the importance of WES analysis in families with undiagnosed genetic disorder to enable PGD. To our knowledge this is the first PGD application for Band like calcification with simplified gyration and polymicrogyria (BLC-PMG).

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Suggested indications of preimplantation genetic screening (PGS)
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Introduction: Although there have been improvements in a process of in vitro fertilization-embryo transfer (IVF-ET), implantation rates are reported to be approximately 30% in women less than 35 years old and less than 10% in women more than 40 years old. Approximately 50-75% of spontaneous miscarriages are due to numeric chromosomal abnormalities of embryos. Recently, preimplantation genetic screening (PGS) seems to be one of the methods in screening chromosomally abnormal embryos. Therefore, in this study, we are to study on indications of women and embryos undergoing PGS for successful pregnancy outcomes.

Methods: Total 51 cases of infertile couples who visited, underwent IVF and agreed to do PGS at Gil hospital in between August 2014 and January 2016 were included in this study. Data were collected by reviewing charts retrospectively. PGS was recommended to couples under following 4 indications; woman's age ≥ 38 years old, abnormal serum karyotyping in at least one parent, history of recurrent pregnancy loss or recurrent implantation failure. From a good quality embryo (grade I, II), embryo biopsy was done on day 3 and whole genomic amplification (WGA) with CMA were done on the same day. Percentages of euploidy status were calculated and analyzed according to each indication.

Results: Total 234 embryos were sent for PGS and 188 (80.34%) embryos were found to be aneuploidy. We analyzed whether each indication has statistically significant effect on euploidy status. A percentage of euploidy status within embryos undergoing PGS among women's age ≥ 38 years old was 13.6 % and it had statistically significant impact on euploidy status (P-value = 0.001). Secondly, a percentage of euploidy status among couples who at least have abnormal serum karyotyping was 11.7 % and it had statistically significant impact on euploidy status (P-value = 0.032). However, having history of spontaneous miscarriages ≥ 2 times did not have significant impact on euploidy status (P-value = 0.30). Lastly, a percentage of euploidy status among couples having history of repeated implantation failure ≥ 2 times was 10.6 % and it had statistically significant impact on euploidy status (P-value = 0.004).

Conclusion: Although embryos are graded as good qualities morphologically, couples having risk factors of advanced maternal age, abnormal serum karyotyping or history of repeated implantation failure ≥ 2 times were found to have statistically significant impact on aneuploidy status. Since these embryos would lead to low potentials of resulting successful deliveries, PGS should be emphasized and recommended especially to these couples for better pregnancy outcomes.

Preimplantation Genetic Diagnosis of Pompe disease using one-step multiplex PCR

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INTRODUCTION: Glycogen storage disease type II (GSD II), or Pompe disease, is classified by age of onset, organ involvement, severity, and rate of progression. Classic infantile-onset Pompe disease may be apparent in utero but more often presents in the first two months of life with hypotonia, generalized muscle weakness, cardiomegaly and hypertrophic cardiomyopathy, feeding difficulties, failure to thrive, respiratory distress, and hearing loss. Without treatment by enzyme replacement therapy (ERT), classic infantile-onset Pompe disease commonly results in death in the first year of life from progressive left ventricular outflow obstruction. The non-classic variant of infantile-onset Pompe disease usually presents within the first year of life with motor delays and/or slowly progressive muscle weakness, typically resulting in death from ventilatory failure in early childhood. *GAA* is the only gene in which mutation is known to cause GSD II. Glycogen storage disease type II (GSD II; Pompe disease) is inherited in an autosomal recessive manner.

Our couple have an affected child by Pompe, caused by two different mutations c.854C>G (p.Pro285Arg) and c.1798C>T (p.Arg600Cys). Primers for direct analysis of mutations were designed anda panel of four polymorphic STRs (D17S1822, CCDC40-20XAC, GAAdowns_20XAC and EIF4A3downs_18XTG) located surrounding the *GAA* gene, was selected to perform indirect molecular analysis of Pompe disease.

MATERIAL & METHODS: Genomic DNA was obtained from whole blood. Supernumerary IVF embryos not suitable for transfer or cryopreservation but suitable for biopsy were used as starting material. During previous segregation analysis we detected an insertion GAAint5+7ins7at exon 5 linked to paternal mutation c.854C>G of *GAA* gene.
After alkaline lysis of blastomeres, PCR mix was added. A one-step multiplex single-cell fluorescent PCR is used for the simultaneous amplification of the 4 markers and insertion linked to paternal mutation, using the QIAGEN® Multiplex PCR kit (QIAGEN, GmbH; Hilden, Germany). PCR products were analysed on an ABI3730 automated sequencer (Applied Biosystem, USA).

RESULTS: A clinical PGD for Pompe disease have been carried out using this method. Patient ovarian stimulation, oocyte retrieval, ICSI and biopsy procedures were carried out by standard protocols. 4 blastomeres from 4 embryos were genetically analyzed and results were obtained from all of them (100%). No ADO was detected. A healthy embryo was transferred on day 5, but not pregnancy was obtained.

CONCLUSIONS: None previously protocols for preimplantation genetic diagnosis for Pompe disease have been published. The co-amplification of insertion with four different microsatellites markers provides direct information about paternal mutation and indirect information for both mutations besides to ensure informativity but also to determine contamination and monosomy, thereby increasing the reliability of the results.

Decreased recombination in non-obstructive azoospermic patients associated with increased risk of sperm chromosomal abnormalities

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During the first meiotic division in spermatogenesis there are two critical events. First, synapsis between homologous chromosomes and formation of the synaptonemal complex (SC), which regulates sister chromatid cohesion and provides the template for localization of recombination machinery proteins. Secondly, recombination between homologous chromosomes which is essential for proper chromosome segregation in Meiosis I. Several studies have suggested that errors in recombination are

a major cause of aneuploidy in gametes. The aim of this study was to assess meiotic recombination in primary spermatocytes, SC length and the correlation with chromosomal abnormalities in testicular spermatozoa from infertile men with idiopathic non-obstructive azoospermia (NOA).

Meiotic progression, SC total length, meiotic recombination and sperm aneuploidy were evaluated in samples obtained from testicular biopsies of 23 non-obstructive azoospermia patients (NOA). The study group was compared with a control group formed of 10 post-vasectomized (OA) patients. Meiotic recombination was assessed using immunocytogenetics with primary monoclonal antibodies: anti-SCP3 directed to axial/lateral elements of the SC; CREST directed to the centromeres of chromosomes and a MutL homolog1 antibody (MLH1), which is a mismatch repair protein that co-localizes to sites of meiotic crossovers. We performed Fluorescence in situ Hybridization (FISH) for chromosomes 1, 4, 6, 13, 16, 18, 21, 22 in primary spermatocytes to evaluate recombination level per chromosome. MicroMeasure 3.3 was used to measure SC length. Aneuploidy and diploidy rates on testicular spermatozoa for chromosomes 13, 18, 21, X, and Y were analyzed by FISH. Chi-square test,t-Welch test and t-test was used for statistical comparisons.

In the NOA group, there was a significantly higher percentage of cells at zygotene stage, before completing sinapsis of homologous chromosomes (42.1% vs. 55.6%; p<0.0001).The mean number of MLH1 foci per cell showed a significant decrease in NOA group compared to the control group (44.9 ± 2.4 vs. 48.7 ± 1.5; p<0.0001). The mean number of MLH1 foci per specific chromosome was significantly decreased in the NOA group compared to the OA group for chromosome 1 (3.24 ± 0.73 vs. 3.66 ± 0.62; p≤0.0001),chromosome 4 (2.42 ± 0.59 vs. 2.67 ± 0.66; p= 0.0015), chromosome 6 (2.19 ± 0.60 vs. 2.64 ± 0.63; p≤0.0001), chromosome 16 (1.67 ± 0.48 vs. 1.88 ± 0.51; p≤0.0001), chromosome 18 (1.84 ± 0.45 vs. 2.06 ± 0.51; p≤0.0001), chromosome 21(0.95 ± 0.21 vs.1.00 ± 0.15; p= 0.0074) and chromosome 22 (1.03 ± 0.37 vs. 1.11 ± 0.34; p= 0.0307). Regarding SC total length, NOA group showed a significant decrease compared to the control group (284.69 ± 15.37 µm vs. 276.14± 21.65 µm; p<0.0001). FISH analysis in testicular spermatozoa showed an increase of chromosomal abnormalities in NOA patients compared to the control group (0.5 vs. 0.1; p<0.0001).

Our study shows a decrease in recombination levels and increased sperm aneuploidy rates in NOA patients. These findings would corroborate the correlation between both parameters and the higher aneuploidy risk for the offspring of NOA patients.These studies are crucial to understand the origin of aneuploidy and to better characterize different phenotypes that may contribute to higher sperm aneuploidy risk.

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Powerful NGS workflow that combines Preimplantation Genetic Diagnosis, informativity testing and chromosomal abnormality

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Introduction: Preimplantation Genetic Screening (PGS) and Diagnosis (PGD) refer to chromosomal and genetic profiling of embryos prior to implantation, and represent a reproductive option for couples at risk of having a child with a chromosomal anomaly or a specific monogenic disorder, respectively. Both involve the use of assisted reproductive technology combined with IVF techniques. PGS was originally performed using fluorescent in-situ hybridization. Its limitations in genome coverage were solved by array-based 24sure™ technology with demonstrated accuracy and improved implantation rates. In this way, NGS is the latest b*reakthrough encouraging method for PGS, offering reliability, higher throughput and personalized assays. As for*, PGD coupled with informativity testing on polymorphic markers become mandatory before IVF. Analysis of Single Nucleotide Polymorphisms (SNPs) linked to gene regions involved by mutation with NGS technology enables an all-in-one informativity testing, PGS and PGD shortly after embryo biopsy.

Material & Methods: This study comprised three phases. Firstly, a blinded PGS retrospective validation tested 20 day-5 embryo biopsies in triplicate by Ion PGM™ platform along with Ion ReproSeq™ as alternative NGS approach, Ion Aneuploidy workflow as traditional NGS (Thermo-Fisher-Scientific), and array-based 24sure™ Microarrays (Illumina). Secondly, a prospective PGS trial with Ion ReproSeq™ tested 2359 biopsies (92 day-3, 2267 day-5) recruited in 428 clinical IVF-cycles (June - December 2016). Thirdly, a PGS/PGD study over 50 day-5 embryos comprised seven couples where one or both members carried a point mutation in *PKD1*, *RET, L1CAM*, *LAMB3* or *UNC13D* genes. A high-

throughput tag-SNP genotyping combined with Ion-AmpliSeq™-targeted-sequencing on Ion PGM™ platformwas implemented to ensure a sufficient informativity. LD Tag-SNP tool (NIEHS, US) provided linkage disequilibrium for SNPs selected in 200,000bp flanking region within each gene.

Results: Along retrospective validation, 27 of 60 day-5 embryos resulted euploid and 12 aneuploid. Almost all triplicates showed the same ploidy status regardless of the PGS-technique employed. This first phase provided a 98.3% concordance (99/100; 95% Confidence Interval: 91.1%-99.9%) for aneuploidy screening by NGS-Ion ReproSeq™ in contrast to well-established microarray-based 24Sure™ and traditional NGS-Ion Aneuploidy workflow. In the prospective study, 2040 of 4718 embryos were euploid (62 day-3; 1978 day-5 embryos). A total of 651 aneuploid embryos presented monosomies (24.31%), 630 trisomies (23.52%), 654 full gain and losses (24.42%) and 743 partial imbalances (27.75%). Moreover, 16 unbalanced translocations were accurately identified from 6 balanced translocation carriers. To date, implantation rate is 60.69% (1238 embryos transferred). A panel of 130 different SNP markers was studied to ensure a sufficient informativity in PGD cases (dbSNP database). Around 15% of tag-SNP studied was fully informative in order to identify linked-point mutations carried by couples studied. No amplification failures in genomic regions involved by mutations, neither false positives nor negatives were detected. The overall allele-drop-out (ADO)rate was $\leq$ 3%.

Conclusions: Ion ReproSeq™ PGS by Ion PGM™ System is more accurate than others technologies and platforms. Turnaround time is substantially reduced to 10 hours in contrast to 24h-protocols. The presented tagging-SNP method combined with NGS-Ion AmpliSeq™-technology is reliable to determine mutation-free embryos avoiding the ADO in successful PGD cycles.

Hemi-NeSTR: Turbo batch primer design for targeted STR genotyping of single cells for PGD

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Background: Standard molecular PGD involves short tandem repeat (STR) genotyping of single cells from preimplantation oocytes or embryos. Accordingly, PGD laboratories generally possess well established PCR primer panels for commonly tested molecular disorders such as cystic fibrosis. However, in this day and age of whole exome sequencing and chromosomal microarray, new pathogenic mutations for molecular PGD testing are identified in uncharted genomic regions at a dizzying pace. To keep up with this demand, PGD labs must expeditiously design new STR amplicon panels on a weekly basis even though this manual batch primer design is overly time-consuming and tedious. Therefore, we aimed to design and assess a batch hemi-nested PCR primer design program to streamline new assay development for molecular PGD applications.

Materials and Methods: The Hemi-NeSTR program was designed for batch combination of primer3-based primer design with in silico PCR (isPCR) primer specificity checking. In the first step, Hemi-NeSTR is programmed to scan multiple STR (with flanking DNA) sequences in a genomic region of interest (usually 4Mb in size) for the longest stretch of di- or tri-nucleotide repeats in each target. Subsequently, primer3 designs multiple primer pairs per input target which are then sent for primer specificity checking to an isPCR server which has already tested every possible primer pair combination for specificity (predicted amplification of single amplicon only) against the human reference genome (hg19). For STR targets in which Hemi-NeSTR successfully identifies one specific primer3 designed primer pair, Hemi-NeSTR continues to design a primer3- and isPCR-certified external forward primer for coupling with the initial designed reverse primer. This feature facilitates automatic hemi-nested primer design of multiple target STR sequences at once.

Results: Over the course of one year, Hemi-NeSTR was used to design an average of 16.6 hemi-nesting STR-flanking amplicons in each of 39 different 4Mb sized genomic regions. For each region, 78.4%+/-18.7% of the designed primer pairs successfully amplified their respective STR targets. After linkage analysis, an average of 46.8%+/-21.4% of the designed STR amplicons were identified as informative for single cell haplotype analysis. Thus far, 17 novel mutation-flanking STR panels were applied in live PGD cases. Generally, each panel consisted of 5-6 informative STR markers for

haplotype analysis of single cell embryo biopsies. Using hemi-nested PCR amplification strategies, 97.2%+/-6.1% of the markers in each panel were successfully amplified with Hemi-NeSTR designed PCR primers. In all PGD cases, a diagnosis was achieved for each biopsy and currently there are 8 ongoing pregnancies from Hemi-NeSTR facilitated PGD.

Conclusions: Hemi-NeSTR completes hemi-nested PCR primer design of 30 different STR target sequences within 5 minutes. The equivalent manual operation requires a minimum of 2 full human work days. Thus, Hemi-NeSTR facilitates rapid new assay development without compromising on single cell amplification efficiency or endpoint embryo diagnostic effectiveness; a welcome addition to the PGD laboratory toolbox.

The reproductive history of PGD couples

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Introduction: Preimplantation genetic diagnosis (PGD) is aimed to couples at risk of transmitting a genetic disease to their offspring, or to carriers of balanced translocations.
In most cases, couples at risk of monogenic disorders are fertile couples. The main reason for them asking for PGD is the desire to avoid transmission of the disease to their child and to avoid the trauma related to a potential therapeutic abortion.
On the other hand, translocation carriers are exposed to implantation failure, (early or late) miscarriages or affected offspring. Their reproductive risk depends on the type of translocation, the chromosomes involved and the length of the translocated segment. In the general population, the incidence of translocations is 0.7%, but in couples who experienced more than 3 miscarriages this value reaches 5.2%.
The reproductive history of PGD couples is often very complex and sometimes traumatic, and this is one of the main reasons for them to ask for IVF with PGD. The aim of this study was to analyse the reproductive history of patients that turned to our Center for a PGD treatment cycle.

Materials and Methods: From 1994 to February 2016, 695 couples with an indication for PGD turned to our Center: 398 for single-gene disorders and 297 for translocations. Of these, 193 were reciprocal and 104 were Robertsonian translocations.

Results: Within the group of carriers of single-gene disorders, 218 couples (55%) had previous spontaneous pregnancies (371 pregnancies in total). In 160 cases diagnosed as affected by prenatal diagnosis, the couples opted for therapeutic abortion. In 126 cases (34 %) there was a live birth resulting in 18 healthy babies (14.3%), 15 healthy carriers (11.9%) and 93 children affected by the disease (73.8%). Of the affected babies, 23 (24.7%) died within the first 5 years. In the remaining 85 cases, the pregnancy resulted in a miscarriage.
Within the group of 193 reciprocal translocation carriers, 103 couples (53.3%) had previous spontaneous pregnancies (264 pregnancies in total). Of them, 208 resulted in a miscarriage. In 36 cases, the couples opted for therapeutic abortion following prenatal testing. In all 20 children (10.3%) were born: 5 of them were healthy with a normal karyotype, 7 were healthy carriers of the balanced translocation, while 8 children inherited an unbalanced translocation and 2 of them died as a consequence of it.
Within the group of Robertsonian translocation carriers, 41 couples (39.4%) had previous spontaneous pregnancies (91 pregnancies in total). Of them, 72 miscarriages occurred, and 9 couples opted for an abortion following prenatal testing. Only 10 pregnancies (11%) resulted in a live birth: 6 children were healthy with a normal karyotype, 2 were carriers of a balanced translocation and 2 inherited an unbalanced translocation.
Of the 398 couples at risk of transmission of a single-gene disorder, 82 underwent a PGD cycle at our Center resulting in 37 clinical pregnancies (45%) with a take-home baby rate of 36%
Among translocation carriers, 140 couples underwent PGD with the vast majority of the translocation diagnoses done by FISH (87%). In all, 42 clinical pregnancies were generated of which 31 were on term, accounting for 22% take-home baby rate per couple. Only 57% of the PGD cycles presented here received an embryo transfer. According to our data, the incidence of clinical pregnancies was 37% in the subgroup of patients that could perform at least one embryo transfer.

Conclusions: Although IVF with PGD is certainly a very demanding approach especially for fertile patients, it can represent a valuable reproductive option to prevent the birth of affected children and to avoid the experience of abortions, which is physically and emotionally burdensome for couples.
In translocation carriers, fertility is often significantly reduced and the incidence of miscarriage is very high. PGD can improve the reproductive performance of these couples, but due to the high frequency of unbalanced rearrangements, a good response to hormonal stimulation is crucial to have a good number of embryos for analysis.

Diagnostic and Clinical Outcomes:634 Cycles Using Karyomapping For Preimplantation Genetic Diagnosis Of Gene Disorders

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Introduction: Karyomapping is a linkage-based single nucleotide polymorphism technology proven to be highly efficient in preimplantation genetic diagnosis (PGD) diagnosis of inherited gene disorders. This technology can be applicable to most single gene disorder (SGD) patients undergoing IVF treatment. One of the advantages of karyomapping is that less optimization is needed prior to PGD case which decreases the waiting time for patients undergoing IVF and consequently shorten the time of achieving a pregnancy free from the inherited gene disorder. When requested, comprehensive chromosome screening (CCS) can be performed simultaneously with Karyomapping.

Materials and Methods: Between January 2014 and March 2016, Karyomapping was performed on 634 cases with a total of 3884 embryos biopsied at the blastocyst stage with subsequent embryonic cryopreservation. Each single trophectoderm sample was whole genome amplified and analyzed using Karyomapping (Illumina, USA). Additionally, a total of 553/634 cases (87.3%) requested comprehensive chromosome screening via microarray comparative genome hybridization (aCGH; 24sure, Illumina) or next generation sequencing (NGS; VeriSeq, Illumina). From the 634 cases performed, follow-up clinical data was collected for 453 PGD cycles with embryos suitable for transfer (free from the SGD and/or euploid). At the time of data collection, a total of 212 PGD cycles had embryonic transfer.

Results: Karyomapping was performed for different indications including 98 different gene disorders, small deletions or duplications that could not be detected by other methods such as aCGH and NGS and human leukocyte antigen (HLA) matching. Diagnostic results were available for 97.5% (3788/3884) of blastocyst biopsy samples. The implantation rate was 74.5% (140/188) for PGD+CCS (average maternal age: 34.0 years) and 64% (16/25) for PGD alone (average maternal age: 32.3 years). Single thawed blastocyst transfers were performed for most transfers with only 18 having two embryos transferred at one time. The average number of embryos suitable for transfer per cycle was 2.12 and 1.76 for cases undergoing PGD and PGD+CCS respectively. A total of 24 live births and 91 ongoing pregnancies were reported during data collection. Confirmatory testing was carried out for 6 cycles achieving pregnancy and results were in complete concordance with Karyomapping diagnosis. No misdiagnoses have been reported to date from the 115 cycles with successful pregnancy outcomes.

Conclusions: With the increase in patient awareness regarding availability of PGD and also, with the rise of pre-conception carrier screening testing, there is a rapidly growing demand for PGD. The use of a technology which will shorten preparation time for PGD of inherited gene disorders and will also provide comprehensive assessment of embryos at risk of inheriting a disorder, is highly beneficial for patients. Due to the high diagnostic accuracy, comprehensive analysis and short preparation time, Karyomapping, is a successful treatment strategy for patients requesting embryonic testing for inherited disorders. Additionally, higher implantation rates were observed for patients who underwent CCS in combination with karyomapping.

PGD experience for inherited breast and ovarian cancer

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INTRODUCTION: Thousands of PGD cycles have now been performed for single gene disorders (SGD), with PGD presently offered for some conditions that have historically not been an indication for prenatal diagnosis, such as late onset diseases with genetic predisposition and preimplantation HLA typing**.** We present here our PGD experience for breast and ovarian cancer risk assessment by *BRCA1* and *BRCA2* mutation analyses, as part of our overall PGD series of 4,501cycles for PGD, which is the world's largest PGD experience.

MATERIALS AND METHODS: Similar to other conditions with genetic predisposition, *BRCA1/2* mutation status is an important candidate for PGD, as couples with *BRCA1* and *BRCA2* mutations have a high risk of producing a female offspring with predisposition to breast and ovarian cancer. Our PGD experience for *BRCA1/2* mutation status includes128 PGD cycles performed for 66 couples at risk for offspring to inherit *BRCA1/2* mutations. PGD was performed either by blastomere or blastocyst sampling, followed by nested PCR, involving mutation and linked marker analysis. This included the analysis for42 *BRCA1* (29 of maternal and 14 of paternal origin), and 22 *BRCA2* mutations (18 of maternal and 4 of paternal origin).The most prevalent mutations were: 187 del AG (*BRCA1*; 25 of 42 *BRCA1* mutations tested), and 6174 del T (*BRCA2*; 12 of 22 *BRCA2* mutations tested), both of which are founder mutations in individuals of Ashkenazi Jewish ancestry. Concomitant aneuploidy testing was performed in 77 of 128 PGD cycles for couples of advanced maternal age, of which 43 cycles were tested by PCR and 34 by array-CGH or next generation sequencing (NGS).

RESULTS: Of a total of 993 embryos tested at the cleavage or blastocyst stage (562 and 431 respectively), 441 free of *BRCA1* and *BRCA2* mutations were detected, of which 117 were pre-selected for transfer in 70 cycles (1.6 embryos per transfer on the average), resulting in 39 (56%) clinical pregnancies and birth of 47 children without mutations predisposing to breast cancer. Despite the fact that only 34% of embryos tested by concomitant 24-aneuploidy mutation analysis were available for transfer, as high as an 80% pregnancy rate was observed, with corresponding overall reduction of spontaneous abortions (SA) rated to as low as 7.6% , with no SA observed in cycles with 24-chromosome aneuploidy testing.

CONCLUSION: A large series of PGD for *BRCA1/2* mutation status is presented, also involving a concomitant 24-chromosome aneuploidy testing for improvement of PGD outcome in at-risk couples with advanced reproductive age. The results support a practical value of PGD for cancer risk reduction in offspring at risk for inheriting parental mutations in cancer predisposition genes by profoundly reducing the likelihood of inheritance of the pathogenic variant and thus reducing the lifetime risk for developing breast, ovarian and other solid tumours in children of parents with pathogenic variants in cancer predisposition genes.

Importance of whole karyotype preimplantation screening for infertility patients with balanced translocations

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Introduction: Robertsonian translocations (ROB) and reciprocal translocations are rearrangements between nonhomologous chromosomes. They occur in 0.1% of the total population and 1% of patients with reproductive problems. Carriers of a balanced translocation are phenotypically normal, as there is no loss of genetic material. The pathological effect occurs when they segregate as unbalanced in the conceptus.This may be a cause of infertility, miscarriages and malformations in the offspring. Preimplantation genetic screening (PGS) is a common method in the assisted reproduction techniques (ART) for aneuploidy detection in human embryos. The main goal of PGS for ROB and reciprocal translocationsis to achieve live birth by either reducing the risk of recurrent spontaneous abortions or

to improve pregnancy rate in infertile couples.We report two cases which show the importance of PGS for couples, where one of the partners is a carrier of balanced translocation.

Materials and methods: In the presented cases we report two infertile couples with recurrent implantation failures (RIF). Karyotyping by G-banded metaphase chromosome analysis of cultured peripheral blood lymphocytes was performed in both couples using standard cytogenetic technique. After analysis, a partner carrier of balanced translocation was detected. The man in the first couple was reported as a carrier of ROB with karyotype 45,XY, t(14;21)(p10;p10). In the second case the woman was found as a reciprocal translocation carrier with karyotype 46, XX, t(1;16)(q12;q13). After genetic counseling, PGS was used according to the patients' will. For detection of chromosomal imbalances a laser-assisted biopsy was performed on cleavage *stage embryos (*day 3) followed by whole genome amplification (WGA) and microarray analysis was performed according to manufacturer's instructions.

Results: In the first case seven embryos were biopsied and analyzed, two of them were with euploid karyotype. The rest were with different chromosome anomalies – 46, XX; del(1)(q25q44), 50 XXY (+2; +5; +6; +X), 46, XX; dup(4)(p16q13); del (13)(q12q34), one of the embryos was with one copy 14th chromosome and the other one was chaotic.
In the second case six embryos were biopsied and analyzed. Two of them were euploid. The rest were with different chromosomal and sub chromosomal abnormalities – 45, X0, 47, XX (+17), 47, XXY (-12; +16; +X) and 47, XY (+16); del(1)(p12q44). On a day 5 Embryo Transfer (ET) was performed.

Conclusions: These case reports are a good example for reproductive failures at carriers of different balanced chromosomal rearrangements. Medical practice suggests the chromosomal translocations as a reason for infertility and low pregnancy rates. In both patients, chromosomal abnormalities, related with the translocations were found, but as seen, aberrations may occur in the other chromosomes, which can be the reason for low pregnancy rate and RIF. Application of the new microarraytechniques allows screening of all 24 chromosomes. It is objective, ensuring a high level of confidence in the results and subsequent selection of euploid embryos for transfer.

Validation and clinical application of NGS for aneuploidy screening in single cell and human blastocysts

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Introduction: This is a validation study of a Next-Generation Sequencing (NGS) platform using certified cell lines, in which whole chromosome aneuploidies were detected by NGS and the results were compared to CGH array. Additionaly, it was evaluated the capability of NGS to detect whole chromosome aneuploidies in single cells and trophectoderm biopsies. The clinical results of the first PGS cycles for different indications are also presented.

Material and methods: validation of NGS was performed firstly using Coriell cell lines with normal female and male karyotypes, 45,X0 and 47,XY,+9 and secondly with 133 single blastomeres and 61 throphectoderm biopsies. In the validation with Coriell cell lines for whole chromosome aneuploidies, 42 single cells and 38 cells pools (4-8 cells) to mimic blastomere and trophectoderm biopsies were selected. NGS results were compared for concordance rates with CGH array technology.
For NGS, samples were processed using an improved, 8 hours length protocol, with WGA and barcoding in batches of 15-24 samples, followed by Isothermal amplification, chip loading, sequencing in a PGM equip and analysis using IonReporter software (Thermo Fisher Scientific, Inc., MA, USA).For CGH array, amplified DNA and reference DNA were labeled and co-hybridized in 24sure arrays. After washing, slides were scanned and analyzed by BlueFuse Multi software (Illumina, Inc.).
After validation of the new technology, the clinical results of 329 PGS cycles performed with NGS from December 2015 to February 2016 were also described. Mean female age was 39.4 years, and indications for PGS were advanced maternal age, repetitive implantation failure, recurrent miscarriage, severe male factor and previous trisomic pregnancy.

Results: Validation studies in Coriell cell lines showed97.5% concordance rates per sample and 99.9% per chromosome between NGS and the expected karyotype. In the embryos, the concordance rates

between NGS and CGH array for single blastomeres were 99.2% per embryo and 99.9% per chromosome. For trophectoderm biopsies, concordance rates were 97.2% per embryo and 99.9% per chromosome.
Clinical results of the 329 PGS cycles showed 81.4% abnormal embryos on day-3 and 56.4% on trophectoderm biopsies. Pregnancy and implantation rates per transfer were 56.3% and 47.4% on day-3, and 52.2% and 42.4% on trophectoderm biopsies. Miscarriage rates were 4.7% on day-3 and 16.7% on trophectoderm biopsies.

Conclusion: NGS allows the detection of whole chromosome aneuploidies in single blastomeres and blastocyst with the same efficiency as aCGH. An improved NGS protocol allows its application to fresh transfers as well as for vitrified cycles. Preliminar clinical results showed high pregnancy and implantation rates, comparable to previous published CGH array results by our group.

Preimplantation genetic screening importance for reproductive genetic counseling.
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Introduction: Preimplantation genetic screening (PGS) has become an important tool for Assisted Reproduction Technology (ART). The high percentage of aneuploid preimplantation embryos observed in several studies contributes to explain the low implantation rates in in vitro fertilization (IVF). PGS allows selection of euploid embryos for transfer in order to reduce the risk of pregnancy failure. Here we present our preliminary experience with PGS in a South Brazilian ART Clinic.

Methods: We have reviewed PGS cycles from 2012 until February 2016 in our ART clinic. PGS were performed by **Comparative genomic hybridization (CGH)**.

Results: Since 2012, a total of 204 cycles with PGS were performed. From the 706 biopsied embryos, only 10.75% were euploid and there was a strong correlation with aneuploidy and advanced maternal age (42,3% in women bellow 35 years of age and less than 10% above 40 years).

Conclusions: Our results corroborate the elevated rate of aneuploidy in human conceptions and the relation with advanced maternal age. These findings reinforce the importance of PGS for reproductive genetic counseling, allowing to explain pregnancy failures and preventing miscarriages and potentially viable aneuploid pregnancies.

Correlation between blastocyst morphology and clinical outcome
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Objective: To investigate of the correlation between the clinical outcome of euploid blastocyst and morphology evaluation of frozen-thawed single embryo transfer (SET) cycles.

Introduction: The key of successful IVF treatment is the selection of a high potential embryo leading to healthy pregnancy and baby. Nowadays, many techniques have been utilized clinically to improve the treatment. For example, single blastocyst transferred is the approach to reduce multiple pregnancies. It is well evident that the selection of the best euploid quality embryo is the important factor for an increased success rate. Only few reports with unclear evidence regarding the correlation between morphology of euploid blastocyst and implantation potential are available to date. Although this knowledge may indeed enhance the selection of potential euploid embryo, we still lack of data based on the proper research methodology.

Material and methods: A retrospective study was carried out by including infertile patients who had undergone ICSI with PGS using next-generation sequencing (NGS) between January - September 2015. Prior to trophectoderm (TE) biopsy for NGS, blastocyst morphology was assessed following standard morphology criteria. Blastocysts biopsied were performed in expanded and hatching

blastocysts on day 5 or 6 and categorized them into three groups: good (≥ 3AB; 3AA, 3BA, 3AB, 4AB, 4BA, 4AA), moderate (2BB, 3BB, 4BB) and poor (≤ 2BC; 2BC, 3BC, 4BC, 3CC, 4CC). After that, all biopsied blastocysts were frozen by vitrification technique. The biopsied cells were processed and analyzed using NGS. In frozen-thawed cycles, only one euploid with good quality was thawed and transferred. Chi-square statistic was used to test the correlation between morphology, developmental and clinical pregnancy rate of euploid blastocyst in frozen-thawed single embryo transfer cycle.

Result: Total 182 blastocysts were thawed and transferred, 64 from good euploid morphology, 84 from moderate euploid morphology and 34 from poor euploid morphology. There was no significant difference in maternal age between three groups; 34.5±5 years, 34.2±4.5, 34.6±3.9 for the good, moderate and poor euploid morphology respectively ($P > 0.05$). The clinical pregnancy rate of frozen-thawed single euploid blastocyst transferred was 60% (109/182),in good morphology group 62% (41/66), moderate group 59.8% (49/82), poor group 55.9% (19/34), $P > 0.05$). Ongoing pregnancy rate in good euploid 15/33 (45%), moderate euploid 28/53 (52%) and poor morphology 8/23 (34%) was found. Interestingly, the highest in live birth rate in poor euploid group was 54%, 48% in moderate euploid blastocyst and good euploid blastocyst was 42%.

Conclusion: Assessment of blastocyst morphology may not be an essential approach for improving the selection of euploid embryo. In conclusion, our data indicate that all blastocysts with poor morphology have to be biopsied as far as FET cycle is concerned.

Keywords: Next-generation sequencing, blastocyst, single embryo transferred

Simultaneous detection of a BRCA1 mutation, gender, and a translocation (including balanced versus normal status).

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Introduction: Karyomapping is a technology that can be used to target multiple loci in one sample, and can theoretically be used to look for structural imbalances and distinguish between balanced and normal chromosome status. In this work, Karyomapping was used clinically to examine the inheritance of a BRCA1 mutation, to identify gender, as well as attempt to determine unbalanced versus balanced versus normal chromosome status of chromosomes 1 and 6 in embryos of a woman carrying a BRCA1 mutation and a t(1:6)(q25.3;p11.2) reciprocal translocation.

Material & methods: Inheritance and breakpoints of the translocation involving chromosomes 1 and 6 were unknown. Chromosome flow sorting was performed to isolate single derivative chromosomes and run on a Human Karyomap-12 beadchip to identify the position of the breakpoints of the translocation. DNA was obtained from the parents of the translocation carrier and this was used alongside the sorted derivative chromosomes to determine the inheritance of the translocation to be used for Karyomapping analysis of the translocation. The patient underwent a cycle of IVF and the test was performed on single trophectoderm samples taken from each of her embryos.

Results: Using Karyomapping we were able to determine the BRCA1 status of all embryos, as well as the gender. Male embryos with the BRCA1 mutation were considered acceptable hence the request for gender selection in addition to the BRCA1 mutation. No segmental trisomies or deletions of chromosomes 1 and 6 were observed using Karyomapping suggesting all embryos were either balanced or normal for the translocation. This was confirmed using array CGH. By interrogating the region of the translocation breakpoints in the embryos we were able to determine the balanced versus normal status of each embryo.

Conclusions: Karyomapping allowed the detection of the BRCA1 status, gender and translocation status in a single trophectoderm sample from each embryo. Importantly, we were also able to show that Karyomapping is a technology that can be used in PGD for this (and probably other) chromosome translocations and, in contrast to array CGH, is able to determine the balanced versus normal status of each embryo allowing the preferential transfer an embryo that has not inherited the derivative chromosomes, if available.

Is PGD an alternative to prenatal diagnosis?

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Introduction. PGD is currently one of the options available for couples at risk of having children with genetic or chromosomal disorders. According to the data from dedicated registries, the number of patients undergoing IVF is constantly increasing. At the same time, the availability of increasing sophisticated technology is expanding potential indications for PGD, thus making this technique more commonly used. The birth of several thousand unaffected children suggests that PGD is accurate, reliable, and safe. Therefore, it is presently a valuable option for carriers of single gene disorders, for carriers of balanced translocations, and for the deselection of age-related aneuploidies in patients at risk of generating aneuploid embryos.
Despite its increasing reliability, we believe that PGD should not be considered as an alternative to prenatal diagnosis, whose accuracy is generally greater than 99%. There is no doubt that PGD reduces significantly the probability of transferring affected embryos, but it does not entirely eliminate the risk. This should be clearly explained to patients, who are then free to decide whether to have prenatal testing to confirm PGD results.
The aim of this study is to follow-up our PGD pregnancies to verify the attitude of patients towards our recommendation to undergo prenatal diagnosis.

Materials and methods. 440 PGD clinical pregnancies were included in the study. They were generated during the years 1996-2015 following PGD for single gene disorders, translocations and aneuploidy. Patients were periodically contacted by phone to update the pregnancy follow-up. All relevant documents regarding prenatal testing, abortions or deliveries were included in their clinical records.

Results. 95 of the 440 clinical pregnancies (22%) ended in miscarriage. Of the remaining 345 pregnancies, 232 decided to undergo prenatal diagnosis (67%). When looking at the indications, all ongoing pregnancies generated by PGD for single gene disorders underwent prenatal diagnosis having previously experienced both therapeutic abortions (31.3%) as well as miscarriages (12.5%). In the group of translocation carriers, 91% of ongoing pregnancies decided for prenatal diagnosis; 25% of them had already had therapeutic abortions and 45% miscarriages. Finally, 61% of PGD for aneuploidy cases opted for prenatal diagnosis.
In all cases, PGD results were confirmed by prenatal diagnosis with the exception of one case in which the analysis was done only on the first polar body. Following amniocentesis, one patient aborted; the fetal karyotype was found to be normal.

Conclusions. During PGD cycles, we recommend to all our patients to undergo prenatal diagnosis in case of pregnancy. Some patients understand that the genetic analysis on the fetus can provide a stronger level of assurance compared to the accuracy of PGD results. This awareness seems to be proportional to the reproductive risk of the couple, that is very high in carriers of single gene disorders and translocations, and lower in patients exposed to the risk of aneuploid pregnancies. Other patients are very concerned about the risk of abortion associated with invasive prenatal testing, and for this reason decide not to confirm the PGD results prior to delivery.
Whether or not to have prenatal diagnosis after PGD is a personal decision. It is our duty to provide patients with all necessary information about the limitations of PGD compared to prenatal diagnosis in order to assist them in taking a conscious decision.

Concomitant Testing of Retinitis Pigmentosa and Stargart Syndrome by Preimplantation Genetic Diagnosis

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Introduction: Preimplantation genetic diagnosis (PGD) is an effective method for couples at risk of transmitting a genetic disorder to their offspring. The use of short tandem repeat (STR) polymorphisms for linkage information coupled with mutation testing can also be used to evaluate two different monogenic disorders simultaneously. Here we present a clinical case where concomitant testing of retinitis pigmentosa and stargart syndrome was performed by preimplantation genetic testing resulting with a successful pregnancy.

Materials and Methods: Female patient was affected with autosomal dominant retinitis pigmentosa type 7 (*PRPH2 – p.Ile32Val*) as well autosomal dominant stargardt syndrome type 4 (*PROM1 – p.Arg373Cys*). After PCR amplification and fragment analysis of STR markers designed for both genes, 6 informative markers were obtained for both genes and linkage information was established by testing of patient's parents for the mutations using restriction fragment length polymorphism (RFLP). Out of 19 oocytes collected, 9 of them were mature and 8 of them were fertilized. Trophectoderm biopsy was performed to 3 embryos followed by mutation analysis using STR marker fragment analysis coupled with RFLP analysis.

Results: Out of 3 embryos analyzed, 2 of them were unaffected for both of the conditions. One of the embryos was transferred fresh to and the other embryo was cryopreserved. Pregnancy was obtained but resulted in missed abortion on week 7. After 3 months, the frozen embryo was thawed and transferred to the patient resulting with ongoing pregnancy.

Conclusion: Multiplex PCR technique has been mainly used to diagnose single-gene disorders in single cells biopsied from embryos of couples at risk of transmitting a monogenic disease (autosomal recessive, autosomal dominant, or X-linked) to their offspring. Almost all genetically inherited conditions diagnosed prenatally can also be detected with this approach. Similarly the method can also be used to evaluate multiple monogenic diseases at the same time when the limitations of the multiplex PCR technique like complexity, low amplification efficiency and sensitivity, are resolved. Especially annealing temperatures for each of the primer sets for both of the diseases must be optimized to work correctly within a single reaction.

Keywords: preimplantation genetic diagnosis, monogenic disorders, short tandem repeat (STR) analysis, restriction fragment length polymorphism (RFLP)

Trends in chromosomal segregation revealed by array CGH examination of embryos from translocation carriers.

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Introduction: Balanced carriers of reciprocal translocation suffer from reduced fertility and are at increased risk of recurrent spontaneous abortions or chromosomally unbalanced offspring. During meiosis I, segregation of the translocated chromosomes and their normal homologues produces a variety of unbalanced gametes. Preimplantation genetic diagnosis (PGD) using array CGH allows not only a selection of normal embryos but also provides unique information about the precise breakpoint location and type of chromosome segregation.

Material & methods: Array CGH (24sure+, Illumina and GenetiSure Prescreen Kit 8x60K Agilent) was used to examine the trophectoderm of 266 embryos from 38 reciprocal translocation carriers (18 female / 20 male carriers). Each translocation was evaluated as the ratio of the sum of centric segments (ΣC) to the translocated (acentric) segments (ΣT). The regression analysis was used to compare $\Sigma C/\Sigma T$ *vs* segregation type.

Results: Each reciprocal translocation had a different segregation mode, but at least one embryo with alternate segregation (producing balanced chromosomal constitution) was found in 34 translocation types (89.5 %). This alternate segregation was found in total of 82 (31 %) embryos. The lengths of acentric translocated segments varied from 1.6 Mb to 140.4Mb (2% to 73% of the chromosome involved, respectively). No statistical difference in chromosome abnormalities in reciprocal translocation between male and female carriers was observed. Comparison of the length of translocated chromosome segment and the type of segregation revealed a trend for lower proportion of alternate chromosomal segregation (balanced embryos) when shorter acentric chromosome segments were involved.

Conclusions: This finding provides a hint of the explanation why some balanced translocation carriers produce more chromosomally imbalanced embryos. Consequently, preconception counselling in these couples might focus more on alternative IVF approaches exploiting donor cells.

Management of poor prognosis patients undergoing IVF: Should we offer PGS in difficult cases?

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Introduction: A number of complex entities, including advanced maternal age (AMA), recurrent miscarriages (RM) and recurrent IVF failures (RIF), may increase the risk of numerical chromosomal abnormalities of the embryos. There is limited data regarding to implantation and pregnancy rates for these poor prognosis patients undergoing comprehensive chromosomal screening (CCS) by array comparative genomic hybridisation (aCGH). The aim of the present study was to analyse if the use of aCGH for PGS improves the implantation and pregnancy rates with reduced spontaneous abortions in poor prognosis patients with complex fertility backgrounds and to establish a generalised management scheme in these complex cases. We further aimed to analyse if there are differences in the aneuploidy rates between embryos cultured in all-in-one time-lapse incubation (TLI) systems and standard incubators (SI) in PGS cycles.

Materials and Methods: PGS was performed for the couples with at least one of the following factors; maternal age of 36 years or more, at least three previous miscarriages, at least three previous IVF failures and normal karyotype. A control group of poor prognosis patients with AMA, RM and/or RIF and who did not choose to undergo PGS was included in the analysis to compare the clinical results with the PGS group. Sixty six patients were included in the PGS group and informed consent was obtained from all the couples prior to each PGS cycle. A total of 205 embryos were biopsied on day 5/6 of development. Whole genome amplification (SurePlex, Invitrogen) and aCGH was performed (24Sure v3 array kit, Invitrogen). The implantation and pregnancy rates for each group were analysed.

Results: PGS results showed that 78% of the embryos obtained from poor prognosis patients were aneuploid. Twenty three patients with transferable embryos had one or two with the mean of 1.3 embryo transfers. The number of embryos transferred was slightly higher in the control group (1.6). The implantation rate for the PGS group was 11% higher compared to the control group (p=0.1). The pregnancy loss rates for both PGS (28%) and control groups (23%) were similar.
The aneuploidy rate was further investigated in these embryos cultured in a TLI system (69) and in a conventional incubator (157). The rate of aneploidy was exactly the same for the embryos cultured in a TLI system (72%) and in a conventional incubator (72%).

Conclusions: In this study, we have shown that poor prognosis patients develop a higher number of chromosomally abnormal embryos. This incidence of high abnormal embryos may explain the low pregnancy rates due to IVF failure and miscarriages. This study showed that offering PGS to poor prognosis patients may lower the psychological distress and reduce the financial difficulties that the patients may undergo. We have shown that applying PGS to these patients to select a euploid blastocyst eliminates the unnecessary embryo transfer that may not result in pregnancy or lead to a miscarriage as well as improving the IVF outcome.

First NGS Based Comprehensive Chromosome Screening Data From Turkey

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NGS has been widely used in all genetic fields and recently it has become an encouring tool for reproductive genetics. As a center with high expertise in FISH analysis we have been observing a switch to NGS based PGS applications recently which has been more dramatic compare to array based technologies we have been performing before. The purpose of this report is to document Mikrogen Genetic Laboratory's experience on NGS based comprehensive chromosome screening and to present the data applied in our center between July 2015 - March 2016.

Material&Methods: All single cells were collected in 2µl PBS solution. Whole genome amplification procedure was performed with SurePlex DNA Amplification Kit (Illumina, Inc). Amplified samples for

NGS were processed with VeriSeq PGS kit (Illumina). Whole procedure was performed according to VeriSeq PGS workflow (Illumina, Inc.). The following bioinformatics analysis was accomplished with a pre-release version of BlueFuse Multi for NGS (Illumina, Inc.) (http://www.illumina.com/products/veriseq-pgs.html).

Results: In total of 110 patients 336 embryos were screened using NGS (Next-Generation Sequencing). 10 of these patients were diagnosed with chromosomal translocations. 108 (%32) of these 336 embryos were normal. 65 (%19) aneuploidies, 125(%37) complex aneuploidies,13 (%4) mosaicism and 9(%2) partial deletion /duplications were observed. 16(%5) embryos were not determined due to amplification failure. Rather than 13,16,17,18,21,22,X,Y chromosomes detected by FISH, NGS identified aneuploidies in other chromomes as well. If aneuploidies were detected by FISH methodology 55% of the embryos would be normal. However, NGS showed that 34% of the embryos were normal. NGS was able to detect chromosomal aneuploidies which can not be detected by FISH in 21% of the embryos. We also observed that NGS is a robust technology for unbalanced translocation detection (Figure 1). NGS technology is valuable for translocation screening as the erros of other chromosomes can be determined at the same time.

Conclusion : Chromosomal copy number assessment based on NGS is very recent technology and offers encouraging advances towards improved PGS. Especially increased dynamic range enabling enhanced detection of mosaicism in multicellular samples (Fiorentino et. al., 2014). Cinical usage of NGS technolgy in IVF field becomes widespread due to robust methodology. Unbalanced translocation screening with NGS technology will be very important milestone in PGD applications.

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First experience with the 24Sure+ in PGD of balanced translocations and other selected structural aberrations

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Introduction: About 9 % of couples worldwide have experience with infertility. Structural chromosome abnormalities occur with frequency of 0, 19 % in the human population. Reciprocal translocations are the most common structural abnormalities in human and they occur with frequency 0, 6 % in newborns. In our study we use aCGH as a screening method in PGD of reciprocal translocation and chromosome rearrangements.

Material and methods: 12 couples with chromosomal rearrangements from 4 IVF centres in Czech Republic were included into study. 63 trophectoderm and 9 blastomere samples (total 72) were analysed by aCGH (24Sure+, Illumina).

Results: From December 2014 to March of 2016 the trophectoderm and blastomere samples from 12 couples were tested for PGD of chromosomal rearrangements. Results were obtained from 64 samples (88,9%), 8 samples were not properly amplified by WGA. 35 samples from analysed samples (54,6%) carried unbalanced translocation, either alone or in combination with other chromosomal imbalancies. A further 15 samples (23, 4%) were normal/balanced for the rearranged chromosomes, but affected by aneuploidy of other chromosomes. Only 9 samples (14, 1 %) were with normal number of chromosomes. Of the 12 patients who completed their treatment cycles, 2 became pregnant. In the first case, the miscarriage occurred. In the second case, the pregnancy is continuing. In one case, couple with complex rearrangement decided to use donor gametes after 4 unsuccessful IVF cycles and patient became pregnant.

Conclusion: In our study, we performed PGD by aCGH for diagnosis of structural abnormalities in early human embryos. It´s evident, that embryos of carriers of balanced chromosomal aberrations have many structural and numerical changes, in addition to possible unbalanced changes of affected chromosomes. In these cases, it is important to detect unbalanced translocations and aneuploidies of all chromosomes and aCGH is useful method.

Validation and implementation of karyomapping at UZ Brussel.

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Introduction: An implementation validation study was performed to assess karyomapping as a generic method for PGD for monogenic disorders at a large tertiary centre for reproductive medicine and PGD. Criteria: >99% accuracy and >90% efficiency.

Materials and methods: Part A Validation study: Embryos that were not genetically transferable after PGD with PCR were collected at D4 or D5. Embryos were dissociated and tubed as single cells (SC; 78 SC from 46 embryos from 13 PGD cycles) or multi-cell samples with 5-7 cells (MCS; 117 MCS from 81 embryos from 18 PGD cycles) and amplified by Multiple Displacement Amplification (MDA; Repli-G kit, Qiagen). Karyomapping (Illumina) was performed on a subset of 45 SC and 73 MCS with amplification.
Part B Clinical cycles: Mean maternal age at oocyte retrieval was 33,6 years. PGD cycles were performed on trophectoderm (TE) biopsies containing 5-7 cells obtained at D5 or D6. All embryos were cryopreserved and analysed by karyomapping. Genetically transferable embryos were transferred in an unstimulated cycle.

Results: Part A Validation study: The efficiency (% embryos diagnosed) of the karyomapping procedure for a single indication was 66.2% for SC samples and 82.3% for MCS. All embryos for which diagnosis was obtained by both PCR and SNP array were found concordant (32/32 diagnoses on SC, 55/55 diagnoses on MCS). Even though efficiency was below our set threshold for MCS, we introduced karyomapping as we expected the efficiency in clinical PGD cycles with TE-biopsy to become higher (better embryo quality and more experience).
Part B Clinical cycles. Out of 30 performed workups 27 were sufficiently informative for karyomapping. From 16 started PGD cycles 15 lead to biopsy. In total 69/74 embryos (93,2% efficiency) were diagnosed (3x no MDA, 1x poor quality SNP array, 1x recombination). Affected embryos and embryos with aneuploidy were excluded, resulting in 29 genetically transferable embryos. For 4 cycles no genetically transferable embryo was available (4/16, 25%). So far, 10 PGD cycles led to 14 transfers of 16 embryos in total. Implantation was observed for 8 embryos resulting in 7 early pregnancies (8/16, 50% Fetal heart beat per embryo transferred).

Conclusions:-MDA amplification followed by SNP array and karyomapping on MCS is accurate as we observed no discordances in 32 diagnoses on SC and 55 diagnoses on MCS. The estimated efficiency was too low for SC samples (66.2%) and MCS samples (82.3%) during the validation study.
-Karyomapping was introduced in the clinic. In clinical cycles efficiency was much higher (93.2%, 69/74 embryos diagnosed), meeting the set criterion. Even though preliminary, implantation rate seems promising.

Preimplantation genetic diagnosis for aneuploidy (PGD-A) increases sustained implantation and reduces miscarriage rates

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Introduction: There is a significant body of evidence that human embryos produced in order to treat infertility carry numerical chromosome abnormalities in a high frequency. One of the biggest achievements in assisted reproduction is the implementation of comprehensive chromosome screening (CCS) to be able to select competent embryos for transfer. Recent meta-analyses show that comprehensive chromosome screening is a useful embryo selection tool that results in higher

implantation and lower miscarriage rates. The aim of this retrospective analysis is to compare the conventional morphology-based selection (MBS) method to the use of PGD-A in case of patients with advanced maternal age.

Material & methods: Data were collected between January of 2013 and December of 2015 at our clinic. All autologous cycles were included with advanced maternal age (AMA; 37-42 years of age) and fresh embryo transfer. Patients with known cromosome rearrangement were excluded.
Two patient groups were retrospectively formed based on the embryo selection method used. In PGD-A group the transferred embryo(s) were euploid, while in MBS group transferred embryos had an unidentified chromosome profile.
The type of fertilization was ICSI or IVF in the MBS group while in PGD-A group only ICSI was used. In case of PGD-A group the embryo biopsies were carried out on day 3 and the samples were analyzed with 24sure BAC microarrays (Bluegnome, Illumina). In both groups embryos were transferred at day 5.
Positive chemical pregnancy was considered if βhCG level in blood serum was above 15 mIU/ml and pregnancy was considered clinical if fetal heart beat was seen. If pregnancy had completed at least 12 weeks of gestation it was considered as ongoing pregnancy. Miscarriage was noted in case of pregnancy loss after detected heart-beat.

Results: In the PGD-A group 78 euploid embryos were transferred in 63 cycles with an average number of 1.24 embryos per cycle. The chemical, clinical and ongoing pregnancy rates were 50,79%, 41,27% and 36,51%, respectively.
In case of MBS cycles the average number of transferred embryos was significantly higher (1,54 vs. 1,24; $p<0,01$) but the chemical (46,05%), clinical (32,89%) and ongoing pregnancy (22.37%) rates were similar compared to the PGD-A group.
Despite the similar pregnancy rates there was a significantly higher ($p<0,05$) miscarriage rate in MBS group. These embryos had a 6-fold risk (95 % CI: 1,18 to 31,25) for miscarriage after a detected heart-beat compared to the euploid embryos ($p<0,05$). The sustained implantation rate were significantly higher in the PGD-A group (34,62% vs. 16,24%, $p<0,01$).

Conclusions: Our results demonstrate that PGD-A is a more effective technique for AMA patients to select viable embryos for transfer than conventional morphology-based selection. Despite the similar pregnancy rates, with the use of CCS not only the number of embryos transferred can be decreased but less patients experience miscarriage that is considered a major benefit compare to conventional IVF/ICSI treatments.

Clinical results of single gene defects PGD included aneuploidy detection by karyomapping

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Introduction: The aim of the retrospective study is to evaluate the clinical implications and results of PGD cycles using Karyomapping integrating the aneuploidy screening and a detection of maternal and paternal origin of aneuploidy in one test.

Material & methods: In period May 2014 – February 2016 a set of 476 embryos were evaluated in 92 couples using Karyomapping (Illumina - HumanKaryomap v12 SNP bead chips, BlueFuse Multi software) were carried out. Totally 38 different single gene defects or concrete loci structural rearrangements were analysed (autosomal dominant, autosomal recessive and X-linked type of inheritance). All 310 blastocysts were vitrified after the laser biopsy.

Results: In total 97,4% of embryos were sucesfully evaluated. An unsuitable hyplotype was confirmed in 31% of embryos (96/310). 24,5% (76/310) of blastocysts were excluded due to aneuploidy. Totally 94% of all aneuploidies were of maternal origin. 37% of blastocysts were available for the embryo transfer and 53 single embryo transfers were performed with the implantation rate (IR) 42%. 22 clinical pregnancies were achieved, other couples are waiting for the transfer. This represents a cummulative clinical pregnancy rate 63 % per couple per single embryo transfer. About 88% of whole chromosome aneuploidies were of maternal origin resp. 12 % of paternal origin. Maternally derived aneuploidies consisted in vast majority of monosomies 93/159 (59%). 66,5% of maternal trisomies were originated

from meiosis I (M I) compared to 33,5% from meiosis II (M II). Paternal trisomies were detected only in 13,7% (3/22) compared to 86,3% of paternal monosomies (19/22). Paternal origin of segmental aneuploidies ratio was 82% by contrast of 16% of maternal ones.

Conclusions: Karyomapping is an optimal, universal, effective and fully proven diagnostic tool enabling a two-step embryo selection covering both a single gene defect detection and an aneuploidy detetion. Besides the improvement in pregnancy rates compared to a preimplantation genetic haplotyping (PGH) there is a benefit to study the origin of aneuploidies. Vast majority of whole chromosome aneuploidies are of maternal original compared to the segmantal chromosome abnormities which are mostly of paternal origin.

A mathematical correction method to accurately determine mitochondrial DNA levels in human embryos

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Introduction: In accordance with the quiet embryo hypothesis (Leese 2002), the level of mitochondrial DNA (mtDNA) has been proposed as a biomarker for implantation potential amongst euploid embryos, and overall differences in mtDNA amounts have been shown between embryos grouped by ploidy and age (Fragouli et al. 2015; Diez-Juan et al. 2015). We developed a mathematical formula resulting in an accurate calculation of mitochondrial levels as established by next generation sequencing (NGS) or quantitative polymerase chain reaction (qPCR).

Materials & Methods: We tested mtDNA content of 833 embryos derived from 181 patients by NGS, and 150 embryos derived from 96 patients by qPCR. For each embryo, the level of mtDNA was determined from a trophectoderm biopsy by whole genome amplification followed by NGS and/or qPCR. The value was subjected to mathematical analysis tailored to the genomic DNA composition of said embryo.

Results: On average our quantitation method changed the conventionally determined mtDNA level via NGS by 1.35% +/-1.58%, with changes ranging up to 17.42%, and via qPCR by 1.33% +/-8.08%, with changes ranging up to 50.00%. Our data also shows a proof of principle how the correction factor can considerably impact the P value when calculating the significance of mean changes between embryo groups substratified by ploidy, age, or implantation potential.

Conclusions: We recommend the implementation of our correction factor to all laboratories evaluating mtDNA levels in their embryos by NGS or qPCR.

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Preimplantation Genetic Screening in Parthenogenesis and Poor Quality Embryos

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Introduction: During in vitro fertilization procedure, not all of the patients had to get the good quality of oocyte nor embryo. Approximately the 3% of embryo cultured in vitro are parthenogenesis oocytes, that may initiate early embryonic development leading to preimplantation embryonic death, implantation failure and spontaneous abortions. In other cases, in IVF programme show some repeatedly abnormal embryonic development as soon as day 2 post intracytoplasmic sperm injection by showing >40% fragmentation or dark cytoplasm leading to recurrent IVF failure. In this study, we investigate the frequency of aneuploidy and mosaicism in embryos obtained from parthenogenesis oocyte and poor morphological embryos using Preimplantation Genetic Screening (PGS).

Material & Methods: Ten embryos under IVF treatment that were found to be abnormal or morphologically insufficient were cultured until day 5, then one of the blastomere was biopsied. Embryos were divided into three groups (parthenogenesis oocyte, poor quality embryos with dark cytoplasm; many vacuoles or fragments in cytoplasm; arrested embryos, and good quality embryos). All of the samples were detected for abnormalities by using array-Comparative Genomic Hybridization (a-CGH).

Results: All embryos were observed until day 5 for morphological and developmental progress. One embryos develop from parthenogenesis oocyte, had a trisomy in 6 chromosome and monosomy in 4 chromosome. In seven poor quality embryos, 5 of them had aneuploidy in more than 8 chromosome while the remaining embryos had aneuploidy in 6 chromosome. Good quality embryos had an incidence of aneuploidy not more than 3 chromosome.

Conclusion: This study reveals that parthenogenesis oocyte and poor embryos quality had a high incidence of aneuploidy and mosaicism compare with good embryos quality.

Genotyping single sperm cells by MALBAC-NGS provides linkage information for pre-implantation genetic diagnosis

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Introduction: Linkage analysis is often required in the PGD process to avoid false positive/negative diagnosis. However, DNA sample from the proband is often not available. In this study, we report a new PGD method and procedure that does not require a proband sample or samples from multiple family members for linkage analysis.

Material & methods: A couple both identified as carriers of beta thalassemia mutation presented to our clinic for PGD. Genetic analysis showed that the male is a carrier of the -28A>G mutation, and the female is a carrier of the CD41-42 mutation.

Whole genome amplification and genotyping of single sperm cells

Semen was collected by masturbation from the male patient. After that we picked each single sperm cell into a PCR tube. The MALBAC single-cell whole genome amplificaion(WGA) method was used for amplifying each single sperm cell. The WGA product was then used for targeted resequencing of the mutations involved with the couple, together with 60 single nucleotide polymorphism (SNP) markers linked with the mutations. Two independent methods were used to detect the mutation, Sanger sequencing and next generation sequencing (NGS) on an Illumina HiSeq 2500 platform. By doing this, we generated only one NGS sequencing library from each sample that enables detecting of the mutation, SNPs linked the mutation and chromosomal aneuploidy screening in a single NGS process.

Embryo biopsy and single cell whole genome amplification with MALBAC

About 3-8 trophectoderm (TE) cells were biopsied from each blastocyst on Day 5 and then following WGA. WGA products were used for NGS on the mutation, SNP markers linked with the mutation and comprehensive chromosomal screening. The analytical procedure was the same with the previously described sperm genotyping.

Results:

Single sperms results

In this experiment we found 7 single sperms in all by combining the mutation, SNP with CNV analysis, including 2 single sperms with -28A>G mutation and 5 normal sperms. The SNP results of single sperms are consistent with that of husband, so we could find the linkage SNP to avoid the positive or negative occurrence when selecting embryos without mutations to implant, so it is practicable to estimate embryos by screening a single sperm haplotype.

Embryosresults

With the help of single sperm haplotype, we demonstrated the detailed results of 6 embryos. Three embryos were heterozygote with -28A>G mutation including one had a chromosome deletion (46XN, -17P). Two were normal and one was homozygous mutant. In conclusion we could find the linkage SNPs to avoid the positive or negative occurrence when selecting embryos without mutations to transplant.

Conclusions: Utilizing the recent development of MALBAC single cell WGA and genotyping method, here we present the first successful PGD procedure by genotyping multiple single sperm cells to obtain SNP linkage information. The PGD procedure is also combined with comprehensive chromosome screening to ensure normal karyotype of the implanted embryo, which results in a successful pregnancy. Our improved PGD/PGS procedure does not require genotyping disease-carrying proband, and is widely applicable to most paternal-carrying mutations in practicing PGD with monogenic disorders.

Non-Invasive Prenatal Testing: A Clinical Validation Experience

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Introduction: The last few years have seen a rapid and dramatic development of Non-Invasive Prenatal Testing (NIPT) for detecting foetal aneuploidies using a variety of testing platforms and methodologies. We have introduced a Massively Parallel Sequencing (MPS)-based assay for detecting foetal trisomies in chromosomes 13, 18, and 21 in circulating foetal cell-free DNA (cfDNA). Previous studies have demonstrated that MPS-based NIPT assays offer unsurpassed sensitivity and specificity, and this study describes the results of our validation experience prior to launching our own clinical testing service.

Material & methods: Our validation study consisted of a round of retrospective testing, followed by a round of concurrent prospective testing.

154 plasma samples from pregnant women that had been previously collected and analysed were provided by a partner NIPT provider. This retrospective cohort contained a mix of euploid and aneuploid samples, the results of which had been previously confirmed. All retrospective samples were de-identified and blinded before being sent to us for analysis. Following our testing, results were then compiled and sent back to the external provider for unblinding, comparison and scoring.

For our prospective study, we obtained two blood samples each from 216 pregnant women, with one tube being processed by ourselves, and the other being sent to another 3rd-party provider will a well-established test. These samples were independently and simultaneously processed. Following testing by both parties, our results were then compared with these external results for accuracy scoring. Collection of clinical outcome data from this cohort is ongoing.

For all samples, aneuploidy status is determined by independently examining the Normalized Chromosome Value (NCV) for each of chromosomes 13, 18, and 21, in accordance with previously published parameters.

Results: When the results of our retrospective study were returned, we had correctly identified 14/14 Trisomy 21 (T21) samples, 8/8 T18 samples, and 1/1 T13 sample. Further, no false-positive autosomal aneuploidies were reported.

Of the 216 samples included in our prospective study, the external provider identified 7-T21 samples and 1-T18 sample. Our analysis also detected all 8 of these autosomal trisomies, with no reported false positives or negatives.

Conclusions: The combined retrospective/prospective results for detecting trisomies 13, 18, and 21 were 100% accuracy (31/31 trisomies detected; 21-T21, 9-T18, 1-T13), with no false positives or negatives reported, though clinical outcome data for the prospective study is still being collected. These results suggest that our NIPT assay operates within previously published performance metrics used for similar validation studies, and give us a high level of confidence in our ability to accurately detect autosomal trisomies. We will continue to obtain and monitor clinical outcome data as part of our ongoing efforts to maintain and continually improve the accuracy and sensitivity of our test.

Fetal reduction or demise after multiple gestation: Implications for Non Invasive Prenatal Testing

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Introduction: Current internal best practice guidelines for Non Invasive Prenatal Testing (NIPT) in cases of a vanishing twin involve delaying the blood draw by 4 weeks from the last ultrasound evidence of the vanished twin. Patient A had IVF with two embryos transferred, resulting in a singleton pregnancy and a blood draw was performed at 10 weeks and 6 days of gestation for NIPT. Patient B had *In vitro* fertilization (IVF) and had three embryos transferred, resulting in a triplet pregnancy. Fetal reduction of two embryos resulted in a singleton pregnancy. A blood draw was performed 4 weeks post fetal reduction for NIPT.
This study aims is to investigate the effect of a fetal demise or reduction on the effectiveness and accuracy of NIPT and improve patient management and counselling.
NIPT was performed using the Genesis Serenity test, powered by the Illumina verifi technology, which involves whole genome massive parallel sequencing of cell free fetal DNA in the maternal circulation to detecting aneuploidies involving chromosomes 13, 18, 21, X and Y. For singleton pregnancies patients can also opt in to have gender disclosed.

Results: Results for patient A were consistent with a male fetus euploid for the chromosomes tested. The patient opted to pursue further testing and gave birth to a female. The original blood draw from patient B (15 weeks and 6 days of gestation) was consistent with two copies of chromosomes 13, 18, 21 and a male profile. Ultrasound evidence however showed a female fetus so a redraw (draw 2) was performed (17 weeks), also consistent with a male profile. The patient consented to regular monthly blood draws, to monitor the values for chromosomes X and Y. Draws 3 and 4 (21 weeks and 24 weeks respectively) were also consistent with a male fetus, but draws 5 and 6 (29 weeks and 34 weeks respectively) showed a female fetus.

Conclusions: The testing and analysis of these serial blood draws from patient B allowed us to monitor how well the test performed in cases of a three to one fetal reduction. The presence of chromosome Y was still detected at 24 weeks, showing good reproducibility of the assay. It only dropped out at 29 weeks, indicating that DNA from the placenta of the reduced embryos is still present in the plasma for up to 29 weeks and can therefore affect NIPT results. Allowing a minimum of 13 weeks post reduction before NIPT for such cases is recommended and appropriate genetic counselling is crucial. After investigation it was revealed that patient A also had an empty sac, which was not recoded on her case history notes. Results from patient A therefore further support that DNA from a demised embryo can affect NIPT results. This demonstrates the importance of taking into account case history when timing a blood draw and performing NIPT analysis.

Euploidy rates of Cleavage Stage Embryos versus Blastocysts following PGS in an Asian Population

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Introduction: In this study, we compare the euploidy rates of cleavage stage embryos versus blastocysts obtained from Asian women following PGS in Alpha International Fertility Centre, Malaysia.

Materials and Methods: Three hundred and four (304) patients with 2027 cleavage stage embryos (Group A) and three hundred and eighteen (318) patients with 1397 blastocysts (Group B) were

biopsied from July 2011 to December 2015. These patients were divided into 6 age groups, ≤30, 31 – 35, 36 – 37, 38 – 39 and 40 – 41 respectively. Of the biopsied samples, 3223 had Microarray Comparative Genetic Hybridisation (MaCGH) and 201 had Next Generation Sequencing (NGS). For MaCGH, the biopsied samples were amplified, labelled and hybridised on microarray slides according to manufacturer's specification (Illumina, USA). Microarray slides were analysed using BlueFuse Software (Illumina, USA), identifying normals, gains or losses of chromosomes. For NGS, biopsied samples were amplified and had DNA libraries constructed for sequencing using Ion Torrent™ PGM benchtop sequencer (Thermo Fisher, USA). Data from MaCGH and NGS were combined for analysis. Cases with known altered karyotype of translocation and inversion; and indeterminate biopsies were excluded from this study. Blastocysts from non-Asian women were also excluded from this study.

Results: The euploidy rates of cleavage stage embryos for patients aged ≤30, 31 – 35, 36 – 37, 38 – 39 and 40 – 41 were 33.2% (221/666), 30.3% (197/651), 26.3% (85/323), 13.4% (29/216) and 10.5% (18/171) respectively. The euploidy rates of blastocysts for patients aged ≤30, 31 – 35, 36 – 37, 38 – 39 and 40 – 41 were 61.7% (351/569), 55.4% (262/473), 51.8% (57/110), 34.0% (50/147) and 34.7% (34/98) respectively. The euploidy rates of each age group between Group A and B were statistically significant (p=0.0001 each). The euploidy rates for patients aged >35 years were significantly lower compared to patients aged ≤35 years in both Group A (p=0.0001) & Group B (p=0.0001) respectively.

Conclusion: This study shows that blastocysts have significantly higher euploidy rate compared to cleavage stage embryos in all age groups in an Asian population. These rates are in keeping with studies done by others on predominantly non-Asian population (Kort et. al, 2015).

Reference:
Kort J, et al. Aneuploidy does not explain the difference in outcomes observed between Asian and Caucasian patients undergoing in vitro fertilization. Asian Pacific Journal of Reproduction; 2015. 4(4):305

Single-cell analysis of low frequency mutations in the mitochondrial DNA by massive parallel sequencing

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Introduction: Massive parallel sequencing (MPS) gives an unprecedented opportunity for the analysis of heteroplasmies in the mitochondrial DNA (mtDNA). Conversely, no protocol is currently available to accurately simultaneously quantify single nucleotide variations (SNVs) and large deletions on single cells. This type of method is key to using this technology in preimplantation genetic diagnosis (PGD), or in research, for instance, into cellular diversity.

Materials and methods: We validated a single-cell deep MPS-based approach for the analysis of the mtDNA, and sequenced six single fibroblasts from a healthy individual, two single muscle fibers from a patient with mtDNA deletions, and 11 oocytes from three different patients treated at our center (5 GV, 3 MI, 3 MII). The cells are lysed in alkaline lysis buffer and the mtDNA is enriched by long-range PCR. SNVs are called with CLCBio Genomic Workbench and large deletions were identified using split reads. Mutation load is established using the sequencing depth.

Results: Although ultra-deep MPS allows for the detection of very low frequency events, we found that the signal over noise ratio depends on the number of PCR cycles needed for the mtDNA enrichment. To avoid false positives, the thresholds are set to loads of 0,5% and 1,5% at 35 cycles (oocytes), and 0,5% and 2% at 45 cycles (single fibroblast and muscle fibers), for deletions and SNVs respectively.

In the muscle fibers we detected a small number of large deletions in each cell, with high individual mutation loads; some of these where also identified in the blood of the same patient.
The single fibroblasts showed no large deletions but revealed somatic mosacism at the SNV level, with two variants appearing in 1/6 of the cells at high loads, and absent in the other cells.
In the oocytes, one large deletion at 0,51% was found in one out of the 11 oocytes. SNVs were found in 9/11 oocytes, at frequencies ranging from 1,5% to 20%. In one patient we observed the same variant in 3/ 4 oocytes at loads of 5%, 15% and 20%. In the same patient, another variant was detected in 2/ 4 oocytes at frequencies of 5% and 15%. In 2/4 oocytes from a different patient, another SNV was detected at frequencies of 2,5% and 3%. Only one variant at load of 4% was predicted to be pathogenic, and there appeared to be no correlation between the oocyte maturation stage and the presence of variants.

Conclusions: Our protocol can detect mtDNA variants in single cells with high accuracy. Here, we illustrate three cases of mosacism. In the first, we find somatic mosaicism for large deletions in a patient with mitochondrial disease. Next, we detect SNV somatic variation in fibroblasts of a healthy individual. Finally, we that the same SNVs were recurrently appear in oocytes from the same donor but often at different frequencies. Despite that these results should be matched to the somatic tissue of the same patient to fully understand variant segregation, this data gives interesting information about heteroplasmy shifts during gametogenesis.

Expanded PGD Options for Couples with Unavailable Familial Samples, Germline Mosaicism, or *De Novo* Mutations

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Introduction: Preimplantation genetic diagnosis (PGD) for single gene disorders (SGD) has typically required family members beyond the couple presenting for PGD in order to develop and validate a testing strategy for embryos. Historically, if additional family members were not available, the couple could only proceed with PGD if testing of single sperm or polar bodies provided informative results in terms of creating linkage with the mutation locus of interest. PGD options have been particularly limited in cases where one member of the couple has a de novo mutation or in couples who have evidence of germline mosaicism. Now with the increasing utilization of routine and expanded carrier screening (ECS), even more couples are presenting for PGD without any family history or prior affected individuals in the family. Many of these couples do not want to involve additional family members due to added time, cost and privacy concerns. We recently developed a method for SGD PGD that directly targets mutations with the lowest reported allele drop out (ADO) rates, thus eliminating the requirement of single sperm, polar bodies or additional family members for linkage.

Materials and Methods: The cases that were submitted to our laboratory for SGD PGD, which includes comprehensive chromosome screening (CCS) from January 2014 to March 2016 were evaluated, and included if at least one mutation in the SGD PGD plan required “mutation only” testing.

Results: Since offering this testing option clinically we have developed probe strategies for 48 couples. Thirty of the couples presented for PGD after positive carrier screening with no family history, 2 of the couples presented with somatic or germline mosaicism, 11 couples presented with an autosomal or X-linked dominant disorder where no family members were available, 4 patients/partners presented with a *de novo* disorder, and 1 case where the couple had a *de novo* mutation in a child and wanted to address potential increased risks associated with germline mosaicism. To date 24 of these couples have completed at least one cycle and had embryos tested. Of the cycles tested and information available, 131 embryos from 26 couples have been tested, 77% (20/26) of cases were found to have at least one normal embryo, and of six known transfers, five (83.3%) have an ongoing pregnancy or have delivered.

Conclusions: This testing strategy has successfully allowed for more couples to have access to PGD, either in the absence of additional familial samples, or in cases of germline mosaicism or *de novo* inheritance.

Importance of genetic screening for cystic fibrosis, phenylketonuria and SNHL in Belarusian couples undergoing ART.

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Introduction: The aim of this research was to evaluate the necessity and importance of genetic screening for most frequent genetic disorders in Belarusian region in couples undergoing assisted reproductive technologies (ART).

Materials and methods: Molecular diagnostic methods were performed to diagnose such genetic conditions as cystic fibrosis (CF), phenylketonuria (PKU), sensorineural hearing loss (SNHL). All women preparing to undergo 'in vitro fertilization' (IVF) were screened for CF, PKU and SNHL; if they were heterozygous carriers, their spouses were examined for the same condition. All men with severe impairment of spermatogenesis (azoospermia, severe oligoasthenoteratozoospermia) were screened for CF before testicular biopsy.
In total, 6324 analyses for genetic conditions were carried out.
1992 women and 952 men were examined for most frequent CFTR-gene mutations, such as dF508; CFTR2,3del; 2184insA. 1822 patients were examined for most frequent mutations in PAH-gene – R408W, R158Q. 1558 patients were tested for most frequent mutations in gene JGB2 – 35delG (SNHL). There were 3 couples in which both spouses were heterozygous carriers of dF508 in CFTR-gene. They underwent IVF + ICSI and preimplantation genetic diagnostics (PGD), embryos with homozygous dominant genotype or heterozygous genotype were selected for embryo transfer (ET). Obtained embryos underwent biopsy at 8-cell-stage, blastomers were aspirated and conventional PCR for known parental mutations was performed. Each blastomere was transferred into the reaction tube containing 5 µl of Proteinase K lysis buffer and frozen at -20C.The lysis was then completed by 15 min incubation at 45C and 20 min at 95C. Amplification was performed by single-round fluorescent multiplex PCR designed for the most common mutations in Belarus – dF508, CFTRdel2,3(21kb) and 2184delA. PCR products were analysed on ABI3500 automated sequencer.

Results: 1992 women and 952 men were examined for most frequent CFTR-gene mutations, such as dF508; CFTR2,3del; 2184insA. 37 women (1.8%) and 26 men (2.7%) presented heterozygous genotypes; thus, they were CF-carriers. Three couples where both spouses were dF508 carriers underwent IVF + ICSI + PGD. Two pregnancies resulted in the birth of a clinically healthy baby with heterozygous 'carrier' CF-genotype. The third pregnancy resulted in miscarriage; the aborted fetus presented heterozygous 'carrier' genotype.
1822 patients were examined for most frequent mutations in PAH-gene – R408W, R158Q; thus tested for phenylketonuria. 34 (1.9%) of them presented heterozygous 'carrier' genotype.
1558 patients were tested for most frequent mutations is gene JGB2 – 35delG (SNHL). One patient (0.06%) presented recessive homozygous genotype and was clinically deaf. 69 (4.4%) patients were heterozygous 'carriers'.
Combined frequency of heterozygous mutation carrier status for the three aforementioned genetic conditions was 10.8%.

Conclusion: Combined frequency of heterozygous mutation carrier status for CF, PKU and SNHL was 10.8% for Belarusian population. Thus, these families risked giving birth to an offspring with a severe genetic condition. Therefore, genetic screening for CF, PKU and SNHL is strongly recommended for every couple undergoing ART in the Republic of Belarus in order to minimise the risk of giving birth to an offspring suffering from a genetic disorder.