21ST

PGDIS CONFERENCE

PREIMPLANTATION GENETIC DIAGNOSIS INTERNATIONAL SOCIETY

PGDIS

6-8 May 2024
Kuala Lumpur
Malaysia

PGT and BEYOND...

PGDIS 2024

A summary of all session talks

PGT and BEYOND...

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Session 1

Session 1: Advances in PGT

Evolution of PGT Methods- Mark Hughes

Karyomapping and its discontents- How karyomapping will survive (and thrive) in the genomic era- Tristan Hardy

Single Cell Testing for Embryos- Joris Vermeesch

Transformational Technologies Drive the Next Generation of PGT-A Chris Weier

Preimplantation DNA Methylation Screening to Improve ART Outcomes- Jiang Liu

Posters relevant to this session:

24-A-025 Karyomapping: An Effective Tool to Determine the Parental Origin and Types of Aneuploidies
24-A-026 PGT for monogenic diseases and Pre-conceptual Testing of the Infertile Couple. Advancing PGT-M through Integrated Whole Exom
24-A-057 Pre Implantation DNA Methylation Screening to Improve ART Outcomes

Session 1: Advances in PGT

In this session, we heard from Mark Hughes, one of the pioneers in PGT, how PGT and approaches to PGT have changed from the earlier days to now. The key to this change was technology and the support for progress came from collaborations. Mark raised the question though as to whether more is better and the cost is worth the outcome? Have we learned anything from recent controversies where information exceeded understanding? Above all, Mark stressed that while business drove many things, there should not be any compromise to integrity. It is a timely reminder that echoes George Santanaya: “those who cannot remember the past are condemned to repeat it” and yet acknowledges that the future is exciting and waiting.

Next, Tristan Hardy described how one of the new technologies was offering a more complete vision of the future. Karyomapping advanced testing in PGT-M and then added the possibility of PGT-A. Not stopping there, Tristan described how using even more technology on the karyomapping base, offered opportunities that extended the application of PGT-A/M into the most difficult situations, such as de novo or diseases that were not easily mappable because of no reference samples. It is an expensive and highly technical process, suited for special cases but as the cost of technology drops, the potential for this approach makes it more approachable for routine application.

Joris Vermeesch reminded everyone that quality of analysis starts at the very beginning with the amplification- whether using arrays or NGS for final answers. Performed correctly the methods can reliably be used even on single cells. There are alternative approaches to analysis of chromosomes that can combine NGS, SNP typing, copy number analysis and genetic typing. As with the previous speaker, Joris showed how the combination of analyses offers a more comprehensive approach to PGT-M. All approaches though have their limitations and their opportunities. Introduction of alternative newer technologies provide the possibility of plugging some of shortfalls in the more common approaches. With a glimpse of future options, Joris detailed how genome and transcriptome analysis can be a true possibility which reveals, detail at the cell level, for aneuploidy, cell origin, parent of origin, errors of segregation, development state and more.

Session 1: Advances in PGT

Chris Weier reminded everyone of the true goals of PGT- looking at the health of the embryo to improve chances of a pregnancy, reduce risks and end up with a healthy outcome. Getting back to the start, Chris also reminded everyone that the first step, amplification, is critical for a reliable answer. A reliable, comprehensive amplification is of absolute importance for a complete answer- whether for ploidy or genetic testing. A good test combines copy number and polymorphism signatures and when combined with appropriate analysis, can deconvolute the mire of mosaicism and segmental variations- genuine mosaic results can be confirmed and apparent intermediate copy number results can be properly reassigned to euploid or aneuploid status. The combined approach also offers reliable two factor authentication of deletion and duplication syndromes.

Jiang Liu brought everyone's attention back to some known biology and the basis for much of an embryo's developmental potential- DNA methylation. Embryogenesis is a coordinated reprogramming of methylation and failure to follow the program can drastically affect the outcome. Understanding the process lays open the possibility of identifying embryos that have otherwise invisible defects. Preliminary results suggest that looking at DNA methylation provides a window on evaluating embryo developmental potential at a level beyond that of chromosome profiling. As an addition, such analysis offers the possibility of identifying and reducing the possibility of epigenetic based birth defects

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Session 2

Session 2: PGT today and into the future

PGT-M as a Primary tool for Avoiding disease at the Community Level- Svetlana Rechitsky

PGT-M for Recessive Conditions in Populations with High Consanguinity Rates- Karolina Kobus

Cross-Border Reproductive Care- Patchari Sae Lin

Posters relevant to this session:

24-A-001	Cross-Border Reproductive Care: Four Countries and A Healthy Baby Back Home After PGT-M for PEIZO2 Exon 3 Deletion.
24-A-019	Strategic PGT-M approaches for Multiple Congenital Anomalies-Hypotonia-Seizures syndrome type 2 (MCAHS2) using karyomapping and sanger sequencing.
24-A-022	Case report: PGT-M for a family that had a child with Junctional epidermolysis bullosa
24-A-028	Case Series: Preimplantation Genetic Testing for expansion in size of a terminal deletion/duplication chromosome through generations
24-A-031	PGT-M for Fragile X Syndrome – Clinical Benefits of Direct Mutation Analysis
24-A-051	Case Report: Healthy live birth after Preimplantation Genetic Testing for TBCD gene mutation.

Session 2: PGT today and into the future

Svetlana Rechitsky reconnected us to the real world where congenital disorders are a very real thing for many people. The impact of genetic conditions on families including babies, young children and even adults is pronounced and using terms such as “rare” underestimates the scale of impact. Uncovering the predispositions that populations face is now achievable with pan-ethnic carrier screening programs- and now couples have a choice in how they plan their families. Lana showed how many different genetic diseases and difficult decision making are being avoided by PGT. People are also now being offered options for the adult health of their offspring in avoiding conditions that have impacted on them. This understanding is now affecting significantly what is being asked of PGT and how such testing must be approached.

Not all populations are the same in their genetic predisposition to diseases. Karolina Kobus took us around the world highlighting the impact that inherited conditions have on families- 30% of children with a genetic condition will not see their 5th birthday and many others will suffer delay in diagnosis with up to 95% having no treatment options. From a global view to a localized region, the Middle East, Karolina described the change in perspective needed in dealing with these areas and the problems they face. Some conditions have a different genetic etiology and it’s important to recognize this. In all populations, as average lifespan rises, so does the incidence of genetic disease and the healthcare costs associated with managing them.

Session 2: PGT today and into the future

The Middle East is a region quite distinct and very much underrepresented in genome databases- leading to incorrect or inappropriate diagnoses for many conditions. Also, the genetics of this region can have an impact on understanding the genetics of outside populations with many genes having been linked to different conditions and some pathogenic mutations from outside of the region being questioned as to their real status. Karolina discussed how PGT offers assistance in both fertility related conditions as well as in a disease prevention strategy- highlighted by, but not restricted to, populations such as those in the Middle East.

Not all groups can provide the services needed to provide PGT services. Dr Nood showed how patients can successfully be handled by multiple groups in their journey towards a healthy family. In an approach labeled “Cross Border Reproductive Care” we were shown how many obstacles can be overcome by cooperation between groups in different locations or even different countries. Ultimately, we begin to realise that success is for the patients and not just for the clinic(s). Competition is important but cooperation is even more powerful.

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Session 3

Session 3: Critical evaluation of PGT methodologies

SNPs and Karyomapping- Alan Handyside

Next Generation Sequencing- Dagan Wells

Session 3: Critical evaluation of PGT methodologies

In what could be considered a British main event, two of the most influential players in PGT were brought together to consider and discuss what is arguably the most important technologies for PGT today- SNP analysis and NGS (Next Generation Sequencing) applications.

Alan Handyside described how the first comprehensive PGT approach, Karyomapping, has developed even further with a refined analysis. A single test now combines detailed genetics with comparative intensity to give a single approach to reveal all aspects of chromosome mal-segregation(s)- both meiotic and mitotic. At the same time, this approach can dissect out some of the artefacts that plague and confuse current PGT- mainly mosaic embryo designations. Combining all aspects together, Alan described an advanced and comprehensive approach to embryo transfer prioritization.

In reviewing the past, Dagan Wells described the early failures of aneuploid testing but then the emergence and gaining successes of more comprehensive chromosome analyses. Not halting there, Dagan outlined further gains in the NGS approach to PGT-A. Creeping into SNP territory, it was revealed that a targeted approach to PGT-A now involved massive PCR plexes that enabled genetic typing similar to Karyomapping. This new approach utilized the genetics revealable by deeper sequencing at 1000s of selected sites on the genome, to improve accuracy of chromosome description. Unlike the traditional NGS approach developed over 10 years ago, the combination of SNPs now enabled detection of abnormal fertilization of all sorts as well as clearer identification of contamination. Dagan cautioned though that validations still need to improve.

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Session 4

Session 4: Controversies in PGT part 1

Is PGT-A for Everyone? Don Leigh

Only One Embryo: Should it be tested? Semra Kahraman

Are All mosaics Real? Diego Marin

PGT-A: All or None? Mitko Madjunkov

Are RCTs the Best Way to Demonstrate Benefits of PGT-A or Any PGT Approach? Joe Leigh Simpson

Posters relevant to this session:

24-A-027 Comparison of pregnancy outcomes following preimplantation genetic testing for aneuploidy/structural rearrangement among indications.
24-A-030 How to draw on “No Harm PGT-A”: SNP analysis like a part of a future concept
24-A-038 Preimplantation Genetic Testing - Aneuploidies (PGT-A) by Next Generation sequencing - A study from single centre
24-A-044 Utilization of PGT-A in Young Female Age Groups Reveals Better Outcomes

Session 4: Controversies in PGT part 1

Don Leigh raised an interesting question- or questions? It seemed simple enough: Is PGT-A for everyone? The answer though was maybe not as expected. For the patient, it is for young and old, whoever wants it to help manage their own risks. But Don went on to suggest that not every clinic should be offering it because they are maybe not performing well enough to add complexity to their practice- something clinics do not want to hear. He went on to add that not every lab should be doing it because even commercial services are adding confusion to the results by underperforming and over interpreting. Something laboratories do not want hear. Such a simple question but one that all groups need to think about.

In a simple but inciteful study, Semra Kahraman demonstrated the true value of PGT-A- its function of reducing futile transfers. The unusual (over?) response from some critics actually highlights the importance of the study- it cuts at the very base, the criticisms and critics of PGT-A application. It also highlights the bias that some journals have permitted into the literature. The misunderstandings of all criticisms were dealt with simply. The study showed some updated information that now included a group left out of the original report- the intermediate copy number transfers. To the chagrin of the critics, it just reinforced the original findings- PGT-A offers benefit by reducing futile transfers and reducing failures for all stages of transfer outcome. Semra's group showed that, done properly, PGT-A can benefit patients with limited opportunity- even testing 1 embryo can assist patients in their treatment.

Session 4: Controversies in PGT part 1

One question that has tormented the PGT-A world is the possibility that some analyses were faulty. Diego Marin opened up the box and looked inside. Using a combination of copy number and SNPs, Diego proposed that half of the ICN calls were miscalls of euploid embryos (presumably a similar situation can apply to aneuploidy). Further, Diego went on to describe why predictions based on mosaic rates were meaningless- at least in the low to moderate ranges. It was the power of the combined copy number/SNP approach that let Diego look through the smoke and reveal the uncertainty of some current analyses. A word of caution though finished Diego's presentation- segmental aneuploidies hold their own story.

There is a mini conflict going on in the IVF world: to test or not to test?- that is the question. The IVF groups are split as to whether PGT-A is of benefit or not and this has polarized groups around the world. Mitko Madjunkov took us through the realities of the situation: >50% of cycles in the US utilize PGT-A and this has enabled single embryo transfers to predominate the IVF process. Mitko explained how, in spite of its limitations, analysis is >95% accurate with mosaic calls being substantially less accurate. In his own group, Miko revealed that apparent mosaic embryos do have successful outcomes but these are lower than euploid embryo transfers. As with Diego Marin, Mitko observed that nearly half of the mosaic embryos, on rebiopsy were euploid and only a small fraction were genuinely mosaic. The disparity increased when segmental results were retested. As a bonus, Mitko showed how PGT-A can be used to increase available embryos for transfer when abnormal fertilization observations were investigated using PGT-A.

Session 4: Controversies in PGT part 1

Using more recent data, Mitko showed how PGT-A benefited outcomes by reducing twin birth rates in all age groups. As he delved deeper into the data, Mitko revealed how PGT-A strengthened NIPT results. A key point of the discussion though, was the opportunity that PGT-A gives for precision medicine- patients are not all the same and personalizing the approach for each patient is important for getting patients to their goal in the best way possible

Journeying back 15 years or so, Joe Leigh Simpson revisited the beginnings of the controversies regarding PGT-A (PGS back then). With astute dissection of the problem, Joe Leigh suggested that complex RCTs were difficult to analyse and when critical parts of any study were lacking, then no design would necessarily be valid. What options are there then? Joe Leigh showed how predictive value analysis can replace an RCT approach- and many PGT-A studies showed benefit. Deeper understanding of the underlying problem of achieving a sustained pregnancy is important to resolving the problem and potentially an explanation for when use of PGT-A is not as successful. Joe Leigh led us to the conclusion that RCTs are maybe better suited for straight forward questions that do not need to account for procedural issues (surgical or laboratory) and that Predictive Value analysis (eg non-selection studies) gives a clearer vision that avoids potential biases. While some continue to believe that RCTs are a gold standard for any comparison, maybe it’s time to rethink this idea- All that glitters is not gold!

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Session 5

Session 5: Controversies in PGT part 2

PGT for Polygenic Diseases- Diego Marin

Should We Go Down the Pathway of niPGT? Carmen Rubio

PGDIS 2021 Position Statement on Mosaic Embryo Transfers- Behind the Scenes Controversies- David Cram

Transfer of Mosaic Embryos- Current State- Manuel Viotti

Mosaicism and Miscarriages- Richard Choy

PGT-A for Younger Women- Murat Cetinkaya

Posters relevant to this session:

24-A-015 Clinical Outcome of Mosaic Blastocyst Transfer
24-A-044 Utilization of PGT-A in Young Female Age Groups Reveals Better Outcomes
24-A-049 Outcomes of Mosaic Embryo Transfers in Advanced Female Age Group

Session 5: Controversies in PGT part 2

The possibility of diagnosing almost any gene defect was proposed over 30 years ago and with the understanding that many traits are polygenic, the related possibility that many genes could be analysed in embryos was similarly raised. Diego Marin spoke about the realities of polygenic risk assessment. With an understanding based on real world sibling studies, the possibility of reducing relative risk for the future generation was raised. Diego discussed how modern genetics has revealed many different associations amongst genes and how this can be utilized now to offer risk reduction to couples using IVF. He emphasized that such analyses were not the same as PGT-M where embryos are discarded on the basis of disease association but more as a tool to prioritise embryos in their transfer order. As with PGT-A though, there is an unfortunate polarizing of parties where an “all or none” position is often invoked. Using an example of type 1 diabetes reported to be elevated in IVF patients, Diego discussed the possibility of reducing such risk in offspring. With many such opportunities comes the debate about who best benefits from these things with social and racial disparities are being raised. Aren’t such debates though polarizing in themselves. If one cannot have it then none can have it? Surveys however, reveal that the general public agree with the concept and support its availability. Ongoing data accumulation in adult sibling studies suggest demonstrable risk reduction for such approaches.

Less invasive approaches in prenatal testing and in oncology are rapidly being developed and adopted throughout the world. Carmen Rubio described the analogous approach for embryo testing. As procedures advance, so does relative accuracy of any test. Carmen described how concordance rates between biopsy

Session 5: Controversies in PGT part 2

-based testing has improved and the potential for noninvasive embryo testing increased. A number of steps in the overall process have been suggested to be important in these gains as well as better understanding of the impact and resilience of other parts. As with PGT-A, it was emphasized that such testing is for prioritization of embryo transfer order but unlike PGT-A, it is not for embryo exclusion.

All Professional Societies have an opportunity to present expert opinion to the field, to associated personel and to the public for greater understanding about many topics within the Society's field of expertise. This is a long-held tradition supported by their affiliated journals. Dave Cram took us behind the scenes in a disturbing new aspect that appears to be selective in application. Over the last 8 years, PGDIS has issued several statements attempting to clarify the situation of the transfer of "mosaic embryos" and these were readily accepted as a Committee opinion and published by the Society affiliated journal. The most recent statement in 2021 however, ran into editorial interference with a substantial delay in final release. A Committee Position Statement was subjected to a rigor that appears to exceed that required by other Professional Societies. Dave accepted that the final Position Statement was more balanced but went into the key areas of contention. Here he revealed the biases that were being pushed by editorial staff including a conflict of interest where a sub editor was pushing for a paper, they were co author on, to have higher priority in its description. Also revealed were unusual timing events involving groups opposed to PGT-A publishing in other journals. An important aspect raised by Dave was the failure of groups revealing adverse outcomes- this gave credence to the otherwise baseless claims by some critics and needs to be addressed.

Session 5: Controversies in PGT part 2

Diving into the phenomenon of mosaic embryos, Manuel Viotti took us into the biology of how this might occur- genetics or disturbance of natural processes. In an expanded study, Manuel now looked at how methodological approaches may influence the occurrence of ICN results. While some aspects appeared to have an element of influence, this was typically minor and of little overall concern. However, it was observed that the analysis platform and subsequent interpretations of profiles may contribute to artifact. A key idea was the correct use of metrics to assess the reliability of any result. As part of his role in the Committee collating the worldwide outcomes of transfer of mosaic embryos, Manuel reported that mosaic embryo transfers do have significant outcome reductions in many transfer measures. In addition, it was revealed that there can be adverse outcomes where the health of the pregnancy was significantly impacted on. These are important findings and need to be considered by all clinics when they are advising patients.

Returning to the big world, Richard Choy discussed the impact of mosaicism on prenatal health and development as well as its impact on miscarriage. In detailed studies, Richard revealed how mosaicism can occur and how it might reveal itself or even remain partially hidden from current testing approaches. Using molecular approaches, otherwise unexplained miscarriages were revealed as resulting from (or least containing) mosaic abnormalities at a % approaching the otherwise euploid miscarriages. Logically, many of these mosaic events were likely present at the early embryo stage and not simply late, confined placental mosaic events.

Session 5: Controversies in PGT part 2

There has been much discussion about which group of patients might best benefit from the use of PGT-A with many studies suggesting it is only the older, more mature female patient that truly benefits from embryo testing. Murat Cetinkaya discussed a large retrospective single centre study, looking specifically at the <35 years age group. All transfer outcome parameters were better for the PGT-A group of patients with the overall gains more evident the more advanced the pregnancy. Importantly, even in this younger age group, there was a measurable decrease in miscarriages for the PGT-A group compared to the standard no testIVF group - one of the goals of applying PGT-A.

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Session 6

Session 6: Selection of competent embryos

Confocal Imaging of Developing Embryos- Nicolas Plachta

Analysis of Spent Culture Medium- Richard Choy

AI for Morphology Selection- Collin Lee

Single Cell Sequencing and Embryo Mosaicism- Effrosyni Chavli

24-A-021	Poor quality blastocysts can result in healthy live births – a case report
24-A-023	The effect of an additional day of culture on Stage 3 blastocysts – the PGT-A standpoint
24-A-046	How does the Exclusion of Blastomeres Affect the Outcome of Euploid Blastocysts?
24-A-047	May Collapse be a Predictive Factor for Clinical Miscarriage of Euploid Embryos?
24-A-048	How are Time Lapse Parameters Affected by Embryonic Mosaicism

Session 6: Selection of competent embryos

In an amazing and colourful journey, Nicolas Plachta took us into the micro world of a developing human embryo as it divided, shifted cells, budded fragments and changed shape. Using a rainbow of fluorescent colours, Nicolas peered inside individual cells with an embryo to watch their inner workings as they grew and became part of the bigger coordinated structure of a blastocyst. During this development, Nicolas could observe cells as they got things right and wrong with their nucleus and chromosomes. Mitotic errors were observed as nucleus budding and DNA shedding- fortunately though, these were relatively low occurrence events.

Embryos live in an environment and alter that environment as they grow. As an alternative to an invasive biopsy process Richard Choy described how looking at the changes in the environment might be a viable alternative to biopsy-based approaches. Reviewing the current status of cfDNA analysis, Richard discussed its shortfalls and inconsistencies in application. The bigger picture though relies on looking at a greater range of events- a multi omic approach. Some groups have looked at transcriptomics- what appears to be happening but Richard described the approach of metabolomics- what is happening. What the embryo does appears to have a direct relationship to its health and pregnancy potential. In a proof of principle study, Richard explained how, using Raman spectroscopy, the metabolite profile of spent culture medium had a prediction sensitivity equal to that of chromosome profiling. Such indirect analysis may be part of the future of niPGT.

Session 6: Selection of competent embryos

The Gardner scoring system has been a boon to IVF embryo selection but it holds a level of subjectivity, even with extended embryologist experience, that limits its overall effectiveness. Collin Lee discussed how AI can be an adjunct tool that can assist in standardizing and improving embryo selection. Different AI models have taken different approaches to scoring an embryo but many show success relative to standard IVF practices. Collin then gave a personal experience of integrating AI in his own centre where patients benefitted from improved transfer outcomes and staff benefitted from improved training on their embryo choices. Success in embryo transfers is predicated on the ability to avoid choosing aneuploid embryos for transfer- evaluating morphology alone, AI has high probability of selecting a euploid embryo for prioritized transfer. Collin stressed that AI was not a replacement for PGT-A but was a viable option, especially where the invasive approach of biopsy was too costly or not available. In global collaborations, AI is showing benefit in improving patient transfer outcomes. Extending the possibilities, Collin went further and is involved in ongoing research on the AI evaluation of endometrial receptivity.

Effrosyni Chavli took us deep into the embryo, one cell at a time. After disaggregation of blastocysts, single cells were isolated and whole genome sequencing was performed to reveal their individual chromosome profiles. Then embryos were “reassembled” to give a composite picture of their chromosome profile(s). Using this approach, Effrosyni identified meiotic and mitotic errors of chromosome segregation. Several questions that have been confusing the PGT world were answered: 1) no preferential allocation of cells between TE and ICM, 2) TE held more complexity in abnormalities than ICM, 3) many embryos had 1 or more mitotic errors, 4) 3 of 4 mitotic errors occurred after TE/ICM differentiation, 5) structural errors (segmental) were common (69%), 6) Reciprocal errors are observed (whole chromosome and segmental), 7) Most mosaicism cannot be identified with a 20% threshold. Studies like this one go far in explaining anomalous outcomes after the transfer of seemingly normal embryos.

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Session 7

Session 7: Developments in niPGT

Recent Developments in niPGT -Carmen Rubio

How Far Down the niPGT Path Should we go? Luca Gianoroli

NICS- Devopment of a niPGT Process- Sijia Lu

niPGT and Morphokinetics for Improved Embryo Selection- Muhammad Amaluddin

Laboratory Optimisation for niPGT-A Judy Chow

Session 7: Developments in niPGT

Posters relevant to this session:

24-A-004	Novel non-invasive preimplantation genetic testing for aneuploidy algorithm based on cell-free long non-coding RNA expression profiles in spent media
24-A-007	Clinical implications of noninvasive PGT-A (niPGTA) on IVF outcomes in oocyte donation cycles: a blinded prospective non-selection study
24-A-008	Noninvasive PGT: Experiences from 6 years of clinical studies and applications
24-A-010	Blastocoel fluid DNA level as a predictor for selecting viable human embryos
24-A-011	The effectiveness of non-invasive preimplantation genetic testing using spent culture medium or blastocoel fluid.
	Evaluating IVF prognosis through non-invasive preimplantation genetic testing for aneuploidy to predict ploidy status
24-A-034	Morphokinetic parameters assessed by computer-assisted sperm analysis(CASA) and implications for preimplantation genetic testing for aneuploidy(PGT-A)
24-A-035	The impact of spontaneous collapse and cytoplasmic strings on euploidy rates in embryo cultured in time-lapse incubators
24-A-039	A randomized double-blinded controlled trial of non-invasive PGT-A: the genetic results
24-A-040	A randomized double-blinded controlled trial of non-invasive PGT-A: laboratory optimization prior to commencement of the trial
24-A-043	Optimizing Aneuploidy Detection in IVF Embryos: Integrating Morphokinetic Parameters with NIPGT-A for Improved Sensitivity
24-A-048	How are Time Lapse Parameters Affected by Embryonic Mosaicism
24-A-052	Evaluating IVF prognosis through non-invasive preimplantation genetic testing for aneuploidy to predict ploidy status
24-A-054	Comparison of non-invasive preimplantation genetic testing for aneuploidy (niPGT-A) results between single-step culture medium (SCM) and two-step culture medium (TCM)

Session 7: Developments in niPGT

Carmen Rubio started the session with considering where the cfDNA present in medium originates. Using methylation analysis, one group showed substantial maternal DNA contamination while Carmen's group showed similar origins using SNPs. A change in protocol reportedly clears much of this contamination. Using this modified protocol a multi centre study demonstrated good concordance between cfDNA and ICM. Of great interest were the discordant TE/cfDNA embryos where euploid embryos by biopsy but aneuploid by cfDNA, showed lower clinical pregnancy rates and higher miscarriage rates. In trials, niPGT cfDNA results were equal to PGT-A in both good prognosis patients and poor groups, possibly opening up the idea of greater adoption of noninvasive techniques. Carmen also presented some recent work where the possible mechanism of mitotic chromosome instability, potentially linked to mosaicism was revealed- nuclear budding during blastocyst development releases packets of DNA.

Next up, Luca Gianoroli, a pioneer in this area, discussed the advantages of blastocoel fluid as an alternative to spent medium analysis. BF avoided external contaminants and provided a pure sample of embryo secretions with a glimpse into the ICM itself. However, there are some significant literature discordances to biopsy approaches. Also noted were differences in analysis success rates according to expansion status. TE/BF correlations though (when available) were very high (>98%). A high analysis failure rate was noted amongst the best implanting embryos and this correlated with lower BF DNA even when PGT-A revealed euploidy. Luca went on to describe how the observations seemed to fit some proposals for the biology. In considering spent medium analysis, it was suggested that concordance may not be as important as maintaining the embryo viability. Luca also raised concerns that extended incubation for SCM analysis may warrant further study to ensure it does not prejudice embryo vitality. As a conclusion, the recent recommendations from ESHRE were cited: niPGT is currently not recommended for routine clinical use.

Session 7: Developments in niPGT

While niPGT on SCM is new for some groups, Sijia Lu gave us a view of nearly a decade of development. Using the whole embryo as the standard for comparison to TE biopsy, refinements in process led to high correlation with whole embryo analysis. As a prioritization tool, niPGT has been used for recurrent loss and failure patients as well as structural rearrangements. Refreshingly, Sijia did not promote it for universal application (yet) but as a tool to assist transfer prioritization in difficult cases.

Most groups focus on a single approach to niPGT and compare to morphology selection or PGT-A, whereas Muhammad Ikhsan both combined and compared morphokinetics and niPGT for his comparison to PGT-A. The combination improved sensitivity for euploid selection but at a substantial drop in specificity- a telling result for those hoping to avoid any molecular characterization.

Many clinics are eager to try niPGT but Judy Chow discussed the steps needed to try and maximise potential success. Judy described their approach to controlling variables from the very first steps of washing, in order to reduce false starts. With a well-defined protocol starting at the very beginning of the whole process, different centres were able to achieve consistent results and understand which parts were important and which parts a bit less so.

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Session 8

Session 8: Patient management and genetic counselling

PGT-A an Ethical Dilemma? What Should Clinicians be Discussing With Patients? Andreas Schmutzler

The Role of Clinical Genetics in PGT – Li Wang

Clinician Counseling of PGT-A for Patients- Navdeep Singh

Are all Carriers Equal? The Hidden Impact of Carrier Conditions and Implications for PGT-M Lee Shulman

Prenatal and Postnatal Outcomes for Multi Gestation Pregnancies- Joe Leigh Simpson

Hidden Carriers in the IVF System- Alice Weeks

Mosaic Embryo Classification for Reduced Embryo Losses- Steve Grkovic

Session 8: Patient management and genetic counselling

Posters relevant to this session:

24-A-006 Issues with genetic carrier screening revealed through couples seeking PGT-M
24-A-013 A pragmatic approach to mosaic embryo classification and the minimization of PGT-A false positives.
24-A-044 From Here to Fertility; preconception Genetic Testing for the Infertile Couple
24-A-056 PGT-A an Ethical Dilemma? What Should Clinicians be Discussing With Patients?
24-A-059 Heterozygote Status as Health Risk: Changing the Role of Carrier Screening in Preconception Care and How Couples and Individuals Should be Counseled About Results

Session 8: Patient management and genetic counselling

The first talk by Andreas Schmutzler gave a comprehensive breakdown on what clinicians should (must?) be discussing with patients. Discussions start before any treatment commences and must be honest and open about risks as well as options and alternatives. Andreas took us through the full understanding of informed consent, both from a legal and ethical standpoint. In completing the process discussions about options such as PGT-A are important and necessary for more complete patient understanding. Without introducing personal bias, Andreas discussed many different scenarios where PGT-A can be considered and where no testing may be a better approach- such decisions being with patient understanding, respecting their wishes, values and preferences. Ultimately, being responsible, ethical and fair, the discussions move towards a personalized medicine approach.

Coordination and cooperation were key points brought out by Li Wang. IVF is no longer a single specialty show and other specialties, such as Clinical Genetics, are important contributors to the appropriate patient journey. Every patient has their own risk profile and Li showed how often these comparative risks were being overlooked or ignored. Using her own data, it became apparent that all patients were being exposed to elevated transfer risks to a level that in other medical areas would warrant some form of intervention. As an independent from the IVF clinic itself, the Clinical Geneticist also needed to understand the limitations of any single clinic in discussing options such as PGT-A with patients. Assessment of a clinic's ability to successfully perform a further process can greatly influence advice to a patient- this added another layer to what the Clinical Geneticist should understand in order to advise any patient.

Session 8: Patient management and genetic counselling

The clinician and the patient must have an informative and informed conversation. Navdeep Singh Pannu covered all the essential information that a clinician should be discussing with their patients. What was quite revealing though, was the level that the clinician themself needs to be cognizant of. Navdeep described how with evolving processes, the clinician needs increased understanding of the process so that the patient can be appropriately counselled and informed. The laboratory must have access to sufficient resources to to perform PGT analysis and the referrer must have knowledge of the strengths and weaknesses of the laboratory and the test they are using, their clinic and match these to patient desires. The burden for the clinician lies in the proper preparation of the patient for the whole of their IVF journey and advise on what is most suitable for them and in having themselves knowledgeable in many aspects of ancillary treatments.

Lee Shulman gave everyone a glimpse of what constitutes modern genetics. Carrier screening is a new opportunity for populations to get a better handle of disease in their midst. But. . . with it comes the need for better understanding of the implications for carriers (and offspring). Not everything is as simple and as presented previously. The “Not-So-Healthy” heterozygote is being more often recognized now but the molecular basis is still not well understood. Rigid classification of diseases as “recessive” bely the reality of many conditions having a variable phenotype in recessive state. The frontline staff providing counseling for at-risk couples, need to expand their understanding of disease genetics and with it comes a new responsibility for appropriate informing of patients and clinical colleagues to empower them in making the choices that are best for them

Session 8: Patient management and genetic counselling

One of the principal goals for PGT-A is the opportunity for reducing the likelihood of multiple gestations by the transfer of a single embryo. Clinics often hide poorer performances by doing multiple embryo transfers to maintain some level of success. While multiple gestations are not at the same level as earlier IVF attempts, they still present challenges to the patient and medical treating staff. Joe Leigh Simpson revealed that (in the US at least), SET was becoming the norm rather than the exception. He was here with a timely reminder to everyone that multiple gestations were not without increased complications- for both the baby and the mother. Multiple embryo transfers were a direct cause of multiple gestations in a vast majority of cases. IVF itself has potential for increased adverse outcomes and multiple gestations substantially added further to it.

With a new focus on the genetic health of a population, carrier screening programs are becoming more prevalent and yet in some places, this preconception service is not routinely offered to couples seeking fertility treatment. Alice Weeks spoke about developments in Australia where identification of at-risk couples through such programs has increased. Internationally, many major societies have remained silent on which groups might be offered access to some of these programs. Alice then described her own clinic's experiences when routine IVF couples were given opportunity to have carrier testing. Many new carriers were identified as at-risk for chromosome structural problems or for a variety of genetic conditions not indicated by initial referrals. Alice concluded that offering such testing to IVF patients can drastically change the patient's IVF journey and outcome and in some cases will explain a likely cause for their infertility, further benefitting patients.

Session 8: Patient management and genetic counselling

As technologies advance, so do potential problems in understanding and reporting of findings. Steve Grkovic reported on their efforts to simplify and report issues associated with PGT-A ICN (“mosaic”) results. In taking a more pragmatic approach to the reporting of “mosaics”, Steve showed the potential for reducing over-reporting and increasing the number of embryos available for transfer. Possible false positive results were a selected target amongst the segmental mosaic group and so a rationalization of reporting was undertaken. Low level mosaics were now reported as a transfer ranking rather than with detail. Steve estimated that a further ~40 births/year occurred across their clinic group. This approach saved resources and saved embryos. But with any compromise comes some level of increased risk and Steve revealed that some adverse events had occurred.

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Session 9

Session 9: Quality in IVF- a prerequisite for PGT?

Quality in the PGT Laboratory- Assessing the referring Clinic Performances at the Laboratory- Mohammad Saleem

Sample Swap and Contamination Detection in PGT-A Pedro Echave

Combined NGS and SNP Analysis for Screening Aneuploidy and Contamination in PGT-A Jakob Horak

Embryo Rescue- Steven Yap

From Sample to Report: Comprehensive NGS Solutions for PGT-A, PGT-M and Carrier Screening- Alok Tomar

Posters relevant to this session:

24-A-016	Salvaging euploid blastocysts from abnormal fertilised zygote in IVF through biparental testing and ploidy assessment
24-A-020	The effectiveness of preimplantation genetic testing for aneuploidies using the NGS platform by EmbryoMap kit
24-A-023	The effect of an additional day of culture on Stage 3 blastocysts – the PGT-A standpoint
24-A-033	Combined NGS-based copy number and genome-wide SNP analysis for the screening of abnormal ploidy and maternal contamination for PGT-A
24-A-036	Batching Strategy to Improve IVF Outcomes in Patients with Advanced Maternal Age: Accumulation of Blasts with Preimplantation Genetic Testing for Aneuploidy (PGT-A)
24-A-042	A retrospective comparative analysis of ART outcomes: MESA versus TESE
24-A-058	Sample Swap and Contamination Detection in PGT-A

Session 9: Quality in IVF- a prerequisite for PGT?

It's not only the clinic that should be continuously assessing its quality. Mohamed Saleem took us through the process of how a service laboratory can participate in QC programs to maximise its benefit to a referrer while maintaining internal quality. In addition, Mohamed showed how participation in proficiency testing enables external assessment of how the laboratory performs on controlled samples. A good service laboratory though can further assist a clinic by looking at the consistency of its results and its processes. Mohamed discussed how comparing results from different clinics enabled identification of internal issues in the clinic and potential correction processes. There's no point in getting an answer and then reporting incorrectly- the QC extends all the way to the final report. Is understanding the report also a QC item? Do reports need appropriate understanding by the clinician? Should reports also be assessed on this basis?

Getting the right answer for the right embryo is obvious and yet often overlooked. Since all current PGT (especially PGT-A) involves an amplification step, any environmental or operator introduced contamination can have significant consequences. Pedro Echave raised the point that contamination could result in embryo discard or the possible miscalling of aneuploid embryos as mosaic. Most laboratories have practices to mitigate contamination risks but not everything is perfect or always done to the same standard. WGA prior to PGT-A amplifies mtDNA which is typically ignored in subsequent analyses. Pedro showed that polymorphisms within the mtDNA can be useful in identifying external contamination, sample swaps and sibling comparisons. It is a simple process requiring no extra lab work, just some further informatics. It cannot distinguish maternal contamination nor sibling embryo swaps but can be used in most other situations.

Session 9: Quality in IVF- a prerequisite for PGT?

In extending the utility of NGS PGT-A, Jakub Horak showed how utilization of revealed SNPs in the primary analysis could identify abnormal fertilisations. Jakub went further and verified initial results on a different paltform through rebiopsy and demonstrated haploidy, triploidy, polyploidy instances of maternal contamination and suspected contamination. By considering the combination od copy number and inherent SNPs, false triploidy through contamination could be resolved, aneuploid embryos falsely classified as mosaic could be identified and embryos with abnormal PN observations could potentially be rescued.

Reinforcing this latter theme, Alok Tomar described an integrated commercial system that can flip between copy number analysis and SNP interrogation that uses >500 SNP sites. Alok detailed simple workflows that can assist in ploidy analysis, sample swap detection, maternal contamination and abnormal fertilisation events. The option of sibling analysis offers sample swap detection and preludes abnormal fertilization assessment. In commercial systems such as this, the possibility of automated report generation can simplify processes for the lab. Alok's talk gives a vision of how maximizing data and integrating the analysis, can resolve some problems and streamline the whole PGT-A process.

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Session 10

Session 10: Innovations in Preimplantation genetics

Whole Embryo Sequencing- Nick Murphy

Further Advances in DNA Amplification for PGT and niPGT- Chuck Wagar

Maternal Spindle Transfer Coupled with Hyperspectral Imaging. A Promising Strategy to Restore Developmental Competence in Oocytes with Diminished Metabolic Profiles- Nuno Costa-Burges

Decentralizing PGT-A Cheng Wan

Genome Editing of PreImplantation Embryos for Research and Potential Clinical Use- Nada Kubikova

Combined CNV and Transcriptomic Analysis for Biomarker Discovery- Linbo Zhao

Innovative Use of SNPs in PGT-A Miroslav Hornak

Session 10: Innovations in Preimplantation genetics

Posters relevant to this session:

24-A-003	Cryptic chromosomal rearrangements detected by Optical Genomic Mapping (OGM) in a couple with recurrent miscarriages undergoing preimplantation genetic diagnosis.
24-A-004	Novel non-invasive preimplantation genetic testing for aneuploidy algorithm based on cell-free long non-coding RNA expression profiles in spent media
24-A-005	Multiomic integration of transcriptome and CNV analysis to enable novel biomarker discovery
24-A-009	Aneuploidy-driven gene expression in human blastocysts independent on aneuploid chromosomes revealed by RNA-seq
24-A-012	Difference of implantation-related gene expression using RT-qPCR between day 5 and day 6 euploid blastocysts
24-A-017	Rapid, efficient, decentralized PGT-A with nanopore sequencing.
24-A-018	Next-generation PGT: combined genome and transcriptome analysis
24-A-026	PGT for monogenic diseases and Pre-conceptual Testing of the Infertile Couple. Advancing PGT-M through Integrated Whole Exome Sequencing in Families with Genetic Histories
24-A-029	Innovative use of SNPs in PGT-A allows to distinguish meiotic trisomies without parental DNA sample support
24-A-032	Study of the chromosomal set of embryos obtained using the 1st polar body transfer technique to double the number of patients' oocytes
24-A-037	New developments and clinical utilization of comprehensive PGT-SR for detection of balanced rearrangements and microdeletions/duplications in embryos
24-A-041	Mapping of pathogenic CNV in LAMA2 gene by long-read sequencing- a new approach to preimplantation genetic testing workups
24-A-050	Validation and clinical application of low pass whole genome sequencing for high resolution PGT-SR in Vietnam with PGT-MAX-1 testing
24-A-053	Whole exome sequencing for direct variant testing in embryo biopsy samples

Session 10: Innovations in Preimplantation genetics

It was pointed out by Nick Murphy that babies born via ART have a higher incidence of congenital abnormalities- the links are uncertain but could relate to age of patients or possibly underlying mutations affecting fertility. Logically, if doing PGT, then why not also look at de novo mutations? The cumulative incidence of rare disorders in the population is upwards of 7%- this is for living people but gametes are not screened. Nick described the particular issue with de novo mutations and their genesis. The opportunity now though, is with new technology, even spontaneous mutations can be identified. Using his own group as an example, Nick described how a whole genome sequence can be performed from amplified DNA. This approach identifies any familial gene mutations as well as any de novo changes in that embryo. Validation steps revealed that a new pathogenic mutation occurs for every biopsy equivalent! The next stage is ascertaining the relevance to the couple, the likelihood of disease for the subsequent child, likely phenotype, onset, strength of evidence, etc. Nick emphasized the importance of the analysis, the informatics and appropriate databases for correct determinations, Added requirements included specialised counselling- due to greater complexity of the process. The possible benefits of the approach though, could benefit outcomes in many areas.

Nearly all current PGT starts with an amplification step- making this first stage often crucial for success of any subsequent stage. A couple of years back Chuck Wagar introduced a new twist to an old amplification approach. This new variation offered a more complete and even genome coverage compared to existing methods- something essential for effective DNA testing, especially for new analysis approaches

Session 10: Innovations in Preimplantation genetics

Chuck's new approach resulted in DNA that more closely resembled genomic DNA- making the new analysis approaches more stable and more consistent. With the greater uniformity came more reliable variant calling and greater stability in copy number profiling. The possibility of whole genome sequencing and identification of de novo variations in embryos became a reality. The high coverage of mtDNA also permitted heteroplasmy calling at high and low levels. While there are still some barriers to niPGT, Chuck has refocused on this issue.

For some couples, the underlying problem can be mitochondrial based. Whether it is mtDNA disease or mitochondrial function, the solution was only available recently. Nuno Costa_Borges showed the technology that enabled the transfer of a genome into a new background- Maternal spindle transfer. While mtDNA disease is an obvious application, Nuno revealed the possibility of improving embryo outcomes for couples with embryo development problems. Using hyper-spectral imaging, less competent oocytes could be identified and potential restored with a "change of clothes". There are many opportunities now for couples to have children using parent genetics and also reducing the psychological/and anonymity concerns of donors since only "less personal" mtDNA is perpetuated.

Session 10: Innovations in Preimplantation genetics

The opportunity for clinics wanting to perform their own PGT is often limited by cost and logistic factors. Cheng Wan showed us how new technologies can decentralize PGT-A, effectively democratizing participation in this area. Long read sequencing, often referred to as Third Generation Sequencing, substantially reduces capital costs, provides a flexibility for performance and can speed up the time for analysis. Cheng discussed the whole implementation process backed up by numerous validation studies. This approach may be of interest to many smaller groups wanting inhouse PGT services.

Not every genetic problem can be overcome by simple selection and avoidance. Not every step of embryo development can be revealed by current approaches. Nada Kubikova discussed the potential use of CRISPR-Cas9 in both areas. There are still some significant considerations for its clinical application but Nada showed how it might potentially be applied in the future to benefit patients with genetic disease as well as currently furthering research into many areas of embryo development. Full understanding of the technology is still incomplete and proper control of the process similarly still in development, but the opportunities that genome editing might offer cannot be ignored.

Session 10: Innovations in Preimplantation genetics

The embryo and its potentials are still substantially a mystery. Any approach that can dig deeper into its makeup can be a boon to improving IVF embryo selection processes. Capitalizing on a large research background, Linbo Zhao took us into the sphere where genome analysis and transcriptome analysis can be combined to give a more full, richer picture of what was happening in a growing and developing embryo. Linbo described how a multi omic work flow could be used to interrogate both the DNA and RNA from a single biopsy sample. Looking at both aspects, DNA and RNA, enabled confirmation of chromosome status as well as its impact on gene expression. Looking at the transcriptome separately offers the opportunity of biomarker discovery, with the potential to identify better performing embryos post transfer.

Many approaches to gain more information often comes with the requirement for more work on related but extra samples- this, in itself, increasing workload and ultimately costs of a process. Using information already gained during the routine analysis, offers improved analysis and better outcomes from the study. Miroslav Hornak showed us how SNP results can be an aid to interpreting chromosome profiles. Recommendations for embryo transfer priority can be with limiting information and any extra detail can make a difference to assigning embryo transfer suitability as well as other requirements such as genetic counselling. Understanding the causes of an aberration in embryo chromosomes can assist in determining transfer potential. Simplifying this analysis can create an opportunity for its application in clinical practice.

THANK YOU

PGT and BEYOND...