RBMO

COMMENTRY

Status of preimplantation genetic testing and embryo selection

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ABSTRACT

At the recent 2018 PGDIS congress, a review of randomized controlled trials of preimplantation genetic testing for aneuploidies (PGT-A) showed improved ongoing pregnancy rates per transfer in experienced centres and in women aged 35 years and older. Young women produce 40% abnormal embryos (20–60% range), but not all centres see a selection advantage; this indicates the need for more emphasis in improving biopsy and case management. Some chromosome abnormalities are iatrogenic; PGT-A could, therefore, be used as assisted reproductive technology (ART) quality control. Great improvements in non-invasive PGT by testing spent media have been reported, ranging from 80–95% concordance with trophectoderm biopsy, probably precluding the need for biopsy soon. Mosaicism was widely discussed, with PGDIS agreeing to update their guidelines, but continuing to recommend prioritizing euploid, followed by mosaic embryos. Techniques to allow simultaneous single sample analysis of aneuploidy and inherited mutations are improving, but this does not extend to de-novo mutations. Convincing data were presented on the efficacy of using endometrial receptivity tests to improve ART outcomes adjuvant or independently of PGT-A. Imprinting, CRISPR and cloning were also discussed, with a concluding presentation on the first extensive data (aneuploidy and morphology) on in-vivo conceived embryos.

INTRODUCTION

As Chairman of the 2018 congress of the Preimplantation Genetic Diagnosis International Society (PGDIS), just held in Bangkok, I had the opportunity to attend most of the presentations; from that perspective I would like to share with you my opinion on the status of preimplantation genetic testing (PGT). The aim of the congress programme was for non-experts to develop a clear understanding of PGT, experts to define areas that need further research and science-entrepreneurs to come up with ideas for new products.

Since last year, preimplantation genetic screening (PGS) and diagnosis (PGD) were re-termed preimplantation genetic testing (PGT), of which 90% of cases performed are for PGT for aneuploidies (PGT-A), for indications such as advanced maternal age, recurrent miscarriage, and, in general, to improve the selection of embryos with the most potential to implant and produce a viable pregnancy. In the USA, we estimate that 40% of cycles are now accompanied by PGT-A, and that number is increasing. Despite increased uptake, questions about PGT-A remain. Discussions at the meeting revolved around four main queries: is PGT-A indicated for every patient? Are we losing normal embryos through the PGT-A process? What is the relevance of mosaicism? And where are we with non-invasive methods of sampling embryos?

IS PGT-A INDICATED FOR EVERY IVF CYCLE?

Richard Scott and others presented a summary of the many randomized controlled trials (RCT) that have used the latest techniques for chromosome screening, which overall have shown significant improvement in ongoing pregnancy rates, dramatic reduction of multiple pregnancies by transferring single euploid embryos, reduction in miscarriage rates, improved ongoing pregnancy rates per transfer, reduced risk of aneuploid pregnancies and shorter time to pregnancy. All these studies, but one, however, were conducted in single centres with extensive experience in PGT. When the technique was applied to 34 IVF centres through the STAR study

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KEYWORDS

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(*Munné et al., 2017*), PGT was found to help only patients aged 35 years and older. In fact, analysis of the SART data produces the same result (*SART, 2015*). The SART data show that implantation rates hover around 50% for PGT irrespective of maternal age, whereas, without PGT, rates decrease after the age of 35 years. This is good and bad news. It is good because it shows that the hypothesis is correct, with advanced maternal age a clear indication for PGT-A; however, it is bad news because it does not help young patients in all fertility centres, suggesting that some less experienced centres may be losing embryos through the process.

HOW COULD EMBRYOS BE LOST THROUGH THE PROCESS?

As Scott and Paulson pointed out, the higher the technical error rate, the more likely it is that normal embryos are incorrectly discarded, and abnormal ones transferred. Fortunately, this is a minor problem because published error rates for the most used PGT techniques range between 0 and 2% (*Gutierrez-Mateo et al., 2011; Scott et al., 2012; Treff et al., 2012; Fiorentino et al., 2014; Wells et al., 2014; Kung et al., 2015; Yang et al. 2015*) and the predictive positive and negative values around 4% (*Scott et al., 2012; Rubino et al. 2016*). What may be a bigger problem is embryo biopsy. Only *Scott et al. (2013)* have studied the effect of blastocyst biopsy, which suggests no detrimental effect, but it was conducted by one of the most experienced PGT laboratories. Even a day-3 biopsy can be non-detrimental in experienced hands, as a RCT presented by *Rubio et al. (2017)* has shown, as biopsy is a developed skill. Skilled centres do little harm during embryo biopsy, but when it is carried out by centres without experience one cannot expect the same outcomes. Without guidelines and standardization of embryo biopsy techniques, you cannot reliably compare outcomes across a range of IVF centres. If the risk of damage is high and embryos are being lost, PGT only improves embryo selection above a threshold of chromosome abnormality rates. At lower technical embryo loss, i.e, less than 10%, it may improve embryo selection even in egg donors with only 20% chromosome abnormality rates, whereas, at high embryo loss (i.e. 30%), the damage is only compensated by selecting against 60% or more chromosome abnormalities, i.e., among women aged 35 years and older. More studies and standardization of embryo biopsy is clearly needed to democratize PGT to the masses. Other explanations criticizing PGT-A have focused on technical artifacts and mosaicism.

MOSAICISM

Mosaicism is the occurrence of several cell lines with different chromosome content within the same embryo but is generally understood here as an embryo containing both normal and abnormal cell lines. As we biopsy only five to 10 cells, the result may or may not represent the rest of the embryo. To further complicate the picture, I presented that, depending on the technique used (4% with array comparative genomic hybridization to 18% with next-generation sequencing), the IVF centre growing the embryos (ranging from 4–44% mosaicism), and maternal age (decreasing from 18 to 8% with age), great variability occurs in the incidence of mosaic results. One factor, however, is crystal clear: with the most precise technique, next-generation sequencing, embryos classified as fully normal or fully abnormal have a close to 0% chance of being misclassified. As a collateral result, we have opened a small rift between normal and abnormal diagnoses, i.e. a mosaic diagnosis. Elpida Fragouli presented a summary of the pregnancy outcomes of transferred mosaic embryos. Indeed, as an in-between result, embryos classified as mosaic have a chance to produce a viable pregnancy (35%) that is intermediate between a normal embryo (65%) and an abnormal embryo (<4%). As such, the consensus in the meeting was that they need to be deprioritized for transfer, but not discarded. They can produce viable pregnancies, and, so far (from about 300 transfers), all babies seem to be normal, which suggests that abnormal cells in the mosaic embryo die out or do not grow as fast as normal ones. It is important to notice that reporting mosaic embryos as fully abnormal or not transferring them when no euploid embryos are available, may contribute to lower than expected pregnancy rates in PGT-A. This is certainly the case in the STAR study (*Munné et al., 2017*) in which mosaic embryos were either not reported or, if reported, forbidden for transfer according to the study. The debate continues regarding which mosaics to transfer and how to counsel patients. To assist, a task force has been established by PGDIS to update its guidelines on mosaicism, whereas, the National Society Genetic Councilors (NSGC) is developing one on counselling and management of patients.

INDUCED CHROMOSOME ABNORMALITIES BY ART

In addition to differences in mosaics rates between fertility clinics, differences in aneuploidy rates have also been reported. One potential source of aneuploidy rate variability is hormonal stimulation, and my co-workers and I presented data suggesting a direct link between the absolute number of normal embryos and the fraction of gonadotrophin administered that comprised human menopausal gonadotrophin. Many other factors have been proposed to affect aneuploidy other than maternal age, such as culture conditions, culture media and contaminants from plastics. One idea I presented is that PGT can be used as quality control and monitoring of chromosome abnormalities, especially in egg donors, to compare new protocols with old ones. Although differences in aneuploidy rates, especially in young patients (ranging from 30–50% depending on the technique), were used in the meeting by Richard Paulson as proof that we do not yet have a standardized PGT method, I would say that it makes more sense to think of it as variability between centres (same technique used, 20–60% range of aneuploidy depending on the centre) and that technique is less important than the fertility centre growing the embryos. Nothing was discussed about IVF laboratory automation, but my opinion is that automation is absolutely necessary to minimize variability between treatments and maximize results.

NON-INVASIVE PGT

If blastocyst biopsy is the most likely culprit in the low success of PGT in young patients in some fertility centres, we may be close to a solution by using non-invasive methods of sampling the embryo. Non-invasive PGT (NI-PGT) is based on the observation that embryos shed DNA into the medium where they are growing. The source of this DNA is unknown, some speculating it comes

from apoptotic cells (not the best cells to analyse as they are probably more likely to be abnormal compared with the rest of the embryo), whereas others speculate that a more dynamic process is occurring, with increased DNA shedding from day-5 onwards. Several teams have shown that NI-PGT results had rates of concordance with blastocyst biopsy above 80%, a dramatic improvement from last year. For example, Dhruti Babariya presented 95% concordance between NI-PGT and blastocyst biopsy PGT and, even more recently (ESHRE 2018), Carmen Rubio presented close to 90% concordance. An error rate of 5% with no embryo damage could mean that NI-PGT could improve results in all couples, and not just those above the age of 35 years. Again, the method should not yet be applied widely as DNA contaminants (sperm, corona cells, culture media), choice of culture media, size of droplets, where to culture embryos, volume needed to sample, culture days needed to be sampled, and DNA amplification methods have not yet been standardized. It is questionable, with today's technology, if we will be able to detect by NI-PGT single gene mutations or mosaicism; however, 3 years ago, nobody was thinking that NI-PGT could even be a possibility.

Not all the meeting was focused on PGT-A. If NI-PGT-A is one of the hottest subjects, a comprehensive PGT technique to analyse at the same time aneuploidy and gene defects is greatly desirable. Currently, most laboratories use karyomaping (single nucleotide polymorphism analysis), a technique developed by Alan Handyside and coworkers, to phase the chromosomes inherited by the embryo and trace which chromosome comes from the father and the mother. Knowing which chromosome(s) carries the mutation(s) allows us to determine which embryos are affected, carriers, or free of the genetic defect. The technique, however, is not suited for quantitative analysis of mitotic abnormalities (mosaicism), it does not analyse directly the mutation, and cannot infer de-novo mutations. Joris Vermeesch presented a technique that has potential improvement over the current one. The aim of most approaches, however, is to use a targeted SNP analysis to detect single mutations or groups of common mutations combined with quantitative haplotype analysis or chromosome count. Although not presented in the meeting, new methods for simultaneous analysis of aneuploidy, single-gene Mendelian defects, and, for the first-time, polygenic diseases such as diabetes are becoming available. Indeed, the field of PGT has been expanding for years outside the traditional indications (PGT-A, PGT-M for Mendelian disease) to cover PGT for cancer and human leukocyte antigens matching, and soon polygenic diseases.

Current technology is probably sufficient for PGT-A for carrier couples of a known single-gene or polygenic defects, but not for de-novo mutations. De-novo mutations increase linearly with paternal age, and are possibly involved in the increase in autism, schizophrenia and other mental health disorders observed in recent decades; coincidentally, within this same period, the age at which a couple (fathers included) have their first baby has also been increasing. Unfortunately, no significant advances in PGT of de-novo mutations were reported in this meeting. A dramatic reduction in sequencing costs and better bioinformatics are probably needed to tackle this challenge.

Not all cases of autism are attributed to de-novo mutations, and some are linked to imprinting. Verana Nordhoff presented an excellent summary of the different ways in which the genome is imprinted and the different factors, including ART, environment and paternal age, which affect imprinting. Most research so far has been conducted on animal models, and we still have not attempted either a screening of abnormal imprinting in embryos nor sufficiently studied how different ART methods change imprinting and what consequences those changes have.

Even if aneuploidy is the major cause of failure of human embryos to implant, this is not the whole picture. The most successful centres achieve close to 65–70% ongoing implantation rates; how do we get to 100%? Carlos Simon presented solid proof that endometrial receptivity is also key. Some women have a few-day shift in their window of implantation, and so if day-5 embryos are implanted the standard 5 days after progesterone trigger they may not implant. Individualizing transfer by using endometrial receptivity testing increases overall ongoing implantation rates by 10%. That takes us to 80% ongoing implantation rates, but still not 100%. What next? Simon also presented data showing that the endometrial microbiome has a significant effect on implantation and miscarriage rates, with some microbiomes being clearly pathological. The best way to analyse these microbiomes is through genomics, as many rare bacteria cannot be cultured, one reason why they were not previously analysed.

Beyond PGT, we find ourselves in the era of clustered regularly interspaced short palindromic repeats (CRISPR); the selection of embryos seems so passé when we could instead edit each one of them. As a reminder, we can select for blue eyes with PGT but we have not done it. I presume there is less demand for genetic trait improvement than predicted by Yuval *Noah Harari (2016)*, and it is not yet around the corner. Certainly, the technology is getting there. The team of Lun Suo presented their experience editing human embryos. It remains to be seen if off-target effects are present, what the rate of conversion is when applying edits at the embryo level, or if we should edit at the egg level.

Finally, the last talk was the one I would say generated most awe. It will seem amazing to lay people, but most embryologists have never even seen an in-vivo conceived and grown embryo, and even fewer geneticists have analysed such DNA. Moses Cesario presented the results obtained with a new device, called Previvo, that can effectively perform uterine lavage and collect in-vivo conceived and cultured blastocysts, which my team had the great pleasure to analyse by PGT. The morphology of these embryos seems to be better than that of in-vitro cultured embryos (more cells), but the rate of chromosome abnormalities, so far, seems to be within the higher range seen in-vitro culture embryos.

All in all, this was a great meeting and I look forward to the next Geneva 2019 PGDIS congress. I foresee that by then, NI-PGT will be ready for prime-time in selected IVF centres, and we will have more research on comprehensive PGT, testing of de-novo mutations, CRISPR, imprinting of embryos, and data from in-vivo conceived embryos as perhaps our next gold standard. A workshop to educate pre-millennials on bioinformatics would also be welcome.

REFERENCES

Fiorentino, F., Biricik, A, Bono, S., Spizzichino, L., Cotroneo, E., Cottone, G., Kokocinski, F., Michel, CE **Development and validation of a next-generation sequencing–based protocol for 24-chromosome aneuploidy screening of embryos.** Fertil Steril 2014; 101: 1375–1382

Gutiérrez-Mateo, C., Colls, P., Sánchez-García, J., Escudero, T., Prates, R., Wells, D., Munné, S. **Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos.** Fertil. Steril. 2011; 95: 953–958

Kung., A, Munné, S., Bankowski, B., Coates, A., Wells, D. **Validation of next-generation sequencing for comprehensive chromosome screening of embryos.** Reproductive Biomed Online 2015; 31: 760–769

Munné, S., Kaplan, B., Frattarelli, J., Gysler, M., Child, T., Nakhuda, G., Shamma, F.N., Silverberg, K., Kalista, T., Oliver, K., Katz-Jaffe, M., Wells, D., Gordon, T., Willman, S. **Global multicenter randomized controlled trial comparing single embryo transfer with embryo selection by preimplantation genetic screening using next-generation sequencing versus morphologic assessment.** Fertil. Steril. 2017; 108: e19(O-43)

Noa Harari, Y **Homo Deus: A brief history of tomorrow.** 2016,Random House

Rubio, C., Bellver, J., Rodrigoa, L., Castillon, G., Guillen, A., Vidal, C. **In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized controlled study.** Fertil. Steril. 2017; 107: 1122–1129

SART https://www.sartcorsonline.com/rptCSR_PublicMultYear.aspx?reportingYear=2015 2015

Scott, R.T.Jr., Ferry, K., Su, J., Tao, X., Scott, K., Treff, N.R.T. **Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study.** Fertil. Steril. 2012; 97: 870–875

Scott, R.T.Jr, Upham, K.M., Forman, E.J., Zhao, T., Treff, N.R. **Cleavage stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial.** Fertil. Steril. 2013b; 100: 697–703, 2013

Treff, N.R., Ferry, K.M., Zhao, T., Ferry, K.M., Su, J., Taylor, D., Scott, R.T. **Development and validation of an accurate quantitative real-time polymerase chain reaction–based assay for human blastocyst comprehensive chromosomal aneuploidy screening.** Fertility Sterility 2012; 97: 819–824

Wells, D., Kaur, K., Rico, A., Grifo, J., Anderson, S., Sherlock, J., Taylor, J.C., Munné, S. **Clinical utilization of a rapid low-pass whole-genome sequencing technique for the diagnosis of aneuploidy in human embryos prior to implantation.** J. Med. Genet. 2014; 51: 553–562

Yang, Z., Lin, J., Zhang, J., Leng Fong, W., Li, P., Zhao, R., Liu, X., Podevin, W., Kuang, Y., Liu, J. **Randomized comparison of next-generation sequencing and array comparative genomic hybridization for preimplantation genetic screening: a pilot study.** BMC Medical Genomics 2015; 8: 30. doi: 10.1186/s12920-015-0110-4

Rubino, P., Dearden, L., Guan, L., Ruiz De Assin, R., Mazmanian, K., Kolb, B.A., Nelson, J., Norian, J.M., Wilcox, J., Tan, T. **Healthy baby after intrauterine transfer of monosomic embryos.** Fertil. Steril. 2016; 106: e160(P-145)

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