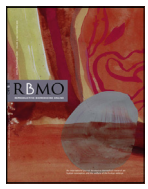
RBMO



COMMENTARY

# Preimplantation genetic testing as a component of root cause analysis of errors and reassignment of embryos in IVF

Megan Bunnell[1], Navid Esfandiari[2,*]

## KEY MESSAGE

Determining the origin of IVF errors is important for future process integrity and error reduction and also for any remaining embryos that need to be correctly identified and assigned to the correct patients. Preimplantation genetic testing with SNP and/or NGS can assist in the mitigation and identification of errors.


## ABSTRACT

The risks of embryo/gamete mix-up are a threat to the integrity of the IVF process, with significant implications for affected families. The use of preimplantation genetic testing through single-nucleotide polymorphism array or next-generation sequencing technology can help to identify, characterize and ultimately help, in some cases, to find the root cause, and to mitigate the extent of these errors for a given patient or laboratory.


## INTRODUCTION

Much has been written about the prevention of errors in the IVF process. Mismatch events in IVF, including switching of oocytes, sperm and/or embryos, are rare but unfortunate errors that have long-lasting consequences. The ability to trace parental samples is crucial to the integrity of the IVF process. To minimize the risk of gamete and embryo mix-ups and provide guidance to assisted reproductive technology (ART) laboratories and clinical sites, protocols from ART-related organizations including the European Society for Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Medicine (ASRM) have been developed (*Daar et al., 2016*). These guidelines require labelling of labware and double-checking procedures for patient identification, as well as a chain of custody for all patients' samples. Novel technological advances have also made use of radiofrequency identification technology, barcodes and, most recently, direct embryo tagging systems with silicon injectables for zygotes/embryos (*Ilina et al., 2019*). Ultimately, while the risks of embryo/gamete mix-up can be minimized by these interventions, they cannot be eliminated.

Little has been written about error mitigation once mix-ups do occur, and many IVF errors lead to the discarding of all embryos and gametes potentially involved in the investigated error. Most errors in gamete/embryo identification have, up to this point, been identified due to phenotypic, often racial, differences (*Sakkas et al., 2018*). It is very likely that, with the increased use of direct to consumer genetic testing, more errors will be identified and require further investigation and workup. Various publications have helped to identify the steps in the IVF process that are subject to error. One comprehensive review by Cimadomo and colleagues used a failure mode and effects analysis to define the various risks across the whole preimplantation genetic testing (PGT) protocol (*Cimadomo et al., 2016*). Through this, they identified five main processes, namely blastocyst biopsy, biopsy tubing, blastocyst cryopreservation, diagnosis and blastocyst warming, that were subject to potential error. It is clear that recognizing high-risk opportunities for error in the

[1] Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Boston, MA, USA
[2] Department of Obstetrics and Gynecology and Reproductive Sciences, University of Vermont Medical Center, Larner College of Medicine, Burlington, VT, USA


*Corresponding author. E-mail address: Navid.Esfandiari@uvmhealth.org (N Esfandiari). https://doi.org/10.1016/j.rbmo.2020.08.031 1472-6483/
Declaration: The authors report no financial or commercial conflicts of interest.


## KEY WORDS

Embryo biopsy
IVF errors
Paternity testing
Preimplantation genetic testing
Single-nucleotide polymorphism

IVF process is an important endeavour; however, it is also critical that, when errors occur, the available technology can be used to mitigate the long-term effects.

This paper highlights a proposal for the use of PGT using single-nucleotide polymorphism (SNP) array and next-generation sequencing (NGS) technologies to identify, characterize and ultimately, in some cases, help to find the root cause, and to mitigate the extent of these errors for a given patient or laboratory.

## THE IVF PROCESS

There are many stages of the IVF process that can result in errors or specimen mix-ups. Oocyte retrieval and sperm processing necessitate accurate labelling and tracking of samples and, at all times during post-fertilization and embryo development, embryos must be accurately assigned to the patient(s) whose original gametes produced them. In some cases, the embryo will be biopsied for PGT, adding another sample that must be accurately labelled and traced back to the original embryo. If embryos are cryopreserved, they must be stored in such a way that labels cannot be detached, destroyed or mixed, as accurate embryo transfer relies on tracing an embryo back to a patient (*Nesbit et al., 2020*).

If an error occurred in the use of unintended spermatozoa to fertilize the intended oocyte, PGT would be able to identify that the intended father was not the father of the embryo in question through paternity testing. There are numerous case reports of the use of wrong spermatozoa to fertilize an oocyte, due either to a mix-up or to the intentional use of spermatozoa by centre employees, as in *United States* v. *Cecil Jacobson* (*Vaughn et al., 2015*).

When mix-ups have happened, they are most often at embryo transfer, where the wrong embryo is transferred to the wrong woman, although many gamete-level and even embryo-level errors may be occurring occultly without phenotypic differences salient enough to prompt investigation (*Sakkas et al., 2018*). The most recent, high-profile, incidence of this is the lawsuit brought about by *Anni and Ashot Manukyan* v. *LA CHA Fertility Center*, in which their embryo (and the embryo of another couple) was transferred into a third, unrelated patient who later gave birth to twin boys, one of whom had the Manukyans as genetic parents (*Zhang, 2019*). Although it is not known if any of these three involved couples had additional cryopreserved embryos, these embryos would need to be discarded or tested via PGT to determine their correct parentage if they were to be used in the future.

In an analysis of errors from a single centre's andrology and embryology laboratories between March 2003 and November 2015, moderate and significant errors (those that negatively affected a cycle to the point that it was nearly or completely lost), found that such errors were predominantly human and equipment errors, with cryopreservation as the most vulnerable cycle aspect (*Sakkas et al., 2018*). In the vast majority of cases, however, the root cause of errors of gamete or embryo mix-up are not determined.

Errors can also occur at the level of the genetics laboratory when specimens undergo screening for genetic disease through PGT. Sample mix-up can occur as biopsies are sent to the genetics laboratory and results are returned to the IVF centre. Errors made by the genetics laboratory include potential errors in diagnosis of aneuploidy and/or monogenic disease. Some of these errors are secondary to the limitations of testing, as there is potential for complications such as allele drop out, which occurs when one copy of a gene (or allele) amplifies and the other copy does not, which can cause a misdiagnosis. For example, in a recessive condition, an unaffected carrier embryo could appear affected, or for a dominant condition an affected embryo could appear unaffected.

It is also possible to have a spontaneously conceived pregnancy while undergoing PGT for monogenic/single gene deficits or aneuploidies during an IVF cycle. This could result in an unexpectedly affected offspring. A case of this has been described by Bettio and colleagues, where a 45,X result was found for products of conception after a spontaneous abortion in a patient undergoing PGT for aneuploidies (*Bettio et al., 2016*). DNA fingerprinting was performed on the products of conception and euploid IVF embryos using 40 SNP, with results showing a similarity rate for the products of conception that was consistent with sibling and non-self relationship.

The ASRM ethics committee opinion "Disclosure of medical errors involving gametes and embryos" focuses on "errors resulting in situation in which the gametes or embryos used in a fertility center are not those originally intended for use in the couple undergoing treatment, potentially leading to the birth of a child with an unplanned genetic parentage" (*Daar et al., 2016*). This document clearly states that "clinics should address medical errors and near misses by conducting a root-cause analysis aimed at revealing system failures". Practically speaking, it can be impossible to identify the root cause without revealing the genetic parentage of an embryo, necessitating embryo biopsy and genetic testing.

## SNP ARRAY AND NGS

SNP arrays are a type of DNA microarray used to detect polymorphisms within a population. SNP are single-nucleotide variations at a single site in the DNA. SNP arrays allow for the study of slight variations between whole genomes. The most important clinical applications of SNP arrays allow a determination of disease susceptibility and are used for genome-wide association studies (*Tobler et al., 2014*). SNP-based analysis can be used to map disease loci and determine disease susceptibility genes in individuals. SNP arrays can also be used to perform paternity and maternity testing on embryos as long as paternal/maternal samples are available. Commercially available SNP platforms include 200,000–300,000-probe microarray platforms, which are fully capable of detecting paternity if such information were needed.

NGS technology encompasses a number of different platforms, all with the general technique of amplification of the entire genome (from a single cell or several cells) and subsequent fragmentation of this material, with comparison to a reference sequence. It allows for the detection of aneuploidy, translocation and single-gene mutations, and is the only technology capable of detecting low-level mosaicism. NGS is also capable of detecting paternity when compared with a parent sequence.

If a patient underwent embryo transfer with an embryo that had been previously biopsied and then there was a question of maternal/paternal inheritance, any other embryos assigned to that couple could be retested using SNP array to determine parental inheritance as long as parental samples were available. It is important to note that, with the current low-pass sequencing methodology used for NGS-based PGT, genotyping is usually not performed; therefore what is proposed would require rebiopsy or re-analysis versus use of previously collected data. Many genetic laboratories do save amplified DNA in case it is needed, for example so that a whole-genome amplification, with appropriate parental samples/amniocentesis or chorionic villus sampling, or a postnatal sample could help to confirm that the correct embryo had been transferred and originated from the intended gametes. A method of trophectoderm qualitative PCR technology has been validated by Scott and colleagues that allows for the discrimination of sibling human embryos, and could be extrapolated to identify embryo origin (*Scott et al., 2014*) .

Until recently, a hindrance to this method would have been the need to test already cryopreserved embryos. There is evidence that the warming, biopsy and revitrification does not result in a statistically significant difference in rate of embryo survival and clinical pregnancy rate compared with fresh embryo transfer (*Wilding et al., 2019*). Thus, if there was a question of the origin of an embryo, instead of discarding all embryos of uncertain paternity and/or maternity, embryos could be warmed, biopsied and revitrified, allowing potential reassignment to the rightful parents or, in the case of mix-ups, identification of the root cause. Similarly, if an error was made at the level of the genetics laboratory (e.g. biopsy sample to original sample mix-up), embryos would need to be rebiopsied to determine the origin of the mishap. For example, if the couple was expecting a female child after PGT and the child born was male, possible errors would include an error at the level of the IVF laboratory (wrong embryo transferred) or at the level of the genetic laboratory (incorrectly managed biopsy).

## PREVENTION OF ERRORS

Various strategies have been proposed to avoid errors within the IVF process. To prevent the transfer of wrong gametes/embryos, ESHRE recommends (*Luca et al., 2000*) that laboratories perform procedures with the use of disposable pipettes and associated materials only. At minimum, containers holding gametes must be labelled with at least three patient identifiers, and with patient and specimen identity confirmed by two staff members at each step of the process. Newer technology has introduced barcodes and radiofrequency identification tags to add an additional layer of tracking capability. These methods are still subject to some degree of potential for human error and technological failure, and also to the introduction an element of potential harm to the embryos/gamete (*Nesbit et al., 2020*).

For errors related to PGT, it is recommended that the name of the genetic abnormality being screened for is present on the specimen label in addition to patient identifiers. It is also recommended that probes be tested on other cells before use on embryo biopsy specimens, and that embryos should be stored in separate dishes or droplets to minimize the chance of an incorrect transfer. Types and examples of errors reported throughout the literature have been well summarized in a recent systematic review from Nesbit, Porter and Esfandiari (*Nesbit et al., 2020*). In a review by SenGupta and colleagues methods are described for internal audits within ART laboratories with recommendations for root cause analysis if any non-conformances are identified, and external quality assessments are conducted as recommended by the ESHRE PGT Consortium (*SenGupta et al., 2016*).

## CONCLUSION

As direct to consumer genetic testing becomes more mainstream, recent and historical IVF errors are likely to be uncovered. Determining the origin of these errors is important not only for the future integrity of processes and error reduction, but also in terms of any remaining embryos that need to be correctly identified and assigned to the correct patients. PGT with SNP paternity/maternity testing and/or NGS can help to clarify many of the questions that can arise regarding IVF mix-ups and can assist in the mitigation and identification of errors.

## REFERENCES


Bettio, D., Capalbo, A., Albani, E., Rienzi, L., Achille, V., Venci, A., Ubaldi, F.M., Levi Setti, P.E. **45,X product of conception after preimplantation genetic diagnosis and euploid embryo transfer: evidence of a spontaneous conception confirmed by DNA fingerprinting.** Reprod. Biol. Endocrinol. 2016; 14: 55. doi:10.1186/s12958-016-0190-9

Cimadomo, D., Ubaldi, F.M., Capalbo, A., Maggiulli, R., Scarica, C., Romano, S., Poggiana, C., Zuccarello, D., Giancani, A., Vaiarelli, A., Rienzi, L. **Failure mode and effects analysis of witnessing protocols for ensuring traceability during PGD/PGS cycles.** Reprod. Biomed. Online 2016; 33: 360–369. doi:10.1016/j.rbmo.2016.06.002

Daar, J., Amato, P., Benward, J., Collins, L.R., Davis, J.B., Francis, L., Gates, E., Klipstein, S., Koenig, B., McCullough, L., Reindollar, R., Sauer, M., Sokol, R., Stein, A., Tipton, S. **Disclosure of medical errors involving gametes and embryos: an Ethics Committee opinion.** Fertility and Sterility 2016; 106: 59–63. doi:10.1016/j.fertnstert.2016.03.018

Ilina, I.V., Khramova, Y.V., Filatov, M.A., Sitnikov, D.S. **Application of femtosecond laser microsurgery in assisted reproductive technologies for preimplantation embryo tagging.** Biomed. Opt. Express 2019; 10: 2985–2995. doi:10.1364/BOE.10.002985

Luca, Gianaroli, Michelle, Plachot, Roelof van, Kooij, Safaa, Al-Hasani, Karin, Dawson, Anick, DeVos, M. Cristina, Magli, Jacqueline, Mandelbaum, Jacqueline, Selva, Wouter van, Inzen **Committee of the Special Interest Group on Embryology, ESHRE guidelines for good practice in IVF laboratories.** Human Reproduction 2000; 15: 2241–2246. doi:10.1093/humrep/15.10.2241

Nesbit, C., Porter, M.B., Esfandiari, N. **Catastrophic human error in assisted reproductive technologies: A Systematic Review.** Journal of Patient Safety 2020

Sakkas, D., Barrett, C.B., Alper, M.M. **Types and frequency of non-conformances in an IVF laboratory.** Hum. Reprod. 2018; 33: 2196–2204. doi:10.1093/humrep/dey320

Scott, R.T., Su, J., Tao, X., Forman, E.J., Hong, K.H., Taylor, D., Treff, N.R. **Trophectoderm DNA fingerprinting by quantitative real-time PCR successfully distinguishes sibling human embryos.** J. Assist. Reprod. Genet. 2014; 31: 1421–1425. doi:10.1007/s10815-014-0315-z

SenGupta, S.B., Dhanjal, S., Harper, J.C. **Quality control standards in PGD and PGS.** Reprod. Biomed. Online 2016; 32: 263–270. doi:10.1016/j.rbmo.2015.11.020

Tobler, K.J., Brezina, P.R., Benner, A.T., Du, L., Xu, X., Kearns, W.G. **Two different microarray technologies for preimplantation genetic diagnosis and screening, due to reciprocal translocation imbalances, demonstrate equivalent euploidy and clinical pregnancy rates.** J. Assist. Reprod. Genet. 2014; 31: 843–850. doi:10.1007/s10815-014-0230-3

Vaughn, M., Hossain, A., Phelps, J.Y. **Liability for mismanagement of sperm specimens in fertility practices.** Fertility and Sterility 2015; 103: 29–32. doi:10.1016/j.fertnstert.2014.09.038

Wilding, M., Terribile, M., Parisi, I., Nargund, G. **Thaw, biopsy and refreeze strategy for PGT-A on previously cryopreserved embryos.** Facts Views Vis. Obgyn. 2019; 11: 223–227

Zhang, Sarah 2019 **IVF Mix-Ups Have Broken the Definition of Parenthood.** The Atlantic


Received 20 May 2020; received in revised form 10 August 2020; accepted 24 August 2020.