ORIGINAL ARTICLE: ASSISTED REPRODUTION

Abnormally fertilized oocytes can result in healthy live births: improved genetic technologies for preimplantation genetic testing can be used to rescue viable embryos in in vitro fertilization cycles

Antonio Capalbo, Ph.D.,[a,b] Nathan Treff, Ph.D.,[c] Danilo Cimadomo, M.Sc.,[a,d] Xin Tao, Ph.D.,[c] Susanna Ferrero, M.D.,[a] Alberto Vaiarelli, M.D.,[a] Silvia Colamaria, M.D.,[a] Roberta Maggiulli, Ph.D.,[a] Giovanna Orlando, M.Sc.,[a] Catello Scarica, Ph.D.,[a] Richard Scott, M.D.,[c] Filippo Maria Ubaldi, M.D.,[a,b] and Laura Rienzi, M.Sc.[a,b]

[a] Genera, Centers for Reproductive Medicine, Clinica Valle Giulia, Rome, Italy; [b] Genetyx, Molecular Genetics Laboratory, Marostica, Italy; [c] Reproductive Medicine Associates of New Jersey, Basking Ridge, New Jersey; and [d] Dipartimento di Scienze Anatomiche, Istologiche, Medico Legali e dell'Apparato Locomotore, Università degli Studi di Roma "Sapienza," Rome, Italy

Objective: To test whether abnormally fertilized oocyte (AFO)–derived blastocysts are diploid and can be rescued for clinical use.
Design: Longitudinal-cohort study from January 2015 to September 2016 involving IVF cycles with preimplantation genetic testing for aneuploidy (PGT-A). Ploidy assessment was incorporated whenever a blastocyst from a monopronuclear (1PN) or tripronuclear zygote (2PN + 1 smaller PN; 2.1 PN) was obtained.
Setting: Private IVF clinics and genetics laboratories.
Patient(s): A total of 556 women undergoing 719 PGT-A cycles.
Intervention(s): Conventional chromosome analysis was performed on trophectoderm biopsies by quantitative polymerase chain reaction. For AFO-derived blastocysts, ploidy assessment was performed on the same biopsy with the use of allele ratios for hetorozygous SNPs analyzed by means of next-generation sequencing (1:1 = diploid; 2:1 = triploid; loss of heterozygosity = haploid). Balanced-diploid 1PN- and 2.1PN-derived blastocysts were transferred in the absence of normally fertilized transferable embryos.
Main Outcome Measure(s): Ploidy constitution and clinical value of AFO-derived blastocysts in IVF PGT-A cycles.
Result(s): Of the 5,026 metaphase II oocytes injected, 5.2% and 0.7% showed 1PN and 2.1PN, respectively. AFOs showed compromised embryo development ($P<.01$). Twenty-seven AFO-derived blastocysts were analyzed for ploidy constitution. The 1PN-derived blastocysts were mostly diploid (n = 9/13; 69.2%), a few were haploid (n = 3/13; 23.1%), and one was triploid (n = 1/13; 7.7%). The 2.1PN-derived blastocysts were also mostly diploid (n = 12/14; 85.7%), and the remainder were triploid. Twenty-six PGT-A cycles resulted in one or more AFO-derived blastocysts (n = 26/719; 3.6%). Overall, eight additional balanced-diploid transferable embryos were obtained from AFOs. In three cycles, the only balanced-diploid blastocyst produced was from an AFO (n = 3/719; 0.4%). Three AFO-derived live births were achieved: one from a 1PN zygote and two from 2.1PN zygotes.
Conclusion(s): Enhanced PGT-A technologies incorporating reliable ploidy assessment provide an effective tool to rescue AFO-derived blastocysts for clinical use. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Ploidy, preimplantation genetic testing, PGT

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Reprint requests: Antonio Capalbo, Ph.D., Genera, Centers for Reproductive Medicine, Via G. de Notaris 2/B, 00197, Rome, Italy (E-mail: capalbo@generaroma.it).

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One of the main rate-limiting steps in in vitro fertilization (IVF) is the availability of viable embryos to be used for transfer in each cycle. This is particularly relevant for patients with poor prognosis due to advanced female age or showing a poor response to controlled ovarian stimulation. In these couples, the limited number of oocytes results in a significant reduction of the chance to achieve a live birth (1).

After oocyte retrieval, insemination is performed with the use of conventional IVF or intracytoplasmic sperm injection (ICSI). In all IVF laboratories, a fertilization check is performed 16–18 hours after insemination and requires the confirmation of the extrusion of the second polar body and determination of the number and shape of the pronuclei (PN) (2, 3). Those zygotes displaying successful extrusion of the second polar body and two even PN are considered to be "normally fertilized" and are cultured further to monitor embryo quality and development. In contrast, those zygotes showing a single or more than two PN are considered to be "abnormally fertilized." Even though the embryos deriving from abnormally fertilized oocytes (AFOs) are capable of normal in vitro development, they are usually discarded because of a higher risk for abnormal ploidy constitution (i.e., haploidy, triploidy, or tetraploidy) (3).

Zygotes with one PN are thought to be at a higher risk of being haploid, and the transfer of these embryos is expected to result in an implantation failure. However, the origin of a monopronucleated zygote after ICSI could be parthenogenetic oocyte activation or an abnormal formation of the nuclear envelope. The latter may result either from the combination of the two genomes into a single PN or from the failure to organize a nuclear envelope around one of the parental genomes. Recently, several articles about the possible origins of zygotes with one PN and the chromosomal constitution of the resulting embryos have been published (4–6). From these reports, it can be assumed that a considerable number of embryos originating from zygotes with one PN could have a normal chromosomal constitution, and as a result they could be considered for reproductive purposes in cases where no embryos deriving from normally fertilized zygotes are available (7–9).

Embryos arising from zygotes with three PN are considered to harbor a polyploid chromosomal constitution, and the transfer of these embryos is expected to result in a higher risk for miscarriage and molar pregnancy with an associated 2.5% risk of developing into choriocarcinoma (10). However, no definitive genetic evidence has been obtained showing that all resulting zygotes are chromosomally abnormal. It is possible that some may be chromosomally normal despite the morphologic defects (10).

Overall, ~10% of inseminated oocytes fertilize abnormally, and the embryos deriving from them are typically discarded in the absence of a reliable approach to monitor their genetic risk for ploidy defects. Indeed, even when aneuploidy testing is performed with the use of conventional comprehensive chromosome testing (CCT), owing to the normalization method ploidy analysis is not possible and embryos showing a normal chromosomal profile can still be tetraploid, triploid, or haploid. In the present study, we report the development, validation, and clinical application of an enhanced CCT protocol for the detection of aneuploidies and parallel ploidy assessment.

After being validated in the preclinical phase, the combined preimplantation genetic testing (PGT) scheme was implemented clinically in IVF cycles undertaken by poor-prognosis patients, with the aim of rescuing the embryos deriving from AFOs, which would have otherwise been discarded.

MATERIALS AND METHODS

Study Design

This is a longitudinal cohort study performed from January 2015 to September 2016 involving 678 consecutive patients undergoing preimplantation genetic testing for aneuploidies (PGT-A) cycles at Genera IVF center in Rome. PGT-A was offered to patients of advanced female age (>35 y) or to younger patients with a history of unsuccessful IVF treatments (more than two failed IVF cycles) or previous spontaneous abortion (more than two miscarriages).

The preclinical validation of the protocol for the combined aneuploidy and ploidy assessment from a single sample was carried out on a set of cell lines with previously established triploid karyotypes (Coriell Cell Repository IDs AG05025, AG06266, GM10013, GM01672, GM04376, and GM04939). Six-cell aliquots of diploid cell lines and triploid cell lines were processed to mimic the approximate number of cells in a trophectoderm biopsy. All samples were processed blindly.

From January 2015, the combined aneuploidy and ploidy analysis was systematically offered to all consenting patients undergoing PGT-A when a blastocyst from a monopronuclear (1PN) or tripronuclear (two evenly sized PN plus one smaller PN; 2.1PN) zygote was obtained (Fig. 1). To start with a more gradual and conservative approach, we decided to analyze only tripronuclear zygotes showing one smaller supernumerary PN, excluding from clinical use the tripronuclear (3PN) zygotes showing three evenly sized PNs. In our standard practice, 2.1PN are still considered to be unsuitable for clinical use owing to similar concerns of ploidy alterations. A specific consent form was provided and submitted to patients undergoing IVF before ovarian stimulation was started. The counseling involved a discussion of the genetic risks associated with the use of AFOs, their clinical fate when no testing is performed, and the possibility to have them rescued for clinical use by means of an improved PGT-A analysis. The limitations of this experimental protocol were also clearly detailed. No extra costs were associated with this additional genetic procedure.

As a general policy, balanced (without chromosome copy number alteration) embryos from 2PN zygotes were always selected first for transfer. Balanced-diploid embryos obtained from 1PN or 2.1PN zygotes were considered to be suitable only in the absence of balanced normally fertilized embryos (Fig. 1). The infertility treatment protocols, including hormonal stimulation, oocyte retrieval, IVF, embryo culture, embryo morphologic evaluation, biopsy, transfer methods, and clinical outcomes assessment applied in this study have