Molar pregnancy after in vitro fertilization with euploid single embryo transfer

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Objective: To describe a case of molar pregnancy after in vitro fertilization (IVF) resulting from the transfer of a euploid embryo derived from a monopronuclear zygote.
Design: Case report and review of the literature.
Setting: Private practice IVF center.
Patient(s): A 42-year-old woman, gravida 3 para 0, with advanced maternal age and infertility who underwent IVF.
Intervention(s): Preimplantation genetic testing for aneuploidy using next-generation sequencing, single frozen euploid blastocyst transfer, and medical management of suspected missed abortion.
Main Outcome Measure(s): Genetic examination of products of conception and correlation with embryonic preimplantation genetic testing for aneuploidy results.
Result(s): Transfer of the euploid embryo derived from an abnormally fertilized oocyte (monopronuclear zygote) resulted in a clinical pregnancy suspected to be a missed abortion. Products of conception collected after medical management of the suspected missed abortion were analyzed using next-generation sequencing with the report "46,XX complete molar pregnancy".
Conclusion(s): To our knowledge, this is the first account of a complete molar pregnancy resulting from the transfer of a reported euploid embryo, highlighting the importance of understanding the limitations of genetic testing platforms in the setting of abnormally fertilized oocyte-derived embryos. (Fertil Steril Rep® 2021;2:146–9. ©2021 by American Society for Reproductive Medicine.)
Key Words: Assisted reproductive technology, preimplantation genetic testing, molar pregnancy

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INTRODUCTION

One of the limiting steps to individuals and couples who are seeking to expand their families via in vitro fertilization (IVF) is the availability of embryos that are capable of resulting in a healthy live birth. After oocytes are retrieved, fertilization occurs in the laboratory via conventional IVF (cIVF) or intracytoplasmic sperm injection (ICSI) and is confirmed hours later by the embryologist by the presence of 2 polar bodies and 2 symmetric pronuclei (PN), 1 from each gamete. Abnormally fertilized oocyte (AFO)-derived em-bryos are those that arise from zygotes displaying an abnormal number of PN with reported rates varying between 1% and 7% (1). Parthenogenetic activation and asynchronous PN have been proposed as mechanisms that lead to the 1PN morphology (2), whereas dispermic fertilization of 1 oocyte with cIVF or nonextrusion of a second polar body with ICSI have been identified as phenomena leading to 3PN (3). Traditionally, AFO-derived embryos have not been considered for transfer because of their abnormal chromosomal makeup; rates of haploidy among 1PN-derived embryos have been reported to be 69%–100% with increased rates of aneuploidy among those fertilized via ICSI compared with that of those fertilized via cIVF (4–6). However, emerging reports on live births resulting from the transfer of blastocysts derived from 1PN zygotes (1), especially among embryos found to be euploid with biparental inheritance (7), provide promising potential in the use of these zygotes, particularly in patients for whom no other embryos are available. As detection rates and availability of preimplantation genetic testing (PGT) have improved, many case reports on the analysis of ploidy status in AFO-derived blastocysts have since been published in an effort to determine which embryos are best to transfer. The aim of this case study was to highlight principles and limitations to the interpretation of preimplantation

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genetic testing for aneuploidy (PGT-A) results in the setting of AFO-derived blastocysts.

CASE REPORT

A 42-year-old woman, gravida 3 para 0, presented to our clinic with a 1-year history of secondary infertility ascribed to decreased ovarian reserve and mild male factor infertility. She underwent an ovarian stimulation cycle using an antagonist protocol with the use of adjuvants and high-dose gonadotropin stimulation along with clomiphene citrate (100 mg/day). After an uneventful stimulation, final oocyte maturation was triggered on stimulation day 10, at which time the estradiol level was 2,908 pg/mL. A total of 10 oocytes were retrieved, all of which were mature metaphase II oocytes. ICSI using her husband's fresh semen sample was performed, and evidence of normal fertilization with 2 PN and 2 polar bodies was observed in 8 of 10 oocytes. One oocyte was not fertilized, and another was found to have 1 pronucleus and 2 polar bodies. This monopronuclear zygote was kept in culture and developed into a "fair" (BB) hatching blastocyst on day 6 of embryo development. An additional 5 of the 8 correctly fertilized oocytes developed into day 6 blastocysts (4 graded "fair" and 1 "poor"). All 6 of the day 6 blastocysts underwent trophectoderm biopsies that were sent for PGT-A via next-generation sequencing (NGS) by Igenomix (Torrance, California). The only "euploid" test result was reported for the blastocyst arising from the monopronuclear zygote; the results for all other blastocysts were aneuploid. This euploid embryo was reported as 46,XX after PGT-A testing using whole genome amplification. The raw data plot for this embryo is shown in Figure 1. An important test limitation stated on the PGT results was that haploidy and triploidy cannot be detected with this platform. Single-nucleotide polymorphism (SNP) analysis was not performed. The patient was counseled regarding the outcomes from transfers of embryos arising from monopronuclear zygotes, in particular the remote risk of triploidy, and she desired to proceed with embryo transfer. A programmed frozen single embryo transfer according to standard clinic protocol using transdermal estradiol and intramuscular progesterone supplementation was performed.

RESULTS

The patient reported a positive home pregnancy test 14 days after embryo transfer. The initial ß-human chorionic gonadotropin (ß-hCG) level was 367 IU/mL; a repeat ß-hCG level measurement 2 days later was 840 IU/mL. A transvaginal ultrasound was performed at an estimated gestational age of 6 weeks and 3 days, and it was notable for a gestational sac with irregular contours and diffuse echogenic material (Fig. 2). No clear fetal pole was visualized. A repeat ß-hCG level measured that day was 24,714 IU/mL. A repeat ultrasound was performed at an estimated gestational age of 7 weeks and 2 days which remained unchanged. The patient was counseled regarding management of her nonviable pregnancy, suspected to be a spontaneous abortion, and desired to proceed with medical evacuation with misoprostol. Products of conception were collected at home and sent for NGS with

short tandem repeat (STR) testing. The result was reported as "46,XX complete molar pregnancy", with a single set of paternally derived alleles confirmed with the use of STR testing. The patient was notified of the results, and weekly ß-hCG levels were drawn until the ß-hCG levels were <5 IU/L. Weekly ß-hCG levels were repeated for an additional 4 weeks and found to be undetectable. Written informed consent was obtained from the patient for inclusion in this case report.
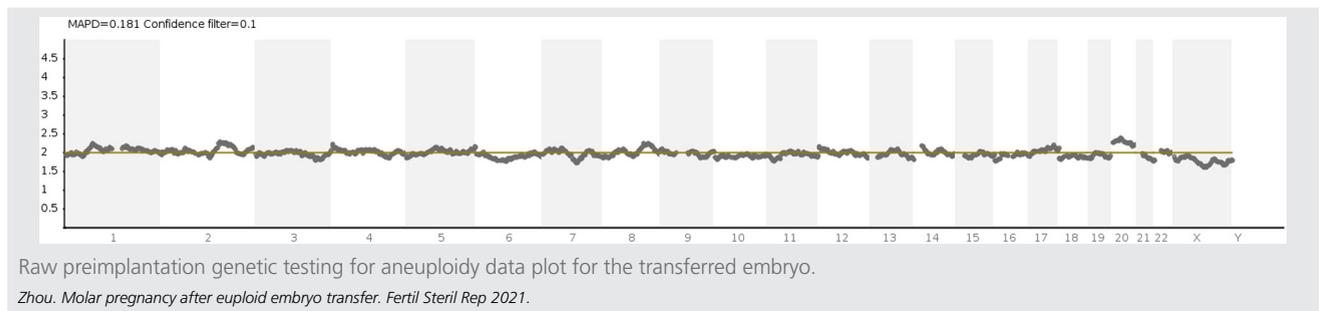
DISCUSSION

In some IVF cases, a 1PN zygote as opposed to the normal 2PN zygote is observed to form after the oocyte is inseminated or injected with sperm, with parthenogenetic activation and asynchronous PN being the proposed mechanisms that lead to such a morphology. The latter mechanism has been observed in normal diploid embryos (2) with the identification of the Y chromosome historically serving as proof of male contribution of genetic material (8). This possibility of having a normal euploid embryo with biparental contribution has called into question the traditional practices of discarding such AFO-derived embryos, which has since progressed to consideration of transfer of such embryos in clinical practice, especially with the evidence that some such embryos develop from zygotes that exhibit differences in morphokinetic development that may not be captured at the fertilization check. In a case series of 33 1PN-derived blastocysts via conventional IVF or ICSI, Itoi et al. (1) reported that although a significantly lower rate of 1PN embryos compared with 2PN embryos progressed to the day 5 blastocyst stage (18.5% vs. 52.6%, respectively, $P < .05$), the respective implantation rates (33.3% and 41.2%), clinical pregnancy rates (33.3% and 37.4%), abortion rates (18.2% and 20.9%), and ongoing pregnancy rates (27.3% and 29.5%) were comparable between the 2 groups. This suggests that once the 1PN zygote reaches the day 5 blastocyst stage, the embryo can be considered for transfer, especially with the adjunct use of PGT.

The use of PGT in IVF cycles has increased since its introduction into the field, and is primarily recommended because of its association with improved ongoing pregnancy rates and live birth rates compared with those of the traditional use of embryo morphology (9–11), particularly in women >37 years of age. Improvements in efficiency, precision, costs in PGT methods, and shifts toward elective single embryo transfers have also led to increased uptake in practice (12). However, PGT is not without its controversies, particularly in the interpretation and guidance on selecting an embryo for transfer. Furthermore, PGT with fluorescent in situ hybridization, comparative genomic hybridization arrays, and NGS platforms each have their own benefits and limitations that should be understood by each clinician. The use of DNA sequencing with NGS, in particular, has increased in use because of higher sensitivity and higher accuracy in assessment of subchromosomal abnormalities compared with those of other methods (13).

Our experience in this case highlights another limitation of the interpretation of NGS results, especially in the setting of a 1PN-derived embryo. In this case, ploidy status was

MAPD=0.181 Confidence filter=0.1

Raw preimplantation genetic testing for aneuploidy data plot for the transferred embryo.

Zhou. Molar pregnancy after euploid embryo transfer. Fertil Steril Rep 2021.

determined via NGS preimplantation. However, NGS performed on the products of conception with the addition of evaluation of STRs demonstrated that the euploid status reported in the PGT results reflected a single paternally derived set of alleles with homozygosity at each locus. This suggests that the molar pregnancy developed from the fertilization of an ovum devoid of maternal genetic material with 1 sperm via ICSI that then endoreplicated (14), a phenomenon that is well-described in the pathogenesis of hydatidiform molar pregnancies. In this case, PGT results could not disclose loss of heterozygosity (LOH) as SNP analysis was not performed; had this been the case, uniparental chromosomal contribution could have been revealed as previously demonstrated with female euploid embryos (15). Collection and comparison of parental genetic material may also be of use to confirm biparental inheritance when considering transfer of euploid AFO-derived blastocysts. With estimated recurrence rates of hydatidiform moles (HMs) in subsequent pregnancies between 1.9% and 9% (16–18), the mechanism to explain the phenomenon of monospermic HMs is currently under investigation.

Recent studies in women with recurrent HMs identified potential genetic mutations that were implicated in early homologous chromosome pairing in oocytes in the mouse model (19, 20). In one of these studies, Nguyen et al. (20) attempted to elucidate the pathogenesis and causative mutations of androgenetic complete HMs by performing whole-exome sequencing on 65 women with recurrent HMs. They identified bi-allelic deleterious mutations in *MEI1, TOP6BL/C11orf80*, and *REC114*, with roles in meiotic double-strand breaks formation. Subsequent experiments in *Mei1*-deficient female mice revealed that 8% of *Mei1*$^{-/-}$ oocytes lost all their chromosomes by extruding them with the spindles into the first polar body, and that 5% of these oocytes produced androgenetic zygotes upon fertilization. Given these findings, the investigators propose that androgenetic complete HMs arise from sporadic or recurrent meiotic dysfunction involving extrusion of all maternal chromosomes and their spindles into the first polar body, followed by endoduplication of the paternal genome (20). It is likely that this proposed mechanism led to the monospermic androgenetic complete HM in our patient.

At the time of diagnosis of pregnancy failure, and because of the low suspicion of a molar pregnancy, we recommended medical management with misoprostol. However, standard of care for gestational trophoblastic disease (GTD) is surgical management with measurement of postevacuation ß-hCG levels. Prior studies demonstrated that medication-induced evacuation of HMs were associated with higher risks of requiring systemic chemotherapy compared with those of surgical management with curettage; this was hypothesized to be related to the risk of embolization and dissemination of the GTD through the venous system with multiple uterine contractions (21). The International Federation of Gynecology and Obstetrics currently recommends measuring ß-hCG levels every 1–2 weeks until they become undetectable, and then obtaining monthly levels for 6 months after (22), the National Comprehensive Cancer Network recommends measuring monthly levels for 3 months (23). Although there has been some suggestion that a reduced postevacuation surveillance period is safe (24), more information is necessary to determine the safety of such modifications of ß-hCG level measurement practices in molar pregnancies managed with medication.

Transvaginal ultrasound at 6 3/7 weeks gestation.

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In conclusion, this is the first published case study, to our knowledge, which demonstrates a complete molar pregnancy as a result of the transfer of a reported euploid embryo. This highlights the importance of understanding the limitations of the genetic testing platforms used in each practice, and to appropriately apply the reported results in the event of AFO-derived embryos. As more data emerges showing that AFO-derived embryos can result in normal pregnancies, rather than discarding these embryos in patients who may not have any 2PN embryos to use, it is more prudent to use adjunct genetic testing to help determine which AFO-derived embryos may lead to a normal pregnancy. Evaluation of LOH in SNPs or of STRs with PGT-A can offer further insight into the genetic material that makes up the AFO-derived embryos to determine ploidy as well as parental contribution. This will aid in deciding whether to move forward with the transfer of an AFO-derived embryo with a potential for a live birth for patients who otherwise may not have any other embryos to use.

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