45,X product of conception after preimplantation genetic diagnosis and euploid embryo transfer: evidence of a spontaneous conception confirmed by DNA fingerprinting

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Abstract

Background: Preimplantation genetic screening (PGS) provides an opportunity to eliminate a potential implantation failure due to aneuploidy in infertile couples. Some studies clearly show that twins following single embryo transfer (SET) can be the result of a concurrent natural conception and an incidence as high as 1 in 5 twins has been reported. In our case PGS was performed on trophectoderm (TE) biopsies by quantitative polymerase chain reaction (qPCR). The product of conception (POC) was cytogenetically investigated after selection of the placental villi by means of the direct method. Molecular cytogenetic characterization of the POC was performed by fluorescence in situ hybridization (FISH) and array-comparative genomic hybridization (a-CGH) analyses. To investigate the possibility of a spontaneous conception, a panel of 40 single nucleotide polymorphisms (SNPs) was used to compare genetic similarity between the DNA of the POC and the DNA leftover of the TE biopsy.

Findings: We describe a 36-year old infertile woman undergoing PGS who had a spontaneous abortion after a single euploid embryo transfer on a spontaneous cycle. The POC showed a 45,X karyotype confirmed by FISH and a-CGH. DNA fingerprinting demonstrated a genetic similarity of 75% between the DNA of the POC and TE biopsy, consistent with a sibling status. All supernumerary euploid embryos were also tested showing a non-self relationship with the POC, excluding a mix-up event at the time of fetal embryo transfer.

Conclusions: DNA fingerprinting of the transferred blastocyst and POC, confirmed the occurrence of a spontaneous conception. This case challenges the assumption that a pregnancy after assisted reproductive technology (ART) is always a result of ART, and strengthens the importance to avoid intercourses during PGS and natural transfer cycles. Moreover, cytogenetic analysis of the POCs is strongly recommended along with fingerprinting children born after PGS to see what the concordance is between the embryo transferred and the resultant child.

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(Continued on next page)
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Abbreviations: a-CGH, Array-comparative genomic hybridization; AMH, Anti-Müllerian hormone; ART, Assisted reproductive technology; FISH, Fluorescence in situ hybridization; FSH, Follicle-stimulating hormone; GnRH, Gonadotropin-releasing hormone; ICSI, Intracytoplasmic sperm injection; IUI, Intrauterine insemination; IVF, In vitro fertilization; LH, Luteinizing hormone; PGD, Preimplantation genetic diagnosis; PGS, Preimplantation genetic screening; POC, Product of conception; qPCR, Quantitative polymerase chain reaction; rFSH, Recombinant FSH; rhCG, Recombinant human chorionic gonadotropin; SET, Single embryo transfer; SNPs, Single nucleotide polymorphisms; TE, Trophectoderm

Introduction

PGS is an early method to detect aneuploidies in preimplantation embryos. PGS can be offered to patients with an increased risk of having a higher percentage of chromosomally abnormal embryos, improving the reproductive outcome. By transferring only chromosomally normal embryos the miscarriage rates are expected to be lower, since about 60–70 % of miscarriages in the first trimester result from chromosomal abnormalities in the fetus [1].

Some studies clearly show that twins following SET can be the result of a concurrent natural conception [2, 3] and an incidence as high as 1 in 5 twins has been reported [4].

We describe a rare case of a 36-year-old infertile patient with recurrent implantation failures. PGS was performed and an euploid blastocyst transferred. However, a spontaneous abortion occurred after 13 weeks of gestation, ascertained to be 10 at ultrasound examination. Cytogenetic analysis revealed a 45,X karyotype. DNA fingerprinting analysis of POC and the euploid embryo transferred excluded a self-relationship confirming the occurrence of a spontaneous conception in concomitance with SET.

Materials and methods

A 36-year-old woman experiencing 6 years infertility with a diagnosis of severe endometriosis was referred to Humanitas Fertility Center. She had a miscarriage at 8 weeks after an intrauterine insemination (IUI), other 2 negative IUI cycles and 3 negative in vitro fertilization (IVF) cycles with the transfer of 11 cleavage stage embryos from fresh and frozen cycles performed in other centers. A normal uterine cavity was confirmed at hysteroscopy while a transvaginal ultrasound imaging showed a 18 mm small endometrial cyst of the right ovary and 12 mm recto-vaginal lesion. Despite a high follicle-stimulating hormone (FSH) level of 14 IU/L, ovarian reserve assessed to be normal with an anti-Müllerian hormone (AMH) level of 3.8 ng/ml and the existence of 10 antral follicles. Her partner sperm analysis showed 12 million total motile sperm with 2 % normal forms and 80 % vitality. After counseling and obtaining informed consent, the patient was enrolled for a first attempt intracytoplasmic sperm injection (ICSI) with PGS cycle. Controlled ovarian hyper-stimulation was started with 225 IU recombinant FSH (rFSH) in a gonadotropin-releasing hormone (GnRH) antagonist analog cycle. Recombinant human chorionic gonadotropin (rhCG) (250 mcg) was administered on day 13 of the induction cycle after a total rFSH dose of 3325 IU.

A transvaginal ultrasound guided under deep sedation oocyte retrieval was performed 36 h after the rhCG trigger and 9 metaphase II oocytes were retrieved. In 8 metaphase II oocytes ICSI was performed and placed in an incubator with an integrated time-lapse system, where the embryos were cultured individually in 25 μl microwells and monitored for 24 h. At 16–18 h post-ICSI, the 8 oocytes were assessed for the presence of 2 pronuclei and cultured until blastocyst stage. Three expanded blastocysts underwent biopsy of TE cells day 5 and 4 day 6 as previously described [5], 1 embryo degenerated after development to morula. Blastocyst quality was assessed immediately before TE biopsy [6], and blastocysts vitrified according to the protocol described by Kuwayama et al. [7]. Of the 7 biopsied and cryopreserved blastocysts, 4 were euploid. TE biopsies were analysed by qPCR based comprehensive chromosome diagnosis [8, 9]. This method has been extensively validated for chromosome copy number analysis of TE biopsies in preclinical [8, 9] and clinical studies [10, 11] and was reported to have a recognizable error rate of 0.2 % in clinical pregnancies [12].

The embryo transfer was scheduled on a spontaneous cycle. The patient was monitored from day 6 of the cycle by ultrasound and a urinary luteinizing hormone (LH) kit. On cycle day 15 an endometrial thickness of 12.2 mm with 2 follicles of 18 and 22 mm were observed with a positive LH determination. Seven days after LH surge a single euploid blastocyst (embryo number 3) was selected for transfer based on morphological score, warmed and cultured at 37 °C until transfer, performed under ultrasound guidance.

A positive hCG determination was performed 14 days after the embryo transfer (1765 IU) and a 6 weeks ultrasound showed an intrauterine gestation with an embryo with normal fetal heart rate.

(Continued from previous page)
| Assay ID | Villi gDNA | Embryo 3 | Embryo 6 | Embryo 7 |
|---------|------------|----------|----------|----------|
| Assay01 | 11         | 11       | 22       | 11       |
| Assay02 | 11         | 22       | 22       | 22       |
| Assay03 | 11         | 22       | 22       | 22       |
| Assay04 | 11         | 22       | 22       | 22       |
| Assay05 | 11         | 22       | 22       | 22       |
| Assay06 | 11         | 22       | 22       | 22       |
| Assay07 | 11         | 22       | 22       | 22       |
| Assay08 | 11         | 22       | 22       | 22       |
| Assay09 | 11         | 22       | 22       | 22       |
| Assay10 | 11         | 22       | 22       | 22       |
| Assay11 | 11         | 22       | 22       | 22       |
| Assay12 | 11         | 22       | 22       | 22       |
| Assay13 | 11         | 22       | 22       | 22       |
| Assay14 | 11         | 22       | 22       | 22       |
| Assay15 | 11         | 22       | 22       | 22       |
| Assay16 | 11         | 22       | 22       | 22       |
| Assay17 | 11         | 22       | 22       | 22       |
| Assay18 | 11         | 22       | 22       | 22       |
| Assay19 | 11         | 22       | 22       | 22       |
| Assay20 | 11         | 22       | 22       | 22       |
Results and discussion
The couple's karyotype was normal. Cytogenetic analysis on 20 Q-banded metaphases obtained from the POC showed a 45,X karyotype. This result was confirmed by FISH analysis showing one signal for CEPX in 94.9% of the nuclei. In 5.1% of nuclei 2 signals were present, probably due to a small maternal cell contamination that is difficult to avoid in a POC sample even after placenta vili selection. The 45,X karyotype observed in the metaphases obtained after 24 h of incubation excluded a mosaic karyotype in the embryo.

Array-CGH analysis performed on DNA from uncultured vili confirmed chromosome X monosomy.

Allelic discrimination analysis of 40 SNPs of the excess DNA from all the euploid blastocysts produced during the present IVF cycle, revealed that none of them had a genotype consistent with the POC. In particular, locus dropout (no amplification of either allele) was observed in only 4 assays of embryo number 3, with an overall call rate of 97.5% (156/160) (Table 1). All the euploid embryos showed a similarity rate to POC below 80%, clearly consistent with a sibling genotype and a non-self-relationship (Table 2). This analysis confirmed the hypothesis of a spontaneous conception in the course of the natural frozen embryo transfer cycle.

To our knowledge this is the only case of PGS and euploid SET resulted in a 45,X POC as consequence of a natural pregnancy. A similar event has been reported in a large study about twin births after SET where the authors demonstrated that about 1 in 5 twins is due to a concurrent spontaneous conception [4]. Another report described a case of dizygotic twins of different-sex delivered after SET demonstrated to be the consequence of a natural fertilization [2].

Conclusions
Our case report demonstrates the occurrence of an aneuploid spontaneous conception, resulted in a miscarriage, in the course of a natural SET cycle. This case highlights the importance of avoiding intercourses and natural transfer cycles for couples with reproductive genetic risk, such as those undergoing preimplantation genetic diagnosis (PGD)/PGS cycles, in order not only to reduce the incidence of multiple pregnancies but especially to avoid the risk of aneuploid natural conceptions.

Table 2 Genetic similarity of the 3 euploid embryos obtained during the IVF cycle with the genomic DNA from the aneuploid POC

| Sample ID | Similarity to POC gDNA |
|-----------|------------------------|
| Embryo 3  | 74 %                   |
| Embryo 6  | 55 %                   |
| Embryo 7  | 75 %                   |
Moreover, cytogenetic analysis of the POCs and fingerprinting of the children born after PGS are strongly recommended.

Furthermore, with this study we confirm that parental DNA information is not necessary since discrimination was achieved based on a clear genotypic heterogeneity among samples, without incorporating selection of informative SNPs from parental genotype. Therefore, the additional time and expense of evaluating parental DNA could be avoided with this methodology as also previously reported [15].

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Availability of data and materials
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Authors’ contributions
PELS is the head of the Fertility Center who personally managed and counselled the patients. EA is the embryologist who managed the ICSI laboratory procedures. DB, VA and AV are the cytogeneticists who performed the karyotype, FISH and a-CGH analyses. AC, LR and FMU are the molecular biologists who managed the POCs and DNA fingerprinting analysis. DB and AC were involved in the study design, DB in supervision and paper drafting. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Written informed consent for publication of the clinical details and/or clinical images was obtained from the patient. A copy of the consent form is available for review by the Editor of this journal.

Ethics approval and consent to participate
Ethics approval not needed. The patient signed our institutional consent for genetic analyses, and gave the consent to use the clinical data.

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