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CASE REPORT *Reproductive genetics*

Two clinical case reports of embryonic mosaicism identified with PGT-A persisting during pregnancy as true fetal mosaicism

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ABSTRACT: The health risks associated with transferring embryos classified as mosaic by preimplantation genetic testing for aneuploidies (PGT-A) are currently unknown. Such embryos produce PGT-A results indicating the presence of both euploid and aneuploid cells and have historically been deselected from transfer and grouped with uniformly aneuploid embryos as 'abnormal'. In recent years, numerous groups have reported the intentional transfer of mosaic embryos in the absence of uniformly euploid embryos, largely observing births of seemingly healthy babies. However, it remains to be understood whether the embryonic mosaicism invariably becomes resolved during the ensuing pregnancy, or whether the placenta and/or fetal tissues retain aneuploid cells, and if so to what potential clinical effect. Here, we report two cases of mosaicism persisting from the embryonic stage to the established pregnancy. Case 1 involved an embryonic low-level segmental mosaic loss in Chromosome (Chr) 1, which was confirmed in amniocentesis as well as in brain tissue of the products of conception. This pregnancy was terminated due to the chromosomal pathologies associated with 1p36 deletion syndrome, such as severe intellectual disability. Case 2 involved a low-level mosaic Chr 21 trisomy, which was confirmed with chorionic villus sampling and amniocentesis. The ensuing pregnancy was terminated after ultrasound identification of severe abnormalities in the placenta and fetus. Together, these two cases should be taken into account for risk-benefit assessments of prospective mosaic embryo transfers.

Key words: mosaicism / embryo / aneuploidy / PGT-A / prenatal testing

Introduction

Embryonic mosaicism can exist as a mixture of chromosomally normal (euploid) and abnormal (aneuploid) cells within a single embryo, a phenomenon that has recently become highly relevant for embryo selection in the IVF clinic. Contrary to meiotic errors, which affect the chromosomal content of gametes and subsequently all cells of the embryo they generate, mosaicism originates from post-zygotic errors of

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mitosis, in which sister chromatids fail to segregate correctly among two daughter cells (Taylor et al., 2014). Mosaicism is thought to be common throughout embryonic development, and although it is hard to quantify accurately, some estimates note a prevalence as high as 70% albeit with a diminishing frequency between the cleavage and blastocyst stages (Fragouli et al., 2011; Capalbo et al., 2013; Harton et al., 2017; Vera-Rodriguez and Rubio, 2017; Starostik et al., 2020). Its occurrence seems to be independent of maternal age (McCoy et al., 2015), which differs from meiotically derived aneuploidies that become significantly more common with the advancing age of the mother (Franasiak et al., 2014).

The root causes for mosaicism-forming mitotic errors are still being defined. In early embryos, mitosis is error-prone, while the cell cycle checkpoint controls are relaxed, and corrective mechanisms are inefficient (McCoy, 2017; Levy et al., 2021). On the one hand, there is mounting evidence for genetic predisposition to chromosomal mosaicism. For example, previous studies have demonstrated that variation encompassing the gene *PLK4* influences rates of mitotic-origin aneuploidy (McCoy et al., 2015; Ottolini et al., 2017; McCoy et al., 2018), and a variant near *TCL1A* is associated with susceptibility to mosaic loss of Chr Y (Zhou et al., 2016). On the other hand, environmental factors could influence the rate of mosaicism in embryos, including culture conditions such as medium, temperature, pH, and gas mix (Taylor et al., 2014).

The distribution and number of aneuploid cells within a mosaic embryo can change in development, and influence its viability. Live-embryo imaging of mosaic embryos from mouse models suggests that when present at low levels, aneuploid cells may be eliminated by apoptosis, leading to a decline in their numbers as development progresses and potentially resulting in a normal placenta and fetus (Bolton et al., 2016). Moreover, extended culture experiments demonstrate attrition of mosaicism in human embryos concomitant with development (Popovic et al., 2019). This is consistent with the low incidence of mosaicism reported in prenatal specimens, being found in approximately 1–4% of prenatal diagnoses performed by chorionic villus sampling (CVS) and in approximately 0.1–0.3% of amniocentesis (Li et al., 2022). Nonetheless, even though an abnormal cell line may become reduced to the point of low-level mosaicism, the developmental consequences of its presence early in pregnancy may still be apparent (Levy et al., 2021).

State-of-the-art methods of preimplantation genetic testing for aneuploidies (PGT-A) (Viotti, 2020) can, in addition to finding uniform monosomies and trisomies, identify intermediate copy numbers for (sub-)chromosomal regions, in a pattern that is consistent with mosaicism in the analyzed multicellular biopsy. As first described by Greco and colleagues, embryos with PGT-A results suggesting mosaicism (hereafter called 'mosaic' embryos) can result in pregnancies (Greco et al., 2015), albeit with lower success rates than euploid embryos (Spinella et al., 2018; Munné et al., 2020; Viotti et al., 2021). One study goes as far as suggesting that low-level mosaic embryos, for which PGT-A results indicate a low percentage of aneuploid cells, have equivalent potential to embryos classified as euploid (Capalbo et al., 2021). Babies born from transferred mosaic embryos have appeared largely healthy by physical examination (Lee et al., 2020; Viotti et al., 2021; Yakovlev et al., 2022). These observations support the notion that self-corrective mechanisms are at play during development. According to that model, abnormal cells become diluted out of the mosaic mix between the embryonic stages and later pregnancy, to the point of clinical irrelevance. To date, there has only been one report of mosaicism persisting from the blastocyst stage to the newborn (Kahraman et al., 2020). In that case, the baby was healthy without presenting any symptoms, but amniocentesis during pregnancy and postnatal chromosomal analysis confirmed a low incidence of the aneuploidy detected in a mosaic pattern with PGT-A.

Here, we report two clinical cases in which the mosaicism identified with PGT-A at the blastocyst stage was reflected in gestation by prenatal chromosomal testing as true fetal mosaicism. Both pregnancies were terminated; one because the chromosomal abnormality is associated with severe intellectual disability and additional health problems, and the other because the ultrasound presented severe abnormalities of the placenta and fetus.

Case reports

Case 1

In April 2021, a 40-year-old woman with a 3-year history of secondary infertility was referred to the Next Generation Clinic IVF center in Moscow, Russia. Her menarche occurred at 13 years of age. The patient reported a normal menstrual cycle of 28 days. Her height and weight were 170 cm and 70 kg (BMI: 24 kg/m^2). Due to the presence of the male factor of infertility, in 2014, the patient underwent IVF/ICSI with the live birth of a healthy child. The couple opted to undergo an IVF followed by a PGT-A analysis by signed informed consent, due to the presence of male factor infertility and advanced reproductive age.

The participant underwent ovarian stimulation, using the long agonist protocol. Oocyte retrieval occurred 36 h after ovulation trigger, and a total of twelve oocytes were retrieved. After denuding the oocytes, 11 were at the metaphase II stage and 1 was at the germinal vesicle (GV) stage. Mature oocytes were fertilized by ICSI and cultured to the blastocyst stage, which was reached by seven embryos. Zona pellucida laser-assisted hatching was performed on Day 4. Each blastocyst was biopsied by removing five to eight trophoblast cells and subsequently vitrified (Kitazato) on Day 5 or 6 post-fertilization. Preimplantation genetic testing of all samples was performed using the next-generation sequencing (NGS)-based assay VeriSeq PGS following standard protocols and manufacturer recommendations (Illumina Inc., San Diego, USA; SurePlex Summary Protocol, Part #15053626; VeriSeq PGS Library Prep Reference Guide, Part #15052877) in the in-house laboratory (with experience of 7 years and 40 000 NGS examinations). Based on the results of PGT-A, six blastocysts were classified as aneuploid. The seventh blastocyst, which had a Day 5 biopsy and a 4AB morphology (Gardner and Schoolcraft, 1999), was classified as mosaic for a segmental region on Chr 1. Genetic analysis identified a deletion 1p36.33-p31.1 of about 70.9 Mb in 40% of trophectoderm (TE) cells (Fig. 1). The decision to transfer the mosaic embryo was made by the patient after genetic counseling and in the absence of euploid embryos available for transfer. Genetic counseling included the potential risks associated with the transfer of mosaic embryos, and a confirmatory prenatal diagnosis of the subsequent pregnancy was recommended.

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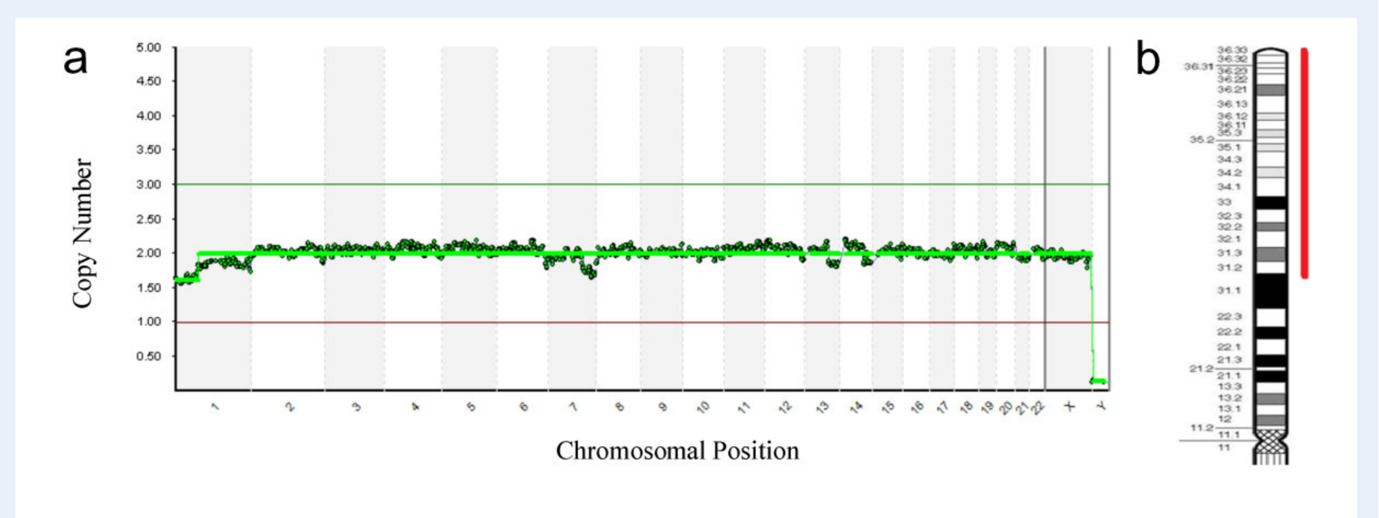

Figure 1. Case 1 results of preimplantation genetic testing for aneuploidies (PGT-A). (**a**) Next-generation sequencing (NGS) karyotype profile indicating mosaic segmental loss on Chr 1p36.33-p31.1 (intermediate copy number 1.6, suggesting 40% aneuploidy). (**b**) Short arm of chromosome 1, indicating the 1p36 region where the mosaic deletion was detected.

The single vitrified-warmed embryo was transferred in a modified natural cycle under ultrasound guidance. Serum beta-hCG was positive 9 days after embryo transfer and progesterone intake was canceled. A transvaginal ultrasound confirmed a viable intrauterine pregnancy at 6 weeks gestation. Subsequently, the patient was recommended to undergo amniocentesis with a chromosomal analysis of amniotic fluids cells and fluorescence *in situ* hybridization (FISH) analysis of Chr 1 to exclude low-level mosaicism.

An amniocentesis was performed in the 17th week of the pregnancy. Amniotic fluid cells were cultured in long-term cell culture for cytogenetic analysis, and slides of interphase nuclei from uncultured amniocytes were prepared for FISH by standard methods. The karyotype of amniocytes was normal 46,XX. FISH analysis of amniocytes was performed with a commercial DNA probe set specific to Chr 1 loci 1pTEL (SpectrumGreen), LSI P58 (SpectrumOrange), LSI 1q25 (SpectrumAqua) (Vysis, Abbott #05J21-020). Deletion of the 1p36 locus in 15% of cells in the amniotic fluid was revealed (Table 1 and Fig. 2A). A control sample of the q25 long arm region of Chr 1 was used to exclude overdiagnosis and/or false positive results. Of the cells in the sample, 85% had two hybridization signals each of the 1p36 locus and the 1q25 control locus of Chr 1. However, in 15% of cells only one hybridization signal of the 1p36 locus and two hybridization signals of the control 1q25 locus of Chr 1 were visualized (Fig. 2A).

Subsequently, the couple consulted a geneticist and was informed of the results of amniocentesis and the possible risks of having a child with chromosomal pathologies associated with a 1p36 deletion syndrome, such as severe intellectual disability (Gajecka *et al.*, 2007). The couple was offered cordocentesis as an additional diagnostic method, which they declined. After extensive consultation with a team consisting of a perinatologists and a geneticist, the couple decided to terminate the pregnancy at 20 weeks of gestation. A written request was received to test the products of conception at the genetic laboratory. Fetal and placental samples (villous tissue) were collected in normal saline (NaCl 0.9%) for subsequent genetic analysis in the pathology department. The results of the FISH analysis of fetal and placental samples are presented in Table 1. No cells with a Chr 1 deletion were found in tissue samples from the villi and heart. However, 1.5% of brain cells contained the 1p36 segmental deletion (Fig. 2C and D). Thigh skin sampling was also performed, but poor quality of this biological material did not allow the analysis of a sufficient number of cells for a qualitative and reliable result.

Case 2

In 2020, a couple with 2 years of infertility presented to our IVF center at Villa Mafalda Clinic in Rome, with advanced maternal age (44 years old), poor ovarian reserve and a normal female karyotype (46,XX). The anti-Mullerian hormone level was 0.75 ng/ml, and the antral follicle count showed few follicles in both ovaries, prompting classification of poor ovarian reserve according to the Bologna Criteria (Ferraretti *et al.*, 2011). A short antagonist protocol was used, and a total 2025 IU dosage of recombinant FSH (Gonal F, Merck Serono, Switzerland) with a daily dosage of 225 IU was administered for 9 days. Ovulation was triggered with one ampoule recombinant hCG (rhCG; Ovitrelle®, Merck Serono, Switzerland). After the ovarian stimulation, three follicles developed and three oocytes were retrieved. Two of these were fertilized by ICSI and developed into two blastocysts, which were biopsied. On Day 3, a hole was made in the zona pellucida of both embryos using a diode laser (RI Saturn 3, England). TE biopsy was performed once the blastocysts were fully expanded on Day 5 of culture, collecting 5–10 cells from each embryo. DNA was amplified with whole genome amplification and analysis was performed by NGS using the commercially available Veriseq PGT-A platform, following the manufacturer's protocol as previously described (Spinella *et al.*, 2018). Biopsied blastocysts were vitrified within 1 h of biopsy, using vitrification media (Kitazato Vitrification Kit; BioPharma, Shizouka, Japan)

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Table 1 Results of FISH analysis of placental and fetal specimens for case 1.

Location	Number of analyzed cells	Results	Mosaicism (percentage of aneuploid cells)
Amniotic fluid	100	nuc ish(1pTELx1,LSI P58x1,LSI 1q25x2) [15]/ (1pTELx2,LSI P58x2,LSI 1q25x2)[85]	15
Villous tissue	100	nuc ish(1pTELx2,LSI P58x2,LSI 1q25x2)[100]	0
Heart	100	nuc ish(1pTELx2,LSI P58x2,LSI 1q25x2)[100]	0
Brain	500	nuc ish(1pTELx1,LSI P58x1,LSI 1q25x2)[7/500]/ (1pTELx2,LSI P58x2,LSI 1q25x2)[493/500]	1.5

FISH, fluorescence *in situ* hybridization.

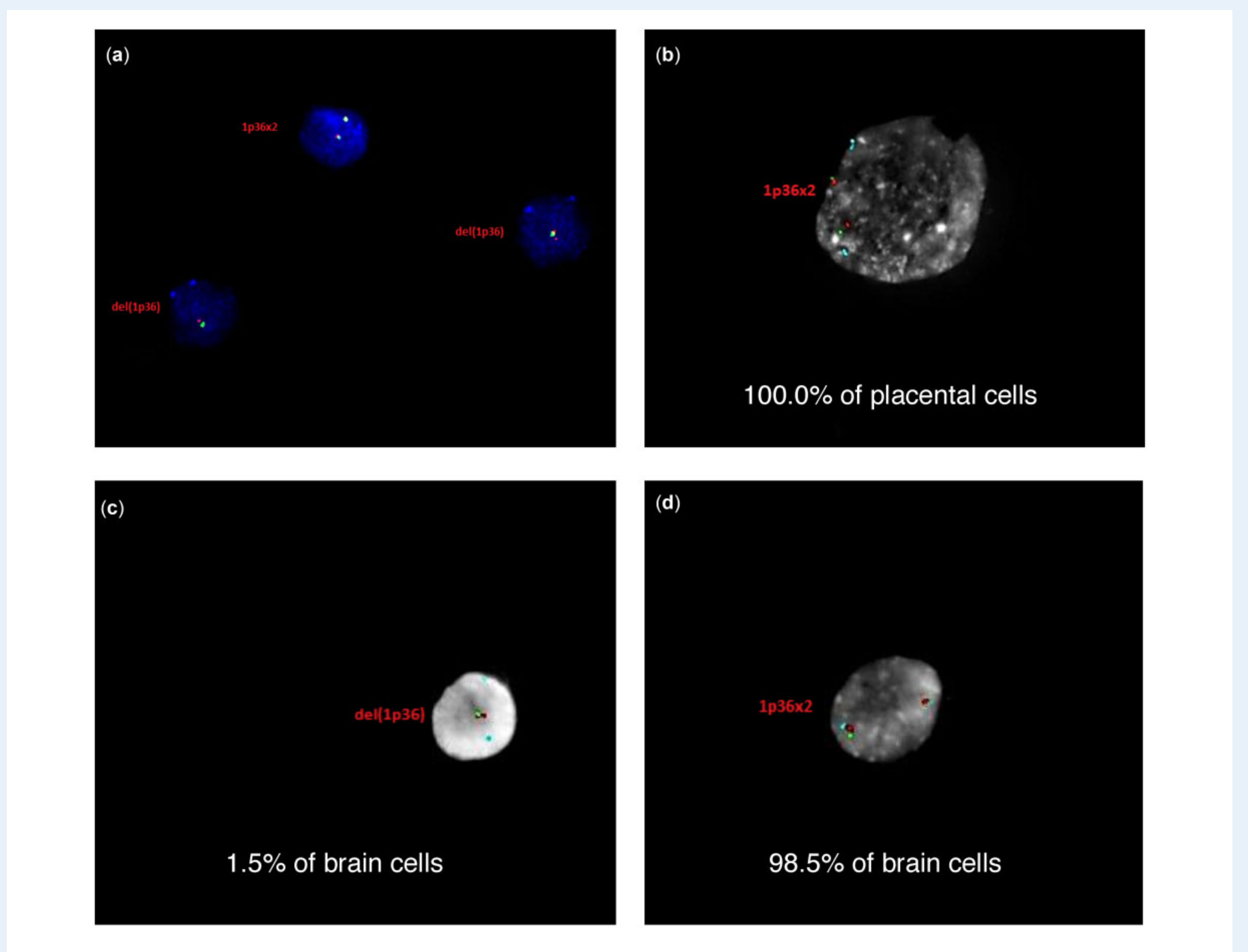

Figure 2. Case 1 fluorescent *in situ* hybridization (FISH) images from prenatal and fetal testing. Specific DNA probes were used for 1p36; the microdeletion Probe Set includes probes to the 1p subtelomere region labeled in SpectrumGreen, p58 (CDC2L1) within 1p36 labeled in SpectrumOrange, and a control probe on 1q25 labeled in SpectrumAqua (Vysis Abbott Molecular Inc., USA). (**a**) Sample image of cells of amniotic fluid with only one fluorescent signal or two signals for 1p36 (see number of green signals); 15% of analyzed cells included only one copy of 1p36. (**b**) Sample image of placental cells with two fluorescent signals for 1p36; all analyzed placental cells include two copies of the region (two green signals present). (**c**) Sample image of fetal brain cells with only one fluorescent signal for 1p36; 1.5% cells contained the 1p36 deletion (only one green signal). (**d**) Sample image of fetal brain cells with two fluorescent signals (in green) for 1p36; 98.5% cells did not contain the 1p36 deletion.

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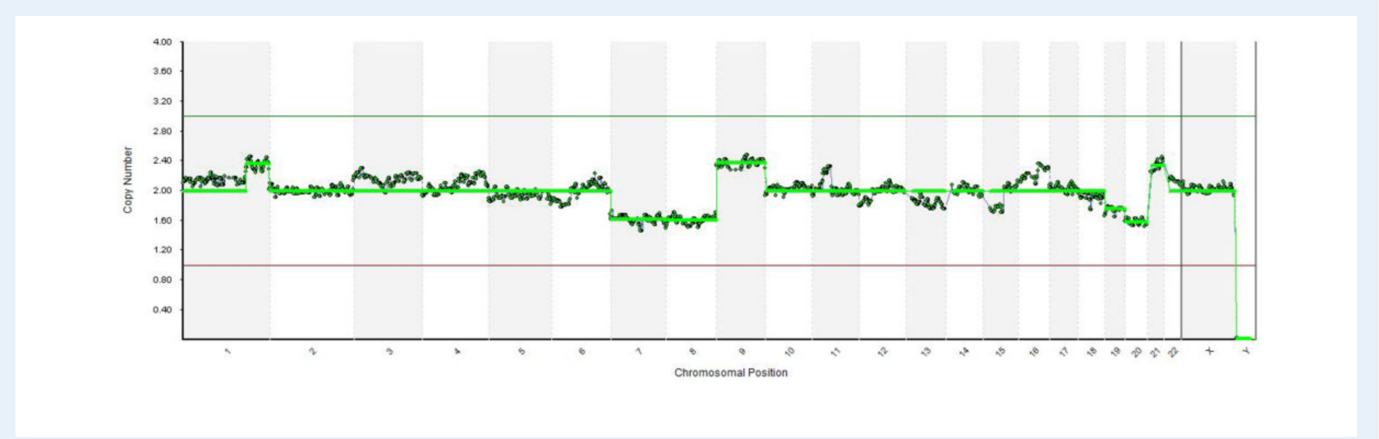

Figure 3. Case 2 results of preimplantation genetic testing for aneuploidies (PGT-A). Next-generation sequencing (NGS) profile of the trophectoderm (TE) sample, showing the following complex mosaic pattern: +1q (40%) −7 (40%) −8 (40%) +9 (40%) −19 (20%) −20 (40%); +21 (40%).

according to the manufacturer's instructions, using a Cryotop® as the carrier.

PGT-A revealed a full trisomy for Chr 11 in one blastocyst, and complex mosaicism (+1q (40%) −7 (40%) −8 (40%) +9 (40%) −19 (20%) −20 (40%); +21 (40%)) in the other blastocyst (Fig. 3), which had a 4BB grade. Following genetic counseling and detailed discussion of the option of a new ART cycle, the couple decided to proceed with a mosaic embryo transfer. An informed consent form, which included information regarding the risk of a mosaic embryo transfer and the necessity of close follow-up in the case of pregnancy, was signed by the couple. The frozen embryo transfer was performed after endometrial preparation using a modified natural cycle with rhCG trigger (Ovitrelle®, Merck Serono, Switzerland). Vaginal progesterone gel with 90 mg (8%) Crinone® (Merck Serono, Switzerland) was administered once a day after rhCG as a luteal phase support. The blastocyst with complex mosaicism was thawed with Kitazato warming media according to the manufacturer's instructions. The mosaic embryo transfer resulted in a pregnancy.

CVS was performed in the 8th week of the pregnancy using standard methods. Samples were processed in parallel using both G-banding for standard karyotyping and array comparative genomic hybridization (aCGH). The karyotype obtained by G-banding was observed to be mos47,XX+21(80)/46,XX(20) in 100 metaphases obtained in two separate culture flasks (Fig. 4A). aCGH confirmed the presence of mosaicism for Chr 21 (arr[GRCh37] 21q11.2q22.3(14 530 938–48 020 049) × 2–3) (Fig. 4B).

In view of the PGT results, testing for uniparental disomy by means of amplification fluorescent gene of short tandem repeat markers on Chr 7 and Chr 20 was also performed, and the results were normal. Noninvasive prenatal testing (NIPT) was performed at the 13th week of pregnancy resulting in a 'normal' result for Chr 21, Chr 13 and Chr 18 with a fetal fraction of 6%.

Following this result, at the 15th week + 6 days, the patient then underwent amniocentesis. Amniocyte culture was performed using standard methods (Howe *et al.*, 2014). The karyotype analysis confirmed the presence of trisomy of Chr 21 in 4 of 25 cells (metaphases) examined on 3 independent cultures. FISH analysis with the specific probe for Chr 21 (Anevysion) of amniocytes was also performed confirming the mosaic presence of trisomy for Chr 21 in 16% of cells analyzed, n = 4.

At Week 19 of pregnancy, the patient subsequently underwent fetal ultrasound which revealed the presence of cerebral ventricles at the upper limits of the norm, corpus callosum with dimensions at the lower limits, hypoplasia of the cerebellar vermis and a thinned antero-posterior diameter.

The couple was extensively counseled. Counseling included the possibility of trisomy 21 at birth. Ultrasound monitoring of the pregnancy was recommended using level II ultrasound and fetal echocardiography. The ultrasound check confirmed, at the level of the posterior cranial fossa, posterior to the brain, the presence of an 8 mm cystic formation, as in the Dandy–Walker Variant. The size of the cerebellar vermis was below the first percentile for the management period.

Following the ultrasound result, the couple decided to terminate the pregnancy with a therapeutic abortion. Microscopic examination of abortion material confirmed a female fetus to be delivered at 22 weeks, with maturation consistent with the 22nd week of gestation, with somatic changes compatible with the reported chromosomapathy, marked laceration at the level of the brain, hypoplasia of the cerebellar vermis and dilatation of the IV ventricle, as observed in the Dandy–Walker malformation. There were also alterations of the placenta and of the amnio-chorionic membranes.

Discussion

We report two cases of mosaicism, detected at the embryonic stage with PGT-A, persisting through the later stages of their respective pregnancies as true fetal mosaicism. Both cases were initially classified as 'low mosaicism' at the blastocyst stage, one involving a only segmental region, the other involving several chromosomes (complex mosaic) of which a mosaic trisomy 21 persisted in gestation.

Studies to date have reported the transfer of mosaic embryos resulting in pregnancies and births of babies that appear physiologically

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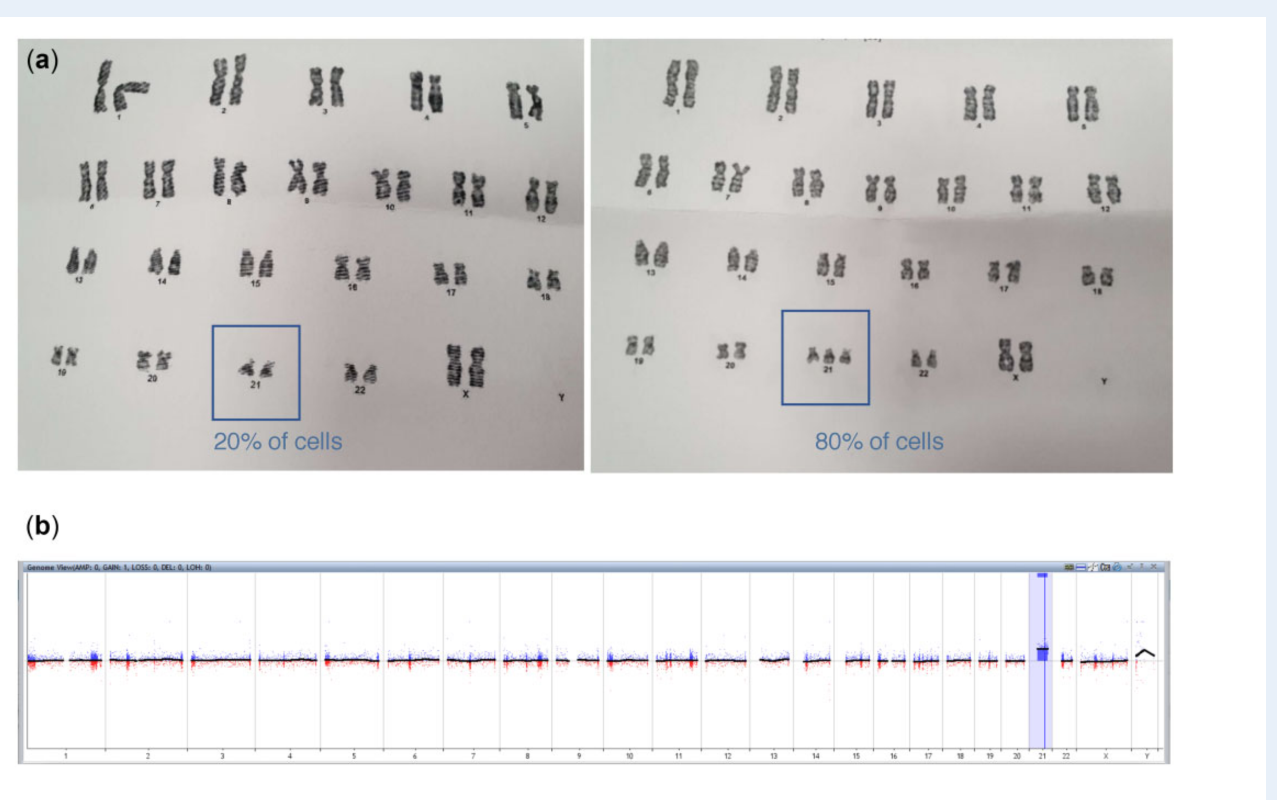

Figure 4. Case 2 chorionic villus sampling (CVS) results. (**a**) Conventional cytogenetic analysis in n = 100 cells showing 46,XX chromosome complement in 20% of cells analyzed, and 47,XX +21 karyotype in the remaining 80% of cells analyzed. (**b**) Analysis by array comparative genomic hybridization (aCGH) produces a plot indicating an elevated shift from the diploid lane for bacterial artificial chromosome (BAC) clones located in chromosome 21 consistent with mosaicism, highlighted in blue.

healthy (Lee et al., 2020; Capalbo et al., 2021; Viotti et al., 2021; Yakovlev et al., 2022). While the data are limited, in instances where prenatal testing was performed, the original chromosomal mosaicism detected at the embryonic stage was not present in NIPT, CVS or amniocentesis (Victor et al., 2019; Viotti et al., 2021). This observation agrees with the notion of mosaic self-correction, in which euploid cells in a mosaic mix gradually outcompete the aneuploid cell compartment by differential rates of cell proliferation and death. Mounting evidence from experimental models supports this concept: (i) euploid-aneuploid chimaeric mouse embryos tend to progressively eliminate the aneuploid cell compartment (Bolton et al., 2016; Singla et al., 2020); (ii) mosaic human embryos in extended culture to Day 12 are frequently converted to fully euploid (Popovic et al., 2019); and (iii) human embryos classified as mosaic show different per cell rates of mitosis and apoptosis compared to embryos classified as euploid (Victor et al., 2019).

The embryo of Case 2 illustrates this point: this complex mosaic had several other mosaic chromosomes besides mosaic trisomy 21, but they were all of normal copy number during the prenatal test. Presumably, their mosaicism resolved itself during pregnancy, but somehow mosaic trisomy 21 evaded the self-correction. Notably, the confirmation during prenatal testing of mosaic trisomy 21 in Case 2, as well as the confirmation of mosaic segmental loss of 1p36.33-p31.1 in Case 1, argue that the original mosaicism detected with PGT-A was a true finding, undermining claims that mosaicism detected with PGT-A is technical noise and an artifact in the procedure (Paulson and Treff, 2020).

One exception was reported in 2020, when an IVF group in Turkey described a case of embryonic mosaicism (low Chr −2) persisting through gestation (Kahraman et al., 2020). Amniocentesis indicated the reciprocal mosaicism (+2), which presumably arose from a non-disjunction event. The pregnancy resulted in a neonate, which produced a chromosomally normal buccal swab but an abnormal blood sample with low-level mosaicism Chr −2. The baby was healthy by physiological inspection. The two cases presented here were terminated during gestation for different reasons, each with important nuances that were considered in the clinical setting.

Regarding Case 1, monosomy 1p36 is the most common terminal chromosomal deletion in humans and one of the most common microdeletion syndromes, with a prevalence of 1:5000–1:10 000 (Di Donato et al., 2014; Jordan et al., 2015; Shimada et al., 2015). The 1p36 deletion syndrome is caused by a partial heterozygous deletion that primarily involves the distal part of the short arm of Chr 1, with breakpoints ranging from 1p36.13 to 1p36.33 (Nistico et al., 2020). The syndrome is characterized by a wide spectrum of features which include intellectual disability, developmental delay, hypotonia,

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congenital heart defects, seizure, facial dysmorphism, and others (Heilstedt *et al.*, 2003; Gajecka *et al.*, 2010; Jordan *et al.*, 2015; Guterman *et al.*, 2019).

According to the Preimplantation Genetic Diagnosis International Society (PGDIS) Position Statement on the Transfer of Mosaic Embryos 2021 (Leigh *et al.*, 2022), patients should be counseled about the benefits of prenatal diagnosis after mosaic embryo transfer. In the case of embryo transfer with 1p36 mosaic deletion, the choice of method and interpretation of prenatal findings is complex. The accuracy of NIPT for deletion syndromes is relatively low (Advani *et al.*, 2017), most NIPT platforms are validated for a subset of chromosomes only, and the placental DNA tested in NIPT and CVS does not necessarily correlate with fetal DNA. In addition, a low fetal fraction in NIPT combined with low level of mosaicism could further impair its detection. Amniocentesis is the preferred method of prenatal diagnosis in such cases, given that the amniotic fluid includes cells derived from the fetus (Gosden, 1983) and the resolution of amniocentesis testing by cytogenetics or microarray can detect segmental chromosomal abnormalities associated with human syndromes. In this clinical case, use of FISH probes specific to the 1p36 deletion in amniocytes revealed that the segmental deletion had persisted during gestation in a mosaic conformation. When the full deletion 1p36 syndrome is detected, the prognosis for the health of the child is severe (Gajecka *et al.*, 2007). Prenatal detection of the mosaic form of the syndrome also has clinical manifestations, but its severity is lower compared to the full deletion (Shimada *et al.*, 2014) and the detected level of mosaicism in the prenatal test is not predictive of the mosaicism level and symptom severity at birth (Liehr *et al.*, 2013). For the purpose of a more detailed examination of the fetus, the couple from Case 1 was offered a cordocentesis. The couple refused the procedure, and in light of the risk associated with the chromosomal findings, decided to terminate the pregnancy.

Regarding Case 2, it is the first report of a case in which mosaic trisomy 21 persisted in the fetus after transfer of a mosaic embryo. Down syndrome, which is caused by an extra copy of Chr 21, is the most common chromosomal abnormality in live-born individuals, occurring at a frequency of about 1/800 live births (Patterson and Costa, 2005). About 2–4% of all cases of Down syndrome are thought to be caused by mosaic trisomy 21 (Papavassiliou *et al.*, 2009). The literature describes cases with variable phenotypic abnormalities associated with mosaic trisomy 21, ranging from traits typically seen in 'uniform' trisomy 21 (people having trisomy 21 in every cell) to that of a near normal phenotype (Papavassiliou *et al.*, 2009). In Case 2, where prenatal testing (CVS and amniocentesis) confirmed mosaic trisomy 21, and detailed ultrasonography detected severe physical abnormalities in the placenta and fetus, the patients felt compelled to opt for termination of the pregnancy.

One important difference between the previous report (Kahraman *et al.*, 2020) and the cases described in our article is the final clinical outcome. The previous report resulted in a physiologically healthy baby. Our case involving mosaic trisomy 21 was terminated after gross abnormalities were found by ultrasound during gestation. Hence, not only did mosaicism persist during the pregnancy, it led to an overt and severe phenotype. Our other case, which involved mosaic loss in 1p also resulted in termination, but for a different reason. No physical abnormalities were detected during the pregnancy, but the phenotype in newborns associated with the detected chromosomal abnormality presented too great a risk for the parents, who opted to terminate. Hence, the two cases discussed in the present paper add new potential outcomes of such pregnancies, which are important when evaluating the risk-benefit of mosaic embryo transfers.

Detection of chromosomal mosaicism in genetic testing is complicated by the intrinsic sampling variability associated with mosaicism. Both clinical cases presented here included a battery of prenatal tests, and while some confirmed the mosaicism observed with PGT-A, others did not. The amniocentesis with cytogenetic analysis in Case 1 produced normal results, as did the NIPT in Case 2, suggesting that the cell samples collected for those respective tests randomly did not contain any aneuploid cell component. The lower the number of analyzed cells in a given prenatal test, the more unlikely it is to capture mosaicism even if it is truly present in the source tissue. In that regard, it is possible that some instances of mosaicism are going undetected in pregnancies from mosaic embryo transfers when prenatal testing is performed. Nonetheless, the two cases presented here argue that performing prenatal testing, especially in the form of amniocentesis followed by microarray, can occasionally detect persisting cases of mosaicism and should therefore be discussed with the patient as a possibly worthwhile procedure following the transfer of a mosaic embryo.

While the vast majority of published data indicates that embryos classified as mosaics by PGT-A experience lower implantation rates and higher risks of miscarriage, the pregnancies that have been profiled by prenatal testing so far have been largely normal and the babies resulting from mosaic embryo transfers have been healthy (Spinella *et al.*, 2018; Victor *et al.*, 2019; Munné *et al.*, 2020; Viotti *et al.*, 2021; Yakovlev *et al.*, 2022). One study has suggested that low-level mosaic embryos might have equal potential to result in live birth as embryos classified as euploid (Capalbo *et al.*, 2021). Notably, both clinical cases presented here, as well as the previously published case from the Turkish group (Kahraman *et al.*, 2020), involved embryos that were classified as low-level mosaics with PGT-A. While they might be rare exceptions, their existence nonetheless highlights a distinction between euploid and low mosaic embryos regarding health risk. Moreover, there might be undetected cases of persistence of mosaicism because of the potential sampling error intrinsic to prenatal testing.

Only additional data from mosaic embryo transfers and paired prenatal tests will allow us to accurately gauge the incidence of cases in which embryonic mosaicism persists and becomes true fetal mosaicism. Meanwhile, the cases presented here should be carefully considered when weighing risks during embryo selection in the clinic.

Data availability

All data underlying this article are available in the article.

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Authors' roles

E.G.: study design, experimental data, manuscript draft; P.Y.: study design, experimental data, manuscript draft, critical discussion; N.K.: experimental data, manuscript draft; S.V., D.B., M.E., Y.T., A.T., A.P.,

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A.B., M.T.S., I.L., C.R. and P.F.G.: experimental data; A.V., F.B. and C.Z.: manuscript draft and editing; F.S.: study design, experimental data, manuscript draft, critical discussion; M.V.: project coordination, manuscript draft, critical discussion.

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Conflict of interest

The authors declare no conflicts of interest.

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