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Monozygotic twins with discordant karyotypes following preimplantation genetic screening and single embryo transfer: case report

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Abstract
Purpose To report a case of monozygotic monochorial diamniotic twins with discordant karyotypes.
Methods and results The pregnancy was achieved following a treatment cycle with intracytoplasmic sperm injection (ICSI) and preimplantation genetic screening (PGS) for chromosomes X, Y, 13, 16, 18, 21, 22. One embryo euploid for studied chromosomes was transferred. Prenatal ultrasonography revealed monozygotic twins. One fetus had growth retardation, multiple organ abnormalities and polyhydramnion. The other twin had normal ultrasound appearance. Delivery on week 29 of gestation resulted in the birth of two females, a stillborn twin with karyotype 45,XX,-13[12]/46,XX,r(13)[3] and a healthy twin with normal karyotype.
Conclusions The discordance in the twins' karyotypes originated from a mosaic embryo. Structural chromosomal abnormality of the affected twin could not be revealed using standard PGS investigation. Embryo splitting occurred probably due to apoptotic process in an early stage of embryo development. Apoptosis represents one of the possible mechanisms which can explain the embryo twinning process globally.

Keywords Monozygotic twins · Preimplantation Genetic Screening (PGS) · Apoptosis · Ring chromosome

Capsule We describe the case report of monozygotic monochorial diamniotic twins with discordant karyotypes following preimplantation genetic screening (PGS) and single embryo transfer. A proposition is made for the mechanism possibly involved in this case where an embryo is divided into twins.

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Introduction

Monozygotic twins (MZT) occur in 0.4% to 0.45% of all births [1, 2]. Numerous articles have reported a significantly higher incidence of MZT following assisted reproduction cycles than after natural conception [1, 3–8].

MZT, as a complication of assisted reproduction, represent a higher risk of adverse outcome than single pregnancies. There is an increased incidence of fetal growth restriction, fetal loss, pre-term delivery and perinatal loss [5, 7]. MZT with monochorionic placentation are often complicated by feto-fetal transfusion syndrome and also play a role in the etiology of cerebral palsy, and

pathogenesis of congenital abnormalities such as cardiac and brain anomalies or other clinical syndromes [9].

Possible etiological factors are still widely discussed and conflicting results were often found in available studies. Based on literature, the etiological factor is not the only factor. A combination of these factors is likely to be responsible for the embryo splitting [7]. Risk factors, most often mentioned in connection with MZT, are controlled ovarian hyperstimulation [3, 5], advanced maternal age [10], zona pellucida hardening after cryopreservation or artificial breach in the zona pellucida due to micromanipulation techniques [4, 5, 11–13]. On the other hand, the zona pellucida manipulation effect was not confirmed by Schachter [3]. Also a recent study did not show an increased incidence of MZT following transfer of biopsied embryos [14]. In some studies, a higher rate of MZT was described after blastocyst transfer when compared with cleavage stage transfer [1, 8, 15, 16]. Although there were no differences in MZT between cleavage stage transfer group and blastocyst transfer group as observed by other authors [2, 17].

We describe the case report of monozygotic monochorial diamniotic twins with discordant karyotypes following preimplantation genetic screening (PGS) and single embryo transfer. A proposition is made for the mechanism possibly involved in this case where an embryo is divided into twins.

Case report

A 29-year-old woman and her 33-year-old husband were referred to our IVF clinic after 1 year of infertility. The patient had one artificial interruption in her personal history. The hormonal profile of the patient was found to be within a normal range. She was a non-smoker and her body mass index was 18.14 kg/m^2. Her husband was diagnosed with oligoasthenoteratospermia gravis. Both partners had normal karyotypes. The couple decided for in vitro fertilization cycle (IVF) with aneuploidy screening due to the factor of severe male infertility.

The patient was stimulated in a long protocol using GnRH agonists in combination with recombinant gonadotropins (Puregon Pen; Organon International Inc., The Netherlands). Follicular aspiration was performed 36–38 h after the recombinant hCG (500 μg of Ovitrelle; MERCK SERONO, UK) administration and 13 oocytes were retrieved.

A sperm sample was obtained by masturbation. Semen parameters were analyzed according to WHO guidelines criteria [18]. The semen sample was processed using a swim-up procedure.

9 oocytes in MII stage were injected. After 16–18 h, signs of fertilization were evaluated and 8 oocytes with two pronuclei were found. Zygotes were transferred to the cleavage medium (Quinn's Advantage Protein Plus Cleavage Medium, SAGE, USA) and cultured till the biopsy. The biopsy was done on Day 3 in the morning. Since all the embryos achieved at least an 8-cell stage, two blastomeres were removed from each. Opening of the zona pellucida was performed mechanically followed by the aspiration of the cells. Biopsied cells were spread using HCl / Tween20 method as described by Coonen [19]. FISH was performed in two-rounds, allowing the detection of chromosomes 13, 16, 18, 21, 22 in the first round (MultiVysion PB kit; Vysis, USA) and X, Y in the second round (CEP X SG/CEP Y(α) SO; Vysis, USA). The standard scoring criteria for FISH analysis were used [20]. FISH results are summarized in Table 1. Three embryos were diagnosed euploid for chromosomes tested in both blastomeres. One of them was selected for embryo transfer on Day 5. The other two were frozen.

After a positive pregnancy test, ultrasonographic examination at week 7 of gestation showed the intrauterine presence of monochorial diamniotic twins. The first trimester ultrasound screening revealed one affected twin (Twin A) with growth retardation, multiple organ abnormalities (heart disease, renal pelvis dilatation) and polyhydramnion. The other fetus (Twin B) had normal ultrasound appearance. The feto-fetal transfusion syndrom was diagnosed, where Twin A was the recipient with paradoxical hypotrophy. During the therapeutic amniocentesis the excess of amniotic fluid was removed from amniotic sac A. The analysis using G-banding performed on the amniotic fluid from sac A revealed mosaic karyotype with two cell lines: the monosomy of chromosome 13 (Fig. 1) in 12 mitoses and the monosomy of chromosome 13 plus a ring chromosome of unknown origin, later specified as chromosome 13 (Fig. 2), in 3 mitoses. Using interphase FISH, aneuploidy of chromosome 13 was confirmed (150 cells evaluated; 105 monosomic / 45 disomic for chromosome 13). The ring chromosome was derived from chromosome 13, which was determined by metaphase FISH using probes LSI13q14 (Vysis, USA) and ToTel Vysion Probe Panel, Mix 6 (Abbott Molecular Inc., Vysis, USA). Monosomy (Fig. 3) was found in 21 mitoses and a monosomy plus ring chromosome (Fig. 4) in 9 mitoses. Thus karyotype of Twin A was determined as 45, XX,-13[12]/46,XX,r(13)[3].

Since the ring chromosome could not be revealed using PGS, all biopsied blastomeres used for aneuploidy screening were subsequently reanalyzed with Sub-Telomere 13qter probe (Kreatech Diagnostics, The Netherlands) in the third round of FISH. Results of the hybridization with subtelomere probe were consistent with the results obtained with a locus specific probe LSI13q14 in the first round of FISH.

Table 1 PGS results

Embryo No	Embryo details	Cells biopsied	Chromosomes investigated							Quality on Day5	
			13	22	21	16	18	X	Y		
1	9cells	a	2	2	2	2	2	1	1	Hatching blastocyst	Excluded
		b	1	2	2	2	2	1	1		
2	9cells	a	2	2	2	2	2	1	1	Hatching blastocyst	Frozen
		b	2	2	2	2	2	1	1		
3	8cells	a	2	2	2	2	2	1	1	Hatching blastocyst	Frozen
		b	2	2	2	2	2	1	1		
4	8cells	a	2	3	2	2	2	3	0	Blastocyst	Excluded
		b	2	3	2	2	2	2	0		
5	8cells	a	2	2	2	2	2	2	0	Hatching blastocyst	Transferred
		b	2	2	2	2	2	2	0		
6	8cells	a	2	3	2	1	2	1	1	Compacted morulae	Excluded
		b	2	1	2	2	2	1	1		
7	10cells	a	1	2	1	N	1	1	1	Morulae	Excluded
		b	Anucleate blastomere								
8	9cells	a	2	1	1	2	2	1	1	Cavitated morulae	Excluded
		b	2	2	3	2	2	1	1		

N=No result

Preterm delivery at week 29 of gestation resulted in the birth of two females, Twin A was a stillborn and Twin B was born alive with normal female phenotype. The twins' zygozity was tested by molecular analysis. The DNA of the affected Twin A was isolated from amniotic fluid while the DNA of Twin B was isolated after delivery. Zygozity of the twins was established by the use of the Identifiler kit (Applera s.r.o., Czech Republic). The identical DNA profiles of tested samples confirmed monozygozity.

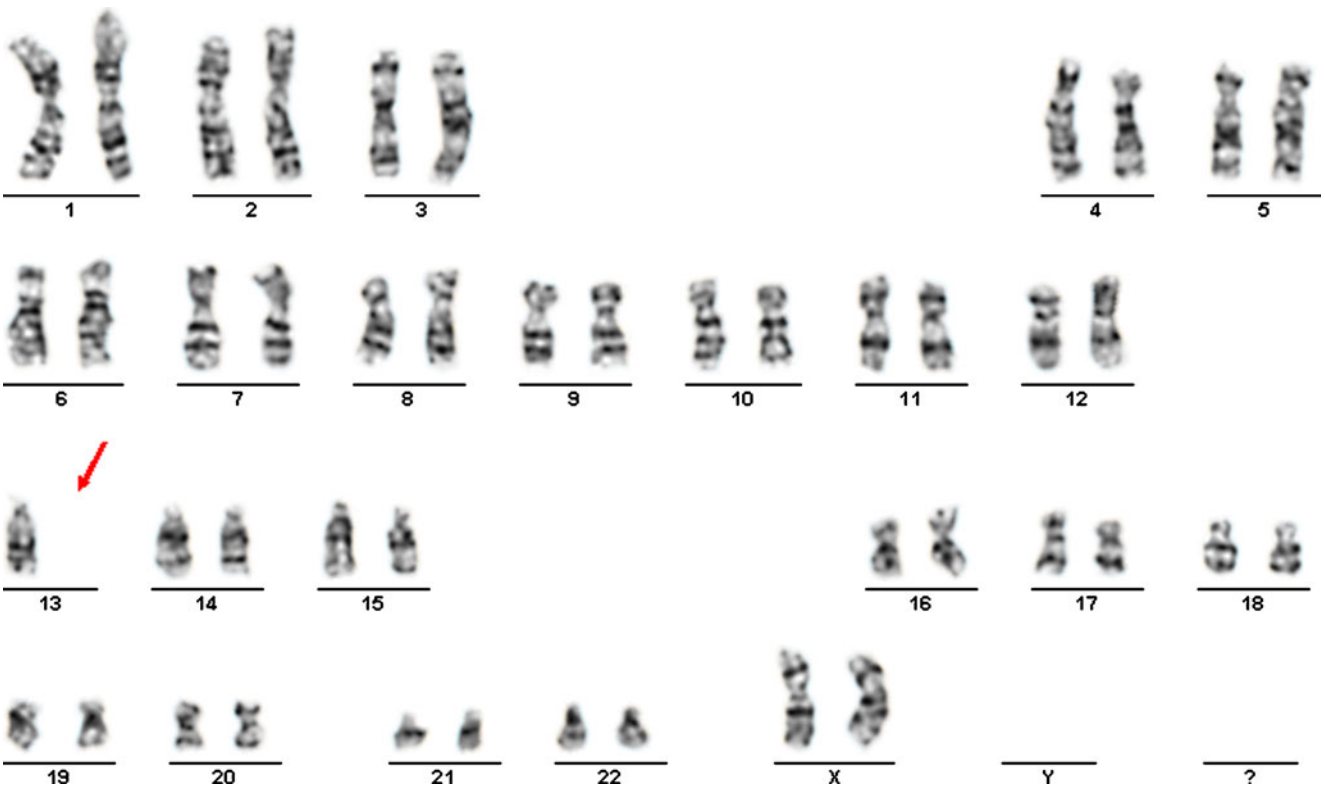

Fig. 1 Karyotype of Twin A, G-banding: 45,XX,-13 cell line

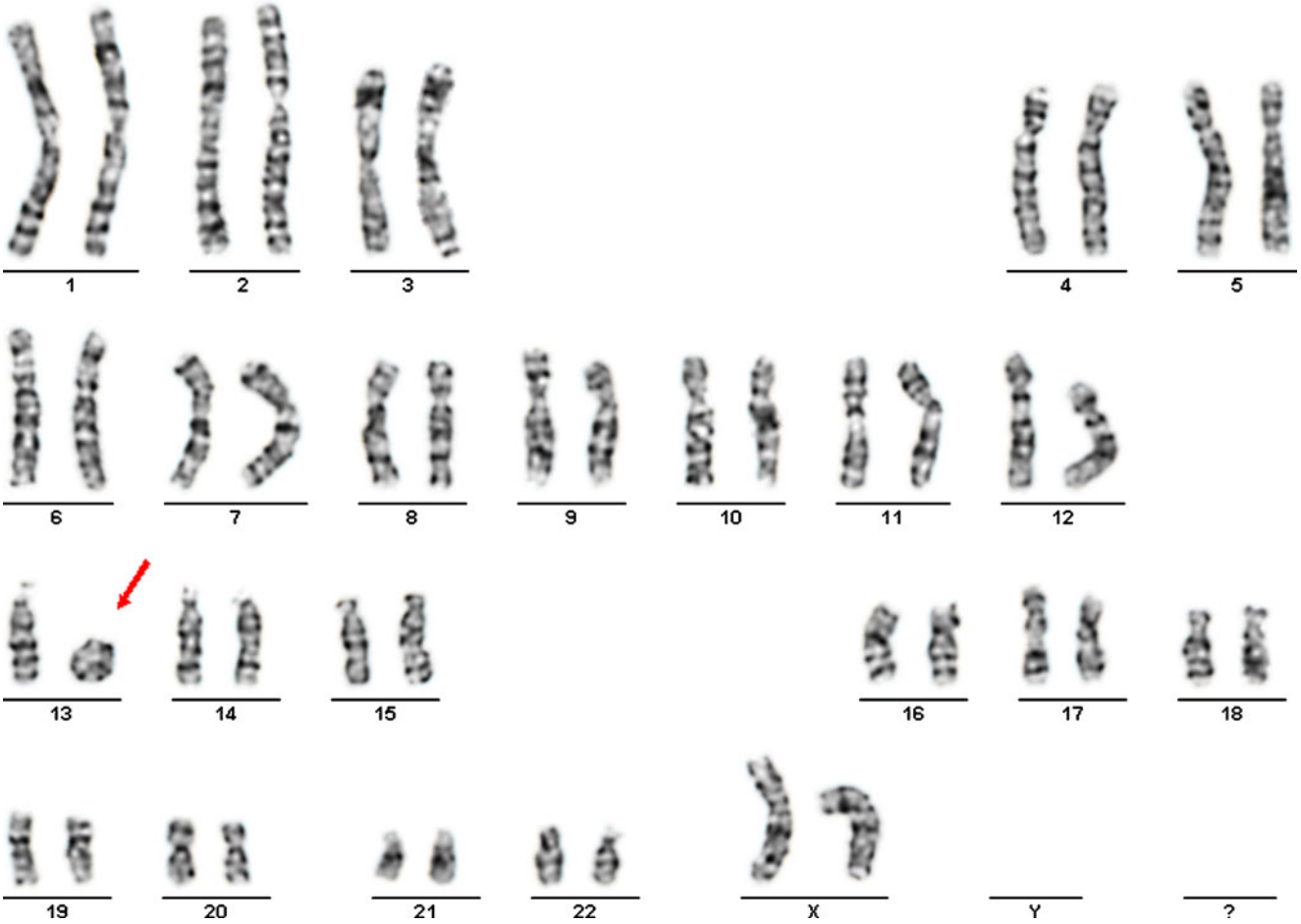

Fig. 2 Karyotype of Twin A, G-banding: 46,XX,r(13) cell line

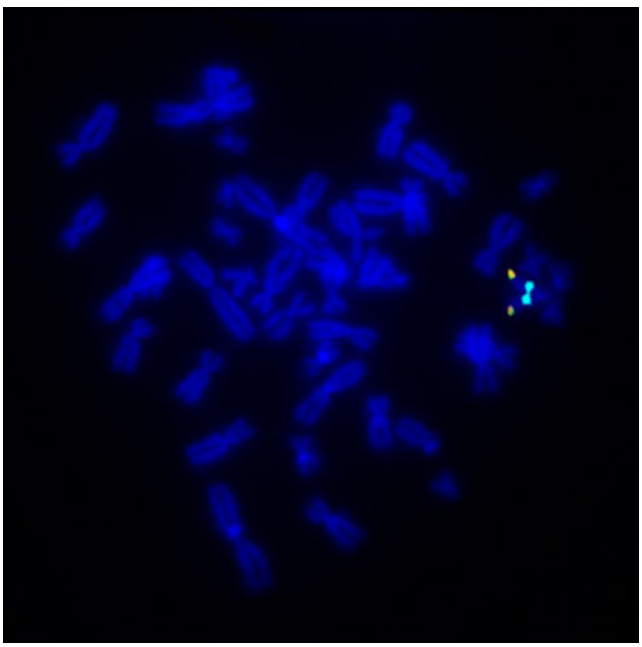

Fig. 3 Twin A, FISH result: 45,XX,-13. Aqua probe = 13q14, yellow probe = 13qtel

The postnatal cytogenetic investigation of blood lymphocytes from Twin B showed normal female karyotype without any numerical or structural aberration in 100 mitoses. Moreover, with respect to the karyotype of

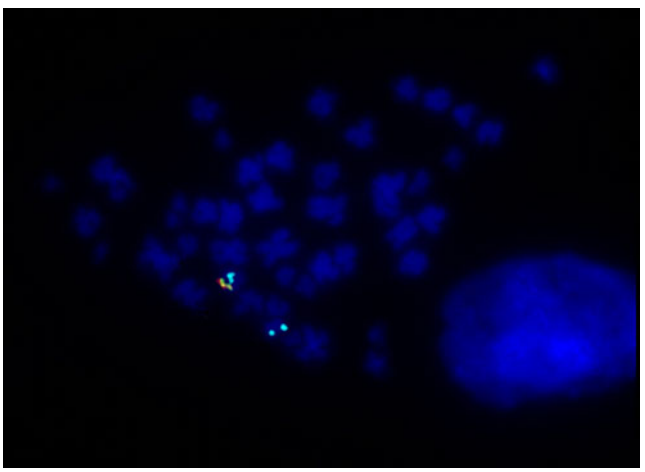

Fig. 4 Twin A, FISH result: 46,XX,r(13). Aqua probe = 13q14, yellow probe = 13qtel; Yellow signals are visible on one of the chromosomes 13 but are absent on the ring chromosome

stillborn Twin A, a detailed FISH analysis of chromosome 13 was completed using FISH with probes LSI13q14 and ToTel Vysion Probe Panel, Mix 6 and no pathological finding was detected in 10 mitoses. The healthy girl is developing normally up to the current day.

Discussion

This article describes a curious case report of MZT with discordant karyotypes, following PGS cycle. One fetus had normal and the second one had pathological mosaic karyotype 45,XX,-13[12]/46,XX,r(13)[3]. MZT with discordant karyotypes were described earlier as an uncommon event [21–28] and chromosomal abnormality in only one fetus of MZT pregnancy is even a more rare constellation [26, 29]. DNA tests confirmed the zygozity of both embryos which was a predictable result because of the fact that a single embryo transfer was performed and the chance of spontaneous pregnancy was limited for several reasons: The main reason was the male partner's compromised semen parameters. In addition to that he had had a car accident few days before the IVF cycle and was hospitalised for several weeks.

The discordance in the twins' karyotypes originated from a mosaic embryo. The question is when the mosaicism had occurred. This finding came about either due to the mitotic error before the twinning, or due to the chromosomal aberration after the twinning. Monochorionic diamniotic twins result from a splitting of an early embryo between Day 4 and Day 8, after inner cell mass (ICM) and trophoblast differentiation, but before the amnion formation [1]. A large amount of abnormal cells, in our case, suggests that mitotic error could occur early, already during the cleavage stage and probably before the embryo splitting.

In the case where one abnormal cell was present at the time of biopsy in 8-cell stage and two chromosomally normal cells were removed, then the abnormal cell volume in the embryo increased from 12.50 to 16.66%, just because of the biopsy. Moreover, even if the ring chromosome was present in the biopsied cell FISH would have evaluated it as euploid. To check up the possibility that the ring was present in biopsied cells, all the blastomeres were reanalyzed in the third round of FISH with subtelomere probe. As a result of reanalysis, no ring 13 was found, even in the cells of the transferred embryo. When we have a look at the PGS result, except for the transferred embryo, mosaicism was discovered in four of eight embryos and monosomy of chromosome 13 in two of eight embryos.

We also considered the option that one of the parents had a structural chromosomal abnormality that was not found by conventional karyotyping but could be the cause of abnormalities in the gametes or embryos. Although this possibility could not be excluded for sure, it is likely that the ring chromosome was developed as a random event just in one mosaic embryo. This theory is supported by the facts that the other twin was healthy and this type of abnormality was not picked up in any other embryo of the couple.

Although the chromosomal status in placental tissue is unknown, it is interesting that abnormal cells were distributed purely in one of the fetuses. Thus there is a theory that incurred chromosomal abnormality could be related also to the twinning process. The mechanism possibly involved in this case of embryo dividing is apoptotic process within ICM.

Ring chromosome 13 could occur as the first chromosomal error. The origin of this ring is most likely connected to the presence of numerous fragile sites on chromosome 13 [30–32]. These sites are specific chromosomal regions where gaps and breaks tend to occur. The presence of chromosome fragile sites was shown to predispose to deletions and chromosomal rearrangements [33]. Interestingly, two of these fragile sites, FRA13A (13q13.2–13q13.3) and FRA13G (13q14) are very close to RB1 gene position (13q14.2; RB1- retinoblastoma 1, GeneID: 5925) and the RB1 gene plays an important role in cell-cycle regulation and apoptosis [34]. Due to the chromosomal instability, the ring chromosome was probably lost during the next mitosis and monosomic cell line occurred. Since the chromosomally abnormal cells derived from one mitotic error, it can be supposed that the cells were gathered in one part of the embryo. Cells in this part of embryo were probably more susceptible to apoptosis than chromosomally normal cells. The enhanced fragility of ICM caused by apoptosis could explain why the embryo divided into a normal and an abnormal twin in the case described.

There are also other explanations of an embryo splitting into twins like the possibility that a serious chromosomal abnormality spread in one part of the embryo caused developmental delay in affected cells and subsequently triggered separate development. Nevertheless, any ultrasound signs of growth retardation of one of the twins in the early weeks of gestation were not observed.

Apoptosis as a possible mechanism of embryo splitting has been mentioned previously in literature. A time-lapse study of blastocyst formation disclosed that some blastocysts are susceptible to repeated collapses. Blastocyst collapse can result in some ICM cells relocating and adhering on the opposite trophectoderm wall which leads in a formation of second ICM and subsequently in formation of identical twins [35]. Frequent blastocoelic collapse can be caused by a failure of junctions between trophectoderm cells due to apoptosis in a specific region of the trophectoderm wall. Another study documented higher sensitivity of ICM to embryotoxic agents, disruption and apoptosis than trophectoderm cells [36]. Moley demon-

strated that expression of an apoptosis regulatory gene is increased in mouse embryos at the blastocyst stage at conditions of high glucose concentration [37]. It has been supposed that not only hyperglycemia but also high glucose levels present in a culture media could activate apoptotic pathway in some embryos. Apoptotic changes in ICM together with mechanical pressure during the hatching process can be related to a higher incidence of MZT in embryos cultured in vitro [38].

Apoptosis in embryos, no matter if it is triggered by internal or external factors, represents one of the possible mechanisms which can explain the embryo twinning process globally.

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