Olgu Sunumu
Case Report

48,XY,+7,+21 And 47,XX,+16 Fetal Karyotypes In A Case With Recurrent Pregnancy Loss

TEKRARLAYAN GEBELİK KAYIPLARI BULUNAN OLGUDA SAPTANAN 48, XY,+7,+21 VE 47,XX,+16 FETAL KARYOTİPLER

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ABSTRACT

Early pregnancy loss is the outcome of approximately 10% of clinically recognized pregnancies and chromosomal abnormalities are the underlying reason in 50%. In this report we discussed aneuploidy mechanisms and management options based on a couple with recurrent aneuploidies. Thirty-five-year-old female was referred with spontaneous abortion. Quantitative Fluorescent PCR was consistent with trisomy 21 but karyotyping revealed double trisomy of 48,XY,+7,+21. During follow-up, another abortion was diagnosed as 47,XX,+16. Peripheral blood analysis revealed a borderline mosaic 46,XX [95]/45,X[5] karyotype. Her physical examination was normal but abdominal ultrasonography revealed accessory spleen and double ureter in left kidney. We excluded Robertsonian translocations, structural aberrations or trisomic mosaicism as a cause but the borderline 45,X mosaicism may be the triggering factor by decreasing oocyte reserve. Presence of urinary malformation indicates that genitourinary mosaicism may be higher, although ovarian biopsy cannot be performed to determine it. Genetic counseling is vital in management of such cases.

Keywords: Aneuploidy, Trisomy, Habitual Abortion, Sex Chromosome Aberrations

ÖZ

Bu yazıda ardışık düşüklerde farklı anöploidiler saptanan bir olgu üzerinden anöploidi mekanizmaları ve takipte düşünülmesi gereken seçenekler tartışılmıştır. Otuz beş yaşında kadın spontan abortus ile başvurdu. Kantitatif floresan PCR, trizomi 21 ile uyumluydu. Ancak kültür süreci sonunda yapılan karyotipleme 48,XY,+7,+21 çift trizomisini ortaya çıkardı. Olgunun takibinde başka bir düşük 47,XX,+16 olarak saptandı. Periferik kandan sitogenetik analizinde sınırda mozaik 46,XX[95]/45,X[5] karyotipi görüldü. Fizik muayenesi normaldi fakat abdominal ultrasonografide sol böbrekte çift üreter ve aksesuar dalak mevcuttu. Tekrarlayan trizomilerin olası nedenlerinden Robertsonian translokasyonlar, yapısal anomaliler veya trizomi mozaikliği dışlandı. Düşük oranlı 45,X mozaikliği ise oosit rezervini azalttığından riski arttırabilir. Olgudaki malformasyonlar mozaikliğin genitoüriner sistemde daha yüksek oranlı olabileceğini göstermektedir. Over biyopsisi invaziv doğası nedeniyle yapılamadığından bu oranın bilinmesi mümkün değildir. Tanı ve tedavi seçeneklerinin sınırlı kaldığı benzer vakaların yönetiminde genetik danışma hayati öneme sahiptir.

Anahtar Kelimeler: Anöploidi, Trizomi, Tekrarlayan Düşük, Cinsiyet Kromozom Aberasyonları

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Early pregnancy loss is the outcome of approximately 10% of all clinically recognized pregnancies and chromosomal abnormalities are the underlying reason in 50% (1). The question remains whether it is possible to predict the risk for aneuploidy before conception. Many different factors have been examined and by far the most significant one is advanced maternal age. In addition, balanced structural chromosome aberrations or trisomic gonadal mosaicism in the family increases the risk of aneuploidy. Environmental factors such as paternal age, maternal smoking, maternal alcohol use, radiation exposure, oral contraceptives, spermicide use, parity or socioeconomic status and genetic factors such as MTHFR C677T variant, consanguineous marriages or racial differences were investigated but no definitive relationship have been found (2).

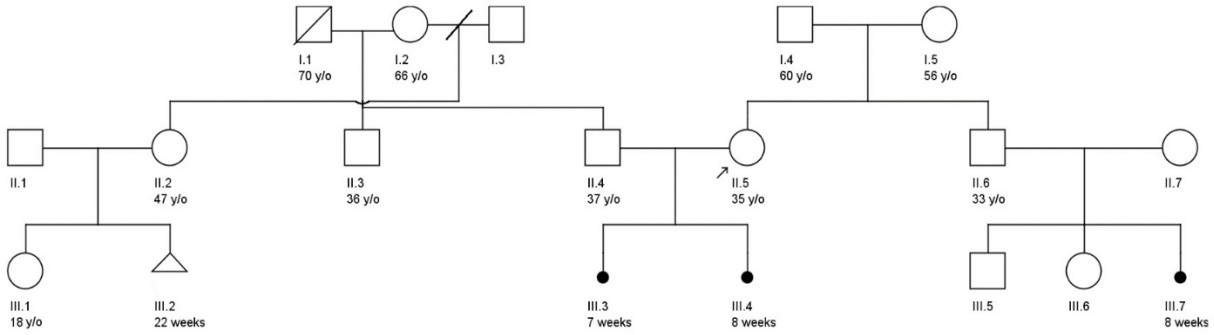
It is a matter of debate whether a personal history of trisomic abortion increases the risk in future pregnancies (3,4). Cases with recurrent trisomies and especially cases with double trisomies are the most appropriate patient populations for the investigation of underlying causes. The exceptionally low probability of two independent nondisjunction events in the same fetus suggests the presence of a causative factor. Double trisomy is rare, accounting for 0.21-2.8% of all karyotyped miscarriages (5). Live births are even rarer and generally involve a combination of sex chromosome and viable autosomal trisomy (6).

Here we present a case with double trisomy and single trisomy in consecutive pregnancies. Trisomy proneness of the family is discussed based on possible risk factors. As far as we know this is the third case of double trisomy in the literature which involves chromosomes 7 and 21.

CASE

Thirty-five-year-old female and her 37-year-old healthy male husband were referred with 8th gestational week (G2A2) pregnancy without fetal heart rate (FHR). The couple previously had an abortion of 7th week pregnancy (G1A1) in which no genetic or pathological examination were done. There was no consanguinity between them. No history of recurrent pregnancy loss or any known chromosomal abnormality were found in their close relatives (Figure-1). The patient's sister-in-law had an 8th gestational week pregnancy loss (Figure-1, case III.7) and her husband's stepsister had a history of 22nd gestational week stillbirth (Figure-1, case III.2).

Figure:1



Pedigree of the family

Rapid aneuploidy screening with quantitative fluorescent PCR (QF-PCR) and cytogenetic culture with G-banded karyotyping were planned. Both patient and her husband have given written informed consent and all work

has been carried out in accordance with the Declaration of Helsinki. To avoid maternal contamination, curettage product is repeatedly washed with Dulbecco's phosphate buffered saline (Biological Industries, Israel) and chorionic villi are dissected form maternal decidua. Chorionic villi are then cultivated in 2 ml of AmnioPlus (Cegrogen, Germany) complete medium following standard protocols. For QF-PCR, total genomic DNA was isolated from some of the dissected chorionic villi with spin column method. Microsatellite patterns specific to 13-15-16-18-21 and sex chromosomes were analyzed with ChromoQuant SuperSTaR Optima QF-PCR kit and ChromoQuant Optima Plus kit (Cybergene AB, Sweden) following manufacturer's protocols. The result was a microsatellite pattern consistent with trisomy 21. After long-term culture, the karyotype analysis revealed double trisomy of 7 and 21 in all metaphases: 48,XY,+7,+21 (Figure-2).

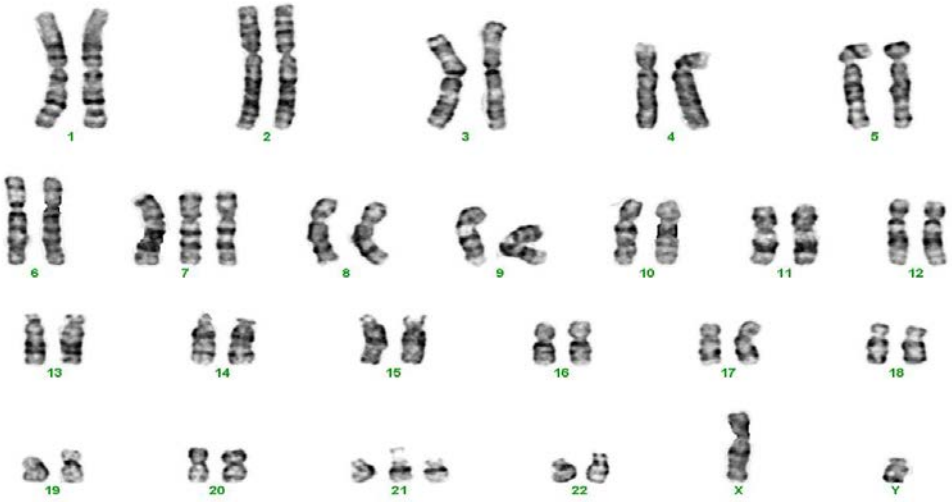


Figure 2. Karyotype of the G2A2 abortion

Peripheral blood cytogenetic culture was performed to the patient. A borderline mosaic structure 46,XX[95]/45,X[5] were seen which was confirmed by two different cultures. Her clinical features were re-examined and found to be normal in terms of Turner stigmata's. But her abdominal ultrasonography revealed an accessory spleen and a double ureter in the left kidney. There were no uterine or ovarian anomalies. Karyotype of her spouse was normal.

Nine months later, the couple presented with another naturally conceived (G3A3) pregnancy. This pregnancy was terminated at 8th gestational week due to absence of FHR. Again, QF-PCR and chromosome analysis were performed. G3A3 abortus karyotype was detected as 47,XX,+16 (Figure-3)

Figure.3

Figure 3: Karyotype of the G3A3 abortion

We determined parental origin of the extra chromosome 21 in 48,XY,+7,+21 fetus and the extra chromosome 16 in 47,XX,+16 fetus by microsatellite marker comparison between the patient and curettage materials. Both were found to be of maternal origin. We were unable to determine the origin of extra chromosome 7 because relevant markers were not included in the QF-PCR kit.

Considering a possible predisposition to trisomy, in vitro fertilization (IVF) and preimplantation genetic screening (PGS) were recommended. The patient's gynecologic evaluation and hormone profile was normal. Her husband's total progressive motile sperm count was $45x10^6$/ml with a normal morphology rate of 3%. They had IVF and PGS performed in a private institution. Twenty-four chromosomes were screened but complex chromosomal aneuploidy were detected in all four embryos obtained (Table-1).

Table 1. Preimplantation genetic screening results of complex aneuploidies

Embryo	Trophectoderm Analysis	Result
1	-8, -13, -15, -18, -20, -22, +1, +2, +4, +9, +19	Complex Aneuploidy
2	-3, -8, -10, -13, -14, -15, -18, -Y, +1, +2, +4, +6, +9, +16, +19, +X	Complex Aneuploidy
3	-8, -10, -13, -14, -15, -18, -21, -22, +1, +2, +4, +5, +12, +19, +X	Complex Aneuploidy
4	-8, -10, -13, -15, -18, -21, -22, +1, +2, +4, +7, +12, +16, +19, +X	Complex Aneuploidy

DISCUSSION

Classic knowledge states that the mechanisms responsible for trisomy, monosomy X, triploidy and tetraploidy are mainly nondisjunction (NDJ), anaphase delay, dispermia and mitotic errors, respectively. NDJ is defined as the failure of separation of homologous chromosomes in meiosis I or sister chromatids in meiosis II, both of which produces an aneuploid gamete. Premature separation of sister chromatids (PSSC) was shown as an additional mechanism in 1991 (7). PSSC was first thought to be a byproduct of IVF protocols. However, further studies have

shown that PSSC is a natural phenomenon and that it is not so infrequent compared to NDJ (8,9).

Recently, a new mechanism has been proposed as a result of information obtained from oocyte polar body biopsies (10). In reverse segregation, both sister chromatids are separated in meiosis I which is followed by a high rate of meiosis II error (Figure-4).

Figure.4

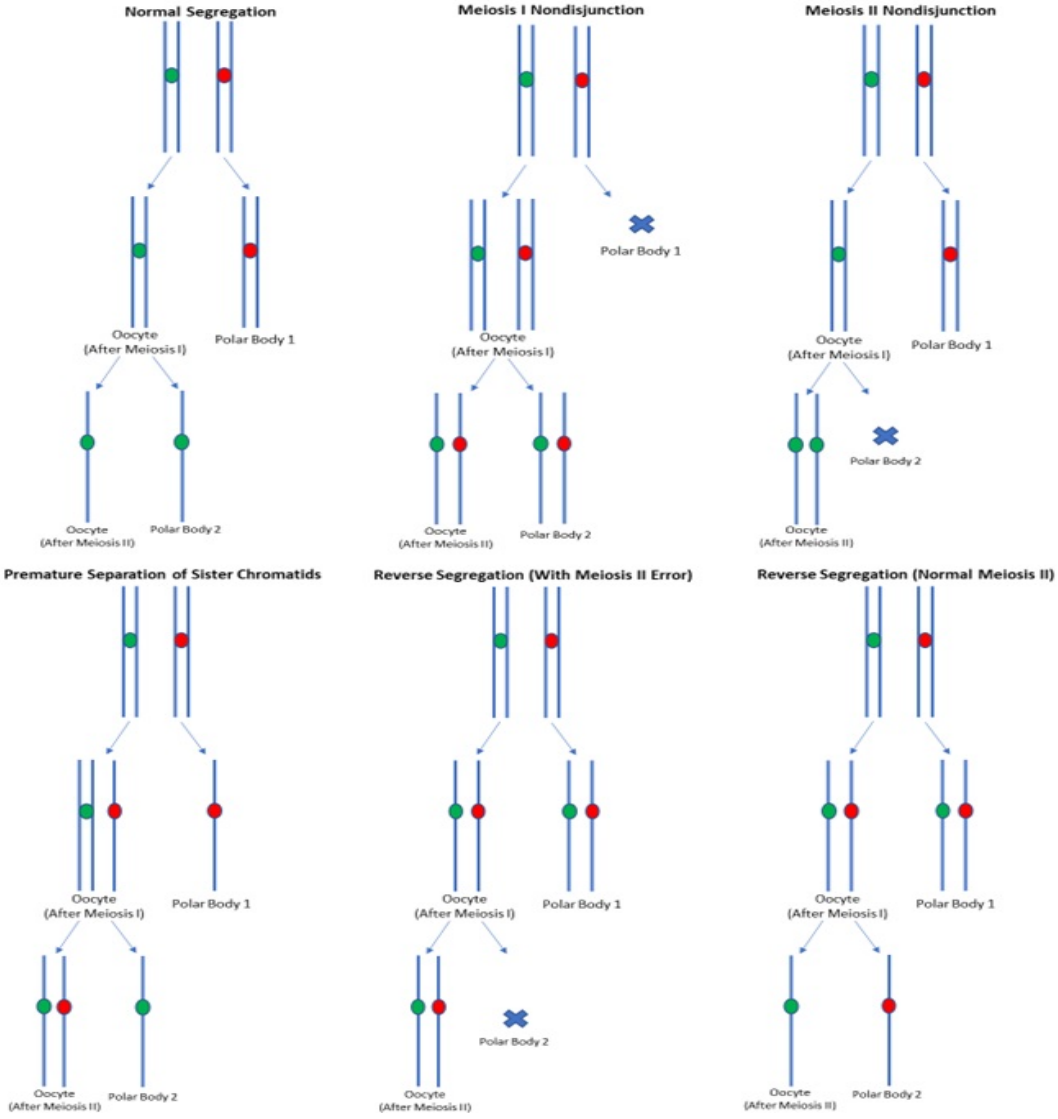


Figure 4. : Four main meiotic error mechanisms. Meiosis I Nondisjunction, Meiosis II Nondisjunction, Premature Separation of Sister Chromatids and Reverse Segregation. Homologous chromosome segregation does not occur in meiosis I NDJ while sister chromatid segregation does not occur in

meiosis II NDJ. Premature separation of sister chromatid is defined as the separation of a sister chromatid couple to oocyte and polar body I in meiosis I when they should have gone to the same pole. In reverse segregation, during the meiosis I, both pairs of sister chromatids separate from each other. Oocyte is normally distributed with two chromatids but of different parental origins. If the meiosis II error is not accompanied, the number of chromosomes would be normal in the oocyte. However after meiosis I, two homologous chromatids remaining in the oocyte are not interconnected, as shown in the figure, and are likely to cause aneuploidy through meiosis II misplacement. Chromosomes marked with the same color in the centromere are sister chromatids while green/red colored ones are homologous chromosomes. Differences caused by crossover events are not shown.

It is impossible to distinguish reverse segregation from meiosis I NDJ by comparing microsatellite markers of the fetus and the mother (Figure-4). Because in both mechanisms, homologous chromosome pairs originating from maternal grandmother and maternal grandfather (or paternal equivalents) are transferred. However, in the case of meiosis II NDJ, sister chromatids from the grandmother or grandfather will be transferred to the fetus. Therefore, meiosis I NDJ and meiosis II NDJ can be distinguished by comparing fetal and maternal microsatellite markers, with the exception of crossover sites. Differentiation between reverse segregation and meiosis I NDJ can only be done by analyzing oocyte polar bodies, which is not possible in naturally conceived pregnancies. In our case, extra chromosome 21 in 48,XY,+7,+21 fetus and extra chromosome 16 in 47,XX,+16 fetus were found to be originated by maternal meiosis I NDJ (or reverse segregation). In 1.000 abortion materials, Hassold et al. reported a frequency of approximately 2% and 2.8% for all double trisomy and trisomy 16, respectively (11). In another study where PGS was performed by analysis of 24 chromosome pairs, complex aneuploidy frequency was found to be around 10% (12).

The probability of aneuploidies in our case being random independent events is $56x10^9$ when calculated using these values (2% x 2.8% x 10% x 10% x 10% x 10%). Although this is a crude method, it is valuable in that it shows the plausibility of thinking that this patient's aneuploidies are not independent events.

There may be several causes for recurrent trisomy in this patient. With cytogenetic evaluation; Robertsonian translocations, structural anomalies and trisomic mosaicism were excluded. A possible gonadal trisomic mosaicism does not explain the patient because in that situation, consecutive trisomies would contain the same chromosome. In addition, there is no history of gonadal damage (e.g. radiation, chemotherapy) to strengthen this possibility. The most important factor known to increase the risk of trisomy is advanced maternal age.

Our case was 35 and 36 years old in respective pregnancies. Decreased oocyte reserve is thought to be the mechanism behind advanced maternal age. With increasing age, the number of oocytes entering the cycle decreases and in parallel the possibility of finding the oocyte with the best response to hormonal stimulation also decrease (13). In our case 45,X mosaicism may have contributed by decreasing oocyte reserve. However, this finding may also be caused by age-related X chromosome loss. The upper limit for age-related loss of X chromosome at 35 years of age were reported to be less than 5% (14). Therefore, our case is not within the normal range. Also, presence of urinary malformation indicates that genitourinary mosaicism may be higher than blood, but the only way to prove this is ovarian biopsy which cannot be applied due to its invasive nature.

Double trisomies are exceedingly rare. Approximately 400 cases have been described in the literature considering all live births and abortions (5). As far as we know this is the third report of an abortus containing chromosomes 7 and 21 in the literature. Two previous cases resulted in spontaneous abortion, and the second case also has monosomy X (5,15).

In conclusion, we gave detailed genetic counseling to the family. Genetic counseling should be a vital part of the clinical management in all similar cases. The advantages and limitations of PGS, the necessity for prenatal diagnosis in all future pregnancies and other options like oocyte donation or adoption should be

discussed. The path to be followed should be decided by the family and healthcare professionals together.

Declaration of Competing Interest

The authors have no conflicts of interest relevant to this article.

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Oral Presentation

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