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## Prenatal diagnosis of triploidy in second trimester of pregnancy: a series of 4 cases over an eleven-year period

### Diagnosticul prenatal al triploidiei în trimestrul al II-lea de sarcină: o serie de patru cazuri depistate în unsprezece ani

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#### Abstract

Triploidy is a numerical chromosomal anomaly characterized by the presence of three sets of haploid chromosomes. The incidence is hard to evaluate, because usually it causes 1<sup>st</sup> trimester miscarriage. At 20 weeks of amenorrhea the incidence of triploidy is estimated at 1/250,000 cases. We present 4 cases of triploidy diagnosed during the decade 2003-2013 in the Prenatal Diagnosis Department of Maternity “Cuza-Vodă” Iasi, Romania, all registered in one year. The analysis of pathological cases identified in the last 11 years by prenatal diagnosis has shown that triploidies represented only 5.7% of numeric chromosomal anomalies, but in 2013 the four cases of triploidy represented 36% of numeric chromosomal anomalies. The karyotypes were recommended after discovering different congenital anomalies by ultrasound scan. In all cases, an intrauterine growth retardation (IUGR) was present but with no placental changes. Also, we discovered anomalies of limbs, congenital anomalies of heart and some dysmorphic features. This series demonstrates that triploidy may be discovered in the 2<sup>nd</sup> trimester of pregnancy and has a heterogeneous aspect at ultrasound scan, which can generate diagnostic difficulties. Therefore, the detection by ultrasound scan, at 18-22 weeks of pregnancy, of complex foetal morphological abnormalities should be an important reason for amniocentesis to search chromosomal anomalies.

**Keywords:** triploidy, chromosomal analysis, prenatal diagnosis, foetal ultrasound scan, congenital anomalies.

#### Abstract

Triploidia este o anomalie cromosomică numerică, caracterizată prin prezența a trei seturi haploide de cromosomi. Incidența triploidiei este dificil de evaluat, deoarece de obicei produce avort spontan în primul trimestru de sarcină. Incidența estimată a triploidiei la 20 de săptămâni de gestație este de 1/250.000 sarcini. În acest articol

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*prezentăm patru cazuri de triploidie diagnosticate în perioada 2003-2013 în Departamentul de Diagnostic Prenatal al Maternității "Cuza Vodă" Iași, România, toate fiind identificate pe parcursul anului 2013. Analiza cazurilor patologice identificate în ultimii 11 ani prin diagnostic prenatal a arătat că triploidia reprezintă doar 5,7% din anomaliile cromosomice numerice, dar raportat la anul 2013 cele patru cazuri de triploidie reprezintă 36% din anomaliile cromosomice numerice. În toate cazurile analiza cromosomică a fost efectuată după descoperirea prin ecografie fetală a diverse anomalii congenitale. În toate cazurile a fost identificată o întârziere de creștere intrauterină, dar nu au fost depistate modificări placentare. De asemenea, au fost găsite anomalii ale membrelor, anomalii congenitale de cord și unele aspecte dismorfice. Această serie de cazuri demonstrează faptul că triploidia poate evolua până în al doilea trimestru de sarcină și că aspectele ecografice sunt eterogene, ceea ce generează unele dificultăți de diagnostic. De aceea, identificarea la 18-22 de săptămâni de sarcină a unui complex de anomalii morfologice fetale trebuie să reprezinte un motiv important pentru efectuarea amniocentezei și a analizei cromosomice.*

**Cuvinte cheie:** triploidie, analiză cromosomică, diagnostic prenatal, ecografie fetală, anomalii congenitale.

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## Introduction

Numeric chromosomal anomalies – aneuploidy and polyploidy – represent a frequent pathology during pregnancy, as an estimated 25% of embryos at conception have this type of anomaly. The majority of these embryos are spontaneously aborted during the first trimester of pregnancy (numeric chromosomal anomalies cause 50-60% of miscarriages in the first trimester of pregnancy). Triploidy, characterized by the presence of three haploid sets of chromosomes, is a severe numeric chromosomal anomaly associated with negative prognosis. Thus, prenatal cytogenetic studies indicate that triploidy has a prevalence of 1:3500 at 12 weeks of pregnancy and only 1:30,000 at 16 weeks of pregnancy (1). It is estimated that >99% of triploidies are spontaneously aborted during the 1st trimester (2), while the discovery of triploidies during 2<sup>nd</sup> and 3<sup>rd</sup> is a rare event (3).

The aim of this study is to identify if there are pathognomonic signs on ultrasound scans in the 2<sup>nd</sup> trimester, and if the ultrasound could bring information discriminating between the type of triploidy (paternal or maternal).

## Material and method

We report a series of 4 cases of triploidy discovered prenatally during an eleven-year period

in obstetrical clinics from Iasi, Romania and we consider that as a peculiar event (the cytogenetic laboratory of Prenatal Department identified only 5 cases of triploidy in the last 11 years, and 4 of them were discovered in a one year period). All pregnant women agreed to participate in the study and signed an informed consent (approved by the Bioethics Commission of Maternity "Cuza Vodă" Iași).

The majority of analyses were performed after amniocentesis and only few analyses followed a chorionic villus sampling. In first years the cytogenetic analysis was made using FISH (Fluorescence In Situ Hybridization), while the complete chromosomal analysis was introduced only in the last three years. In all cases of our 4 case series both chromosomal analyses were performed.

For the FISH method, the fluid was centrifuged, and the cells of the supernatant were hybridized with Aneuvision® probes for prenatal diagnosis. We used centromeric probes (CEP) CEP 18, CEP X, and CEP Y for chromosomes 18, X, and Y, and locus specific (LSI) probes LSI 13 and LSI 21 for chromosomes 13 and 21, respectively. The analysis of hybridization was made with a Zeiss Axiomot 2 epifluorescent microscope, and the images were processed with Isis software. For each case, we analyzed a minimum of 100 cells. In all cases, the analysis

with the epifluorescent microscope showed three blue signals (corresponding to chromosome 18), three red signals (corresponding to chromosome 21), and three green signals (corresponding to chromosome 13). In cases with 69,XXY chromosomal formula we identified two green signals (corresponding to the X chromosome), and one red signal (corresponding to the Y chromosome) (figure 1). In cases with 69,XXX chromosomal formula we identified three green signals (corresponding to the X chromosome) and the absence of red signal (corresponding to the Y chromosome).

The embryonic cells were harvested in vitro using AmnioMAX® medium (Gibco) for 14 days. After this period, we added colcemid to block the mitotic activity, applied a treatment with trypsin, hypotonised with KCl (0.56 M), fixed with methanol and acetic acid (3:1/v:v), prepared the microscopic slide, and finally, analyzed the chromosomes by conventional method using G banding. The metaphases were analyzed using a Zeiss Axiomot 2 microscope with direct lighting. For each case, 64 cells were analyzed, and 12 cells were karyotyped. The karyotype indicated a 69,XXX chromosomal formula (figure

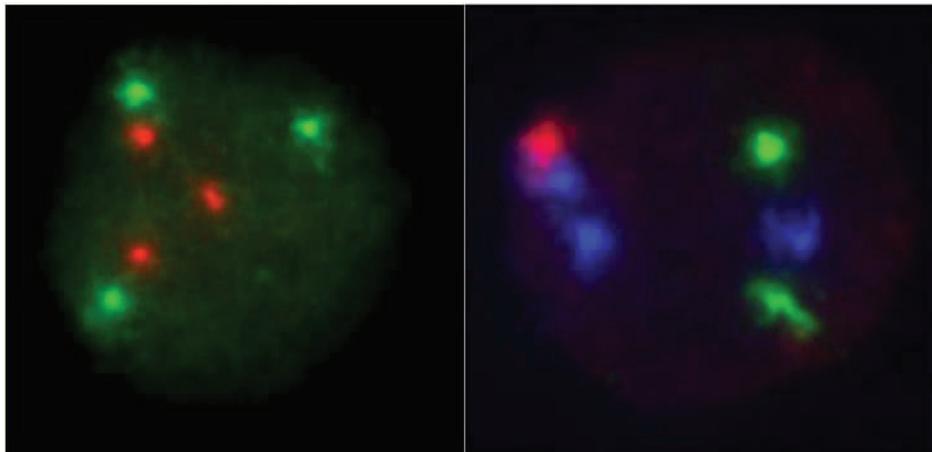
2) in two cases and a 69,XXY chromosomal formula in the other two cases.

According to the Cytogenetic Laboratory statistics over 11 years, there were 5 cases of triploidy in this period. We selected four cases based on the results of karyotyping and the availability of ultrasound data. The other case was excluded because of the lack of detailed ultrasound information.

## Results

### *Case no 1.*

It is a 33 year-old patient with a pregnancy of 18 weeks in which the ultrasound described intrauterine growth retardation, corpus callosum hypoplasia, bilateral cerebral ventriculomegaly, mandibular hypoplasia, big vessels anomaly with interventricular septum defect but without placental changes. The karyotype showed a 69,XXY chromosomal formula. Because the chromosomal anomaly was incompatible with survival, the parental couple decided to interrupt the pregnancy. The clinical examination at necropsy indicated: low implanted ears, dolicocephaly, retrognathia, right tetradactyly and bilateral syndactyly at upper limbs and bilateral syndac-



**Figure 1. FISH features in a case of triploidy 69,XXY. a – three LSI signals for chromosome 21 (red) and chromosome 13 (green); b – CEP signals for chromosome 18 (blue) X (green) and Y (red)**

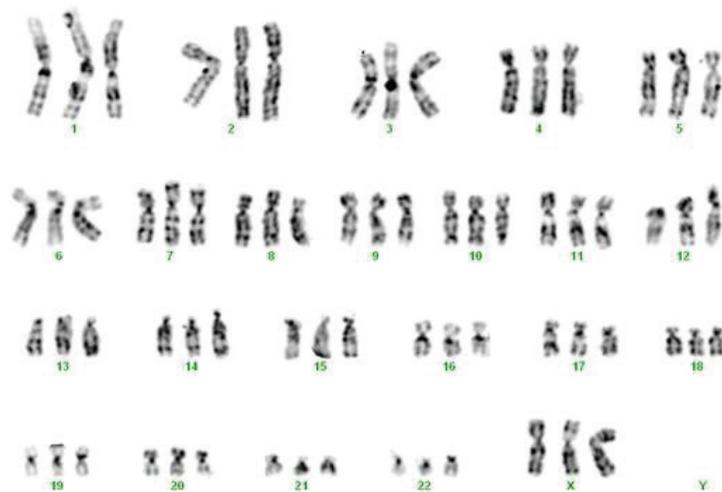


Figure 2. Karyotype 69,XXX (G banding technique; 200 bands resolution)

tyly in lower limbs. The examination of internal organs indicated: hypoplasia of the *corpus callosum*, agenesis of the thymus, and interventricular septum defect (figure 3b).

#### Case no 2.

It is a 23 year-old women with a 21 weeks pregnancy, where morphological ultrasound scan showed: IUGR, macrophthalmia, left ventricle double outlet, left lung agenesis, single umbilical artery, congenital varus equinus, and oligoamnios, but with absence of placental modifications. Amniocentesis was performed and the karyotype was 69,XXY. The necroptic examination confirmed the morphological anomalies identified by ultrasound scan.

#### Case no. 3.

Case 3 is a 21 year-old patient at 22 weeks of amenorrhea, presenting at ultrasound scan only oligoamnios and common arterial duct. Amniocentesis was performed and the karyotype was 69,XXX. The necroptic examination showed: cranio-facial asymmetry, low-implanted ears, bilateral syndactily in upper limbs, and common arterial duct.

#### Case no 4.

It is a 25 year-old patient, at 19 weeks of amenorrhea, where ultrasound indicated the presence of: IUGR, complex cardiac abnormality (interventricular septum (IVS) defect and transposition of great vessels), cheilopalatoschi-



Figure 3. The main ecographic features in cases with triploidy  
a. cheilopalatoschisis; b. hypoplasia of the mandible and *corpus callosum*; c. left ventricle double outlet

sis (figure 3a). The placenta was normal. The amniocentesis was performed and the karyotype was 69,XXX. Pathological examination confirmed the ultrasound description.

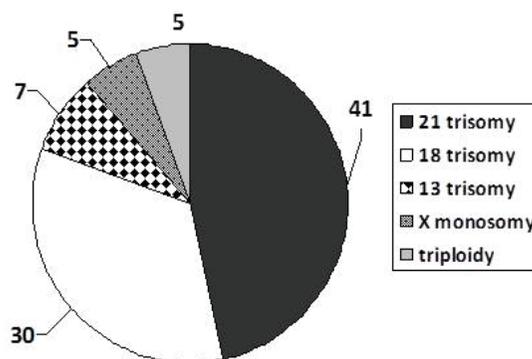
The anomalies described in our cases are summarised in table I. The main ultrasound features found in our cases are presented in figure 3.

Reviewing the data from the cytogenetic laboratory in the last 11 years, we could conclude the following:

We made 1381 cytogenetic prenatal exams during the period 2003-2013

We identified 88 cases of numerical chromosomal anomalies: 5 cases of triploidy, 41 cases of 21 trisomy, 30 cases of 18 trisomy, 7 cases of 13 trisomy, and 5 cases of X monosomy (figure 4).

During 2013 we made 255 cytogenetic exams and we identified 11 numerical chromosomal anomalies: 4 cases with triploidy, 3 cases with 21 trisomy, 3 cases with 18 trisomy and one case with X monosomy.



**Figure 4. The numerical chromosomal anomalies identified in Prenatal diagnosis department of Maternity “Cuza-Vodă” Iași between 2003 and 2013**

**Discussions**

Triploidy is a numerical chromosomal anomaly, characterized by presence of three haploid set of chromosomes. The extra chromosomal set could have maternal (digyny) or paternal (diandry or dispermy) origin (4). This anomaly is relatively frequent in the first steps of ontogene-

**Table I. Review of main features in our cases**

Parameter	Case 1	Case 2	Case 3	Case 4
Age of patient	33	23	21	25
Chronological gestational age	18	21	22	19
Biometrical gestational age	15	17	18	16
Cephalic	Hypoplasia of corpus callosum, retrognathia, Low implanted ears	Macrophthalmia	Low implanted ears	cheilopalatoschisis
Limbs	Tetradactily, syndactily	Varus equinus	Bilateral syndactily	-
Heart	IVS defect	Double ventricle outlet	Common arterial duct	IVS defect, transposition of great vessels
Placenta	Normal	Normal	Normal	normal
Other	Thymus agenesis	oligoamnios	oligoamnios	-
Karyotype	69,XXY	69,XXY	69,XXX	69,XXX

sis, with an estimate frequency of 4.5-12.4% at conception (5). The majority of cases are spontaneously aborted, thus the frequency of triploidy in miscarriages is estimated at 6-15% (4, 6). In newborns, the prevalence of triploidy was estimated at 1.26/10,000 (7). However, the survival of triploid babies is very short, with less than 10 cases that passed the age of 45 days (8).

Usually, the discovery of a numerical chromosomal anomaly during the prenatal period is the result of a prenatal diagnosis after identification of different congenital anomalies by ultrasound exam for foetal morphology. This ultrasound exam is usually performed at 18-22 weeks of pregnancy. This late discovery of chromosomal anomalies is correlated with many factors like low accuracy of biochemical screening or the relative high risk associated with chorionic villus sampling and amniocentesis. For some aneuploidies (trisomies 21, 13, 18) there are biochemical markers able to establish a high risk and therefore to recommend genetic investigations. In triploidies a specific association of biochemical markers is absent. In some cases a biochemical association similar those of trisomy 21 (low alpha-fetoprotein, low unconjugated estriol and high human Chorionic Gonadotrophin (hCG)) was found, while in other cases values characteristic for trisomy 18 (normal alpha-fetoprotein, low unconjugated estriol and normal hCG) were found. Thus, the best indicator for the presence of triploidy is the identification by ultrasound of some congenital anomalies (9, 10).

Our results were similar with the data in other studies. Thus, we found 5 cases with triploidies in 1381 cytogenetic exams (0.36% samples), similar with the frequency found by Wapner et al. (11): 17 triploidies in 4282 samples (0.39%).

The phenotype in triploidy is heterogeneous and depends on the origin of the supplementary set of chromosomes. Thus, one "paternal" and one "maternal" triploid phenotype could be defined. The paternal triploidies could be the result

of a diandry (fertilisation of a normal oocyte by a diploid sperm cell) or a dispermy (concomitant fertilisation of a normal oocyte by two normal sperm cells). In this case, the phenotype is characterized by slight IUGR, and hypertrophic cystic placenta with partial hydatiform mola. The maternal triploidy is the result of digyny (fertilisation of a diploid oocyte by a normal sperm cell) and is characterized by severe asymmetric IUGR, with macrocephaly associated with hypoplasia of the other corporeal segments (4, 12-16). Other anomalies are common to the two forms of triploidy: anomalies of fingers (oligodactily and syndactily of fingers III and IV) different non-pathognomonic heart anomalies, genitourinary defects, lung anomalies and a non-specific facial dysmorphism (4, 12-17).

In our cases, we did not find major differences between cases with 69,XXY and those with 69,XXX chromosomal formula. In all cases we found important intrauterine growth retardation and no changes in the placenta that could be an indirect argument for maternal origin of triploidy. Also, we found different cardiac anomalies and non-specific dysmorphic features in all cases. Other particular aspect could be considered the presence of finger anomalies in two of our cases.

Starting from our clinical data we found several interesting aspects. The fact that all four cases were identified in only one year brings up the question of the real incidence of triploidies, as no explanation is available for specific risks related to triploidy, and the incidence of other aneuploidies is unchanged during the same period. Heterogeneity of ultrasound features could create difficulties in differential diagnosis, and no specific ultrasound markers could be described, excepting maybe intrauterine growth retardation. The identification of "classical" phenotypes (maternal, paternal) is not possible in ultrasound examination or even in necroptic examination. The positive diagnostic is done by chromosomal

analysis, as newer technologies (genomic hybridization, cell-free foetal DNA testing) could not detect triploidy (18, 19).

Our case series is small, although comparable to the literature on the same topic (3). The weakness comes from the unusual presentation of our cases in the 2<sup>nd</sup> trimester, while the majority of triploidies end in first trimester miscarriages. Regarding the ultrasound scan, we could not identify any specific sign for triploidy, although some common features (intrauterine growth retardation, for example) were mentioned. This could also be related to the number of cases.

## Conclusions

We conclude that even if triploidy is a rare event in prenatal diagnosis, more reports are necessary for a better description of the phenotypes that could ameliorate the detection rate on ultrasound scanning. Also, it is important for all specialists that perform morphological examinations at 18-22 weeks to search for some features we found in all our cases, especially intrauterine growth retardation and cardiovascular anomalies that could be associated with triploidy. We would therefore remark that in all cases with foetal anomalies amniocentesis is required, followed by prenatal chromosomal analysis.

No specific sign to identify the type of triploidy (maternal/paternal) has been found. This, although it is described in literature, was not confirmed by our case series, but larger studies are needed to verify this.

## List of abbreviations

IUGR – Intrauterine growth retardation  
 IVS – interventricular septum  
 FISH –fluorescence in situ hybridization  
 CEP – centromeric probe  
 LSI – locus specific probe  
 hCG – human chorionic gonadotrophin

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