PRENATAL DIAGNOSIS OF GONOSOMAL ANOMALIES: LIMITATIONS OF THE FISH METHOD AND GENETIC COUNSELING DIFFICULTIES IN 15 CASES

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(Abstract): Prenatal diagnosis (PD) by FISH or cell culture is today an important tool for the prevention of chromosomal anomalies. A difficult issue is prenatal detection of gonosomal anomalies. Most gonosomal anomalies neither affect life expectancy nor cause psychomotor retardation, but sexualization disorders and the lack of reproductive potential are a constant finding. **Aim:** This study aimed at identifying the medical problems the specialists and the parental couple are faced with at the time of the diagnosis of fetal gonosomal anomalies. **Material and methods:** This retrospective study (2004-2012) was conducted in the Prenatal Genetic Diagnosis Department of “CuzaVoda” Maternity by FISH technique in 1685 pregnancies. The AneuVysion probes were used for identifying and enumerating chromosomes 13, 18, 21, X, and Y via fluorescence in situ hybridization (FISH) in interphase nuclei obtained from amniotic fluid. **Results:** Fifteen fetuses were selected in which we were faced with difficulties interpreting the number of gonosomes: monosomy X (5 cases), pseudomosaicism XX/XY (3), trisomy XXY (3 cases), trisomy XYY (1 case), 45,X/46,XX mosaicism (1 case) and triploidy XXX (2 cases). Later, by repeating the analysis, 2 cases with pseudomosaicism XX/XY were excluded. A case highlighting the limitations of the FISH test was that of a fetus in which the FISH test revealed trisomy XXY, while postnatal karyotyping showed a six cell line mosaicism (marker and ring X chromosomes). **Conclusions:** All parental couples received nondirective genetic counseling, respecting the individuals’ dignity and rights of self-determination. Parents received information on the natural course of the disease, treatment options, and psychological support and were involved in their child's recovery. **Keywords:** PRENATAL DIAGNOSIS, FISH, MONOSOMY X, TRISOMY XXY, TRISOMY XYY.

Chromosomal diseases, with an incidence of approximately 1% newborns, high severity, chronic and disabling course, can be prevented by prenatal cytogenetic diag-
Prenatal diagnosis of gonosomal anomalies: limitations of the fish method and genetic counseling

nosis. It uses minimally invasive, expensive, laborious techniques of embryonic or fetal cells study, requiring a rigorous selection of cases. The indications for the prenatal diagnosis of chromosomal abnormalities are: visceral anomalies at ultrasound examination, advanced maternal age, positive biochemical screening (abnormal double or triple test), family history of chromosomal disease, and a balanced structural chromosomal abnormality in a parent (1). Fetal cells are obtained by chorionic villi biopsy, amniocentesis or cordocentesis. Classical prenatal chromosome analysis requires in vitro culture of fetal cells with a waiting period of 2-3 weeks. An alternative method is the fluorescent in situ hybridization (FISH), based on the hydrogen bonds formed between the DNA of the analyzed chromosome (target DNA) and fluorescently labeled DNA probe (2). Sex chromosome abnormalities are common genetic diseases, affecting 1/400 - 1/500 live births. Increased frequency is correlated with minimal phenotypic changes. Gonosomal abnormalities detected by prenatal diagnosis create difficulties in genetic counseling related to the impossibility of estimating the severity of clinical features, with the decision-making process becoming a complex medical act (3). Prenatal medicine has brought great benefits related to the reproductive choice knowing the particular circumstances, but also generated ethical controversies. Prenatal diagnosis of chromosomal disease puts the prospective parents in a position to choose between recognizing the severity of the genetic disease, understanding and accepting the chronic illness without a pathogen-

ic treatment, or terminating the pregnancy by therapeutic abortion. Severe chromosomal disease (trisomy 13, trisomy 18 or triploidy) is not marked by these dilemmas, because keeping the pregnancy is not put into question. Instead, gonosomal anomalies allow in most cases a fairly normal life. Thus, for the gonosomal anomalies detected prenatal, the genetic counseling must be completed by appropriate support, helping the parents to deal with psychological problems.

The aim of our study was to detect the diagnostic difficulties of gonosomal abnormalities by FISH method in prenatal diagnosis and to identify the genetic counseling issues and bioethical dilemmas in parental couples who have a child with a sex chromosome abnormality.

MATERIAL AND METHODS
This 9-year retrospective study (2004–2012) targeted the gonosomal anomalies identified in the Department of Prenatal Diagnosis of the Iasi "CuzaVoda" Maternity Hospital. Prenatal diagnosis by FISH method in interphase nuclei obtained from amniotic fluid was performed in 1685 pregnant women. Amniocentesis was performed between 16 and 27 weeks of amenorrhea (WA). FISH method used fluorescently labeled DNA probes specific for chromosomes 13, 18, 21, X and Y (AneuVysion Multicolor DNA Probe Kit Vysis CEP 18/X/Y - alpha satellite / LSI 13/21). In 15 cases gonosomal abnormalities were detected or there were difficulties in interpreting the number of fluorescent signals corresponding to the two sex chromosomes: X and Y (tab. I).
TABLE I
Particularities of the study group

<table>
<thead>
<tr>
<th>No.</th>
<th>Patient</th>
<th>Analysis motivation</th>
<th>Gestational age (GA)</th>
<th>Results</th>
<th>Pregnancy outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FE</td>
<td>child with DS</td>
<td>20</td>
<td>46,XX/46,XY denied by repeat test</td>
<td>healthy newborn 46,XY</td>
</tr>
<tr>
<td>2</td>
<td>TI</td>
<td>CPC</td>
<td>24</td>
<td>46,XX/46,XY denied by repeat test</td>
<td>healthy newborn 46,XY</td>
</tr>
<tr>
<td>3</td>
<td>BN</td>
<td>CH, FA</td>
<td>17</td>
<td>45,X</td>
<td>TA</td>
</tr>
<tr>
<td>4</td>
<td>LM</td>
<td>ATT</td>
<td>17</td>
<td>45,X</td>
<td>newborn ♂ ST</td>
</tr>
<tr>
<td>5</td>
<td>GV</td>
<td>HC</td>
<td>16</td>
<td>45,X</td>
<td>TA</td>
</tr>
<tr>
<td>6</td>
<td>GI</td>
<td>ATT</td>
<td>18</td>
<td>47,XXY</td>
<td>newborn ♂ clinically normal</td>
</tr>
<tr>
<td>7</td>
<td>DT</td>
<td>CPC</td>
<td>26</td>
<td>47,XXY</td>
<td>newborn ♂ abnormal, CCM</td>
</tr>
<tr>
<td>8</td>
<td>JI</td>
<td>ADT</td>
<td>17</td>
<td>45,X</td>
<td>SA, fetus with CH</td>
</tr>
<tr>
<td>9</td>
<td>BI</td>
<td>ATT</td>
<td>16</td>
<td>47,XXY</td>
<td>newborn ♂ without KS features</td>
</tr>
<tr>
<td>10</td>
<td>VG</td>
<td>ATT</td>
<td>19</td>
<td>46,XX/46,XY</td>
<td>SA, fetus with IC</td>
</tr>
<tr>
<td>11</td>
<td>CG</td>
<td>ADT</td>
<td>18</td>
<td>69,XX</td>
<td>TA fetal malformations</td>
</tr>
<tr>
<td>12</td>
<td>SE</td>
<td>DH, OH, SUA</td>
<td>27</td>
<td>69,XX</td>
<td>TA fetal malformations</td>
</tr>
<tr>
<td>13</td>
<td>AE</td>
<td>ADT</td>
<td>18</td>
<td>45,XX/46,XX</td>
<td>SA</td>
</tr>
<tr>
<td>14</td>
<td>RŞ</td>
<td>VSD, ASCA</td>
<td>22</td>
<td>45,X</td>
<td>SA</td>
</tr>
<tr>
<td>15</td>
<td>AG</td>
<td>ATT, AMA</td>
<td>18</td>
<td>47,XXY</td>
<td>newborn ♂ without KS features</td>
</tr>
</tbody>
</table>


RESULTS
Six of our cases showed X monosomy. Five were homogeneous forms (single fluorescent gonosomal signal corresponding to chromosome X) and in the sixth case we found a 45,X/46,XX mosaicism (presence of only one gonosomal fluorescent signal corresponding to X chromosome in 60% of the cells). The evolution of pregnancies was the following: one ended with the birth of a baby girl with Turner syndrome (monosomy X confirmed by postnatal chromosome analysis), three ended in miscarriage (one with cystic hygroma), and two ended in therapeutic abortion due to cystic hygroma. In three cases we found both cells with two fluorescent signals corresponding to X chromosomes and cells with a signal...
for chromosome X and a signal for Y chromosome. Pseudomosaicism 46,XX/46, XY was not confirmed by repeat analysis in two cases, proving that the first amniotic fluid samples were contaminated with maternal blood. Both pregnancies ended in normal male babies. In the last case, repeat analysis confirmed the 46,XX/46,XY mosaicism, indicating a true hermaphroditism. The intersex stated was confirmed by anatomopathological examination, the pregnancy ending in miscarriage. In three cases we found a trisomy XXY, the FISH test showing two green signals corresponding to X chromosome and a red signal for Y chromosome. Two pregnancies ended in two baby boys, postnatal chromosome analysis confirming the trisomy XXY. In another case we were faced with one of the limitations of FISH technique in prenatal diagnosis. Thus, although in most fetal cells we detected three fluorescent signals corresponding to gonosomes, we also identified cells with extra small green fluorescent signals. Due to the small number, these cells were interpreted as artefacts. Although the fetus presented choroid plexus cysts, there were no reasons for therapeutic abortion and pregnancy ended with the birth of a baby boy weighing 2750 g and measuring 47 cm in height.

Clinical examination showed microcephaly with scaphocephaly, hypertelorism, epicantus, bilateral cryptorchidism and hypotonia. Postnatal chromosomal analysis revealed mosaicism with 6 cell lines:

- 48,XY,+mar,+mar/ 47,XY,+mar/48,XY,r(X),+mar/
- 49,XY,r(X),+mar,+mar/ 46,XY/50,XY, r(X),r(X),+mar,+mar

The ratio of the six cell lines was: [29]/[14]/[10]/[3]/[3]/[3]. In one case we found a trisomy XYY (a single green signal for chromosome X and two red signals for chromosome Y), the pregnancy ending with the birth of a clinically normal baby boy. Postnatal chromosome analysis disclosed the 47,XYY formula. In two cases we identified three green signals corresponding to X chromosome, but also three fluorescent signals for chromosomes 13, 18 and 21, proving a triploidy: 69,XXX. Both pregnancies were terminated, and the anatomopathological examination revealed fetuses with multiple congenital anomalies and severe intrauterine growth retardation.

**DISCUSSION**

Globally, gonosomal abnormalities have a high incidence, being the most common chromosomal pathology, affecting 1/500 live births. The most common abnormalities are monosomy X, trisomy X, trisomy XXY and trisomy XYY (4, 5).

Monosomy X was the most common gonosomal abnormality in our group. It affects 1/2500 women and causes Turner syndrome, characterized by short stature, primary amenorrhea, infertility and normal intellectual development in most cases. Although phenotypic changes are not very severe, 90% of fetuses with cystic hygroma and monosomy X shows fetal anasarca, causing spontaneous abortion (10% of spontaneous abortions in the first trimester) (4, 6). The prenatal detection of monosomy X is usually accidental and, due to good integration into society, it is not an indication for therapeutic abortion, except for the cases with cystic hygroma (7). In one of our cases the pregnancy had a normal evolution; the girl has a favorable outcome and benefits from growth hormone therapy.

Trisomy XYY affects 1/1000 boys and is a disease diagnosed fortuitously, because unless high stature and possibly hypo fertil-
ity are present, no other particular clinical signs are present (4, 8). In our case the family decided to keep the pregnancy, which was ended by the birth of a clinically normal baby boy.

Trisomy XXY causes Klinefelter syndrome, the most common cause of male chromosomal infertility. The disease affects 1/1000 boys and is characterized by hypogonadotropichypogonadism, but close to normal intellectual development. Two of the trisomy XXY cases detected by us were homogeneous, and at the time of birth the children showed no clinical manifestations. In the third case we identified a complex mosaicism with six cell lines and additional X chromosome fragments (marker and ring X chromosomes). To diagnose such a case is impossible by using only the FISH technique, the anomaly being mosaic and the marker chromosomes representing X chromosome fragments that do not contain centromeric sequences (the region where the probe corresponding to the chromosome X is attached). Polysomy XY with more than two X chromosomes generates mental retardation and birth defects, features identified in our cases. Also, additional ring X chromosomes induce microcephaly, growth retardation, genital abnormalities and mental retardation (4, 9).

Triploidy, characterized by the presence of an extra haploid set of chromosomes, generates a severely altered phenotype, which usually induces miscarriage (4). In the cases of triploidy diagnosed by FISH technique are identified three fluorescent signals corresponding to the gonosomes, and three signals for chromosomes 13, 18 and 21. Both pregnancies with triploidy in our study showed three signals for X chromosome, and ended in miscarriages, the fetuses presenting multiple congenital anomalies.

In all cases there were some genetic counseling issues (related to the type of chromosomal abnormality) and difficulties in providing psychological support. Communication of an abnormal result in prenatal chromosome analysis is a difficult process, inducing various reactions from the parents: confusion, guilt, anxiety related to child’s development and integration into society, helplessness, rejection of the baby. In the first instance, most couples chose termination of a pregnancy with pathological gonosomal abnormalities, even if only minor development features were modified.

Reaction to diagnosis depends on culture, education, religion and economic status of the couple. Overcoming obstacles imposed discussions with parents so that they understood the significance of the diagnosis and took an informed decision about the pregnancy (10, 11, 12). Depending on the evolutionary potential, the pregnancies could be divided into two groups. One group included the cases with trisomy XXY, trisomy XYY and monosomy X without cystic hygroma, characterized by a positive development and satisfactory integration in society. In these cases, during genetic counseling it is explained to the family that there is no reason for therapeutic abortion (10, 11, 12, 13). This strategy was applied by us in our eight such cases. The other group included the cases of triploidy and monosomy X with cystic hygroma. In both situations, the pregnancy has a negative prognosis, ending in a miscarriage with the fetus having multiple congenital anomalies; pregnant women require exceptional attention, particularly when the pregnancy is "special", being obtained after several attempts (7). Thus, the severe progression of the disease should be explained,
with its negative repercussions on the reproductive potential of women, the best option being the therapeutic abortion. In fact, in our cases parental couples have opted for therapeutic abortion, the anatomopathological findings confirming the malformations. In the cases with 46,XX/46,XY pseudomosaicism there were genetic counseling difficulties in the first instance related to accepting the intersex status associated with true hermaphroditism, but repeat tests confirmed the contamination of the first amniotic fluid sample with maternal blood (14).

Another problem we were faced with is the legitimacy of abortion. In Europe there are countries that do not allow abortion (Malta, Ireland), countries that support therapeutic abortion when the fetus has non-viable birth defects or the pregnancy is dangerous for the mother (this approach is applied in Cyprus to 28 WA, 24 WA in Finland and United Kingdom and 22 WA in Spain) and countries that allow abortion on-demand (mostly to 12 WA, except Romania 14 WA, 18 WA in Sweden and 10 WA in Slovenia). In all therapeutic abortion cases, pre-and post-abortion counseling support is necessary (15).

CONCLUSIONS
Our study, aiming to detect gonosomal abnormalities by FISH technique, enabled us on the one hand, to highlight the limits of this type of diagnosis, and on the other hand, to reveal the genetic counseling difficulties. The main limitations are related to the impossibility of correctly quantifying the gonosomal mosaics and interpretation difficulties for some 46,XX/46,XY mosaicism types. Genetic counseling in prenatally detected gonosomal anomalies faces difficulty in convincing the couple that the fetus has a chromosomal abnormality with minor changes in phenotype that allows a normal integration in society, so that they can get over the psychological trauma induced by prenatal diagnosis and choose to maintain the pregnancy. On the other hand, when non-viable abnormalities or with a minimal development potential are identified, the possible negative consequences to maintaining the pregnancy should be objectively presented.

REFERENCES
CORTICOSTEROIDS IN TUBERCULOSIS THERAPY

Tuberculosis (TB) is a major public health problem which determines 1.4 million deaths in each year. The steroids as an associated treatment with anti-TB drugs are routinely used for reduce inflammation, only in certain types of TB. Professor Critchley and collaborators from St George's, University of London have made a systematic review and meta-analysis to estimate the steroids capacity for reduced mortality in all forms of tuberculosis. The researchers examined results from 41 previous trials on the efficacy of corticosteroids therapy in some forms of TB (pericarditis, meningitis, peritonitis, pleurisy and pulmonary TB also). Patient’s age, the comorbidty, the types, the doses and the duration of corticosteroids therapy have varied. The results show that steroids therapy has reduced mortality by 17%, regardless of the form of tuberculosis or type of anti-TB therapy. However, researchers say that further studies are necessary for the evaluation if the benefits of steroids therapy for all TB forms would outweigh the risk of side effects, especially for multidrug resistant TB cases (Critchley JA, Young F, Orton L. Corticosteroids for prevention of mortality in people with tuberculosis: a systematic review and meta-analysis. The Lancet Infectious Diseases, 2013; 13 (3): 223).