METAPHASE OBSERVED IN DIRECT AMNIOTIC FLUID ANALYSIS BY FISH; AN UNEXPECTED FINDING

DOMINIQUE BERUBE, ROSELLA MANZO, GUY ISABEL, SAMIR KHALIFE, ANNIE MICHAUD, ISAAC JACQUES KADOCH
OVO CLINIC (OVO PRENATAL, OVO LABO, OVO R&D), MONTREAL, QC, CANADA.

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ABSTRACT

To report an unexpected finding of XY metaphases in uncultured amniotic fluid leading to an unusual case of XY/XX mosaicism detected at amniocentesis following a positive prenatal screening. To detect chromosomal abnormalities, fluorescent in situ hybridization technique (FISH) was performed on uncultured amniocytes followed by conventional karyotype and FISH with X and Y probes on cultured amniocytes. FISH with 13 and 21 chromosome probes gave normal results . FISH with 18, X and Y probes showed two signals for X chromosome in 87% of cells and 11% of cells showed one signal for X chromosome and one signal for Y chromosome. Amniotic fluid cell culture gave an XX karyotype and FISH with X and Y probes on cultured cells showed two signals for chromosome X and no signal for chromosome Y. The patient delivered a healthy baby girl. The presence of metaphases in direct amniotic fluid analysis has never been described neither observed. The potential presence of XY cells in this analysis may be explained by an undetected degenerating dizygotic twin.

INTRODUCTION

Amniocentesis for chromosome analysis is routinely offered to women at risk for carrying a child with chromosome abnormalities. This technique is long but may be helped by FISH.

However, uncultured amniotic fluid cells have higher chance of maternal cells contamination and it is regularly observed in pregnancies with an anterior placenta. So, finding XY/XX cells in uncultured amniotic cells is not a rare event and most often results in maternal contamination of a XY sample. Other rarer events can explain the presence of two cell lines: chimerism and vanishing twin. That finding is always worrying because of the variable phenotypic spectrum of 46,XX/XY chimeric patient. It ranges from normal male or female genitalia to different degrees of ambiguous genitalia.

Presence of metaphases in uncultured amniotic cells has never been reported. Here, we describe a case of XY metaphase cells in uncultured amniotic cells

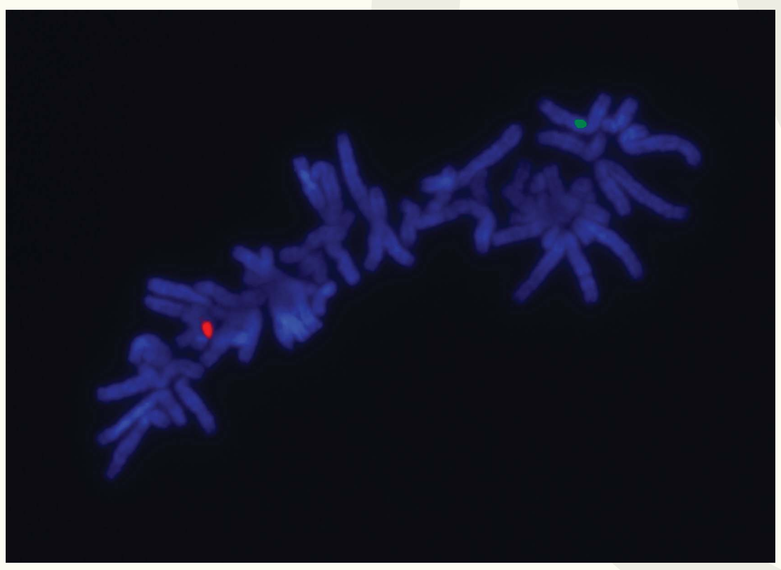
MATERIAL AND METHOD

A 32 years old woman, gravida 2 para 1, one previous female baby, has been referred at amniocentesis after a positive maternal screening of 1/50 for having a child with Down syndrome. PAPP-A and free bHCG are normal but nuchal translucency is 3.8 m (2.33 MoM). Amniocentesis has been done under ultrasound by an experimented clinician. The placenta is anterior. 20 ml of amniotic fluid have been dispatched in three tubes: two for cell culture and one for FISH.

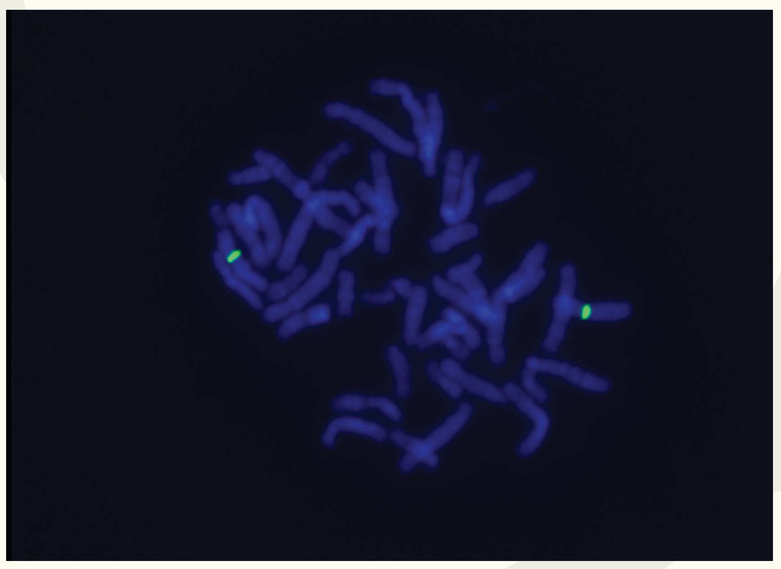
RESULTS

FISH on uncultured amniotic fluid gave 97-99% of cells showing 2 signals for chromosomes 13,18 and 21. 87% of cells showing 2 signals for X chromosome and - 11% of cells showed 1 signal for X and 1 signal for Y chromosome. From 11% of cells, 3% are metaphase cells (fig 1). On cultured amniotic fluid, 500 cells and metaphases have been analysed: 2 signals corresponding to X chromosome and no signal for Y chromosome have been observed (fig 2). Karyotype analysis confirmed female gender. Patient gave birth to a normal female baby as predicted by the complete chromosome analysis.

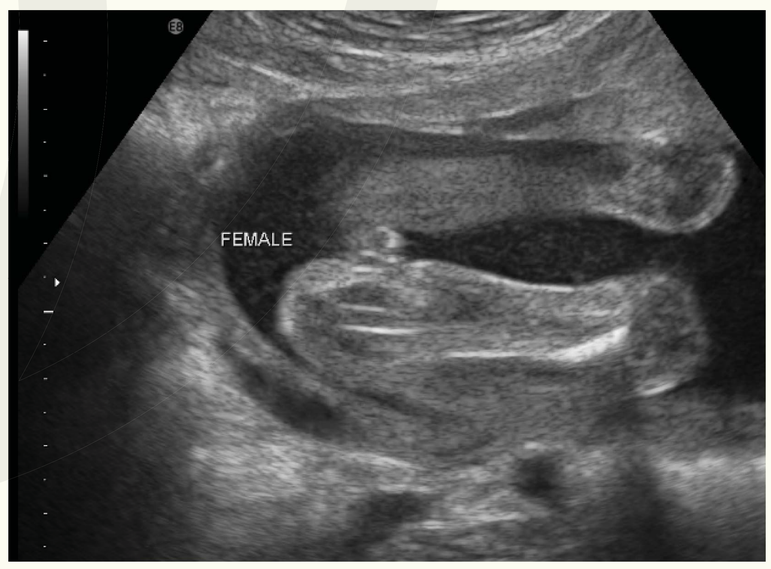
FIGURES



FISH on uncultured amniotic fluid with X (green) and Y (red) centromeric probes: Example of Metaphase chromosomes from direct amniotic fluid examination



FISH on cultured amniotic fluid with X (green) and Y(red) centromeric probes: Example of Metaphase chromosomes from cultured amniotic fluid examination



Ultrasonographic examination at 22 weeks:
- Normal female external genitalia

DISCUSSION

Presence of XX/XY cells in uncultured amniocytes can been explained by maternal contamination, by mosaicism (mitotic error in a single zygote) or by chimerism: true chimerism as true hermaphrodism or confined chimerism as confined blood chimerism (CBC) and confined placental chimerism (CPC). It seems that confined placental chimerism is more frequent than observed, may interfere with prenatal diagnosis with CVS but this discordance can extend to amniotic fluid where presence of XY/XX cells is explained as maternal contamination which may be wrong in some situation.

Finally, two different cell populations, XY/XX, may be the result of dissociated cells from a residual chorion frondosum belonging to a "vanished" dizygotic twin. At the start point, independently from where these cells come from, in each case, even if we want to be reassuring, there is always a risk or a doubt for something wrong and that needs more investigation, as ultrasounds examination for external genitalia, placental analysis and sometimes cord blood chromosome analysis. In fact, that leads to an anxious situation for patient and clinician because this can be a chromosome abnormality leading to a variable and unpredicted sexual phenotype of XY/XX.

In this analysis, it seemed evident that the finding of XY cells is not a maternal contamination: majority of XX cells, XY metaphases and female genitalia and finally, only XX cells on cultured amniotic cells. Cultured amniotic cells have a lower chance of maternal contamination because during culturing processus and finding XX cells in cultured amniotic cells give a good idea of the gender. However, this finding could be a true chimerism with female genitalia but this hypothesis can be eliminated because we found XY metaphases in direct amniotic fluid analysis not in cultured amniotic fluid analysis. There are only few tissues they are spontaneously dividing: bone marrow, gonadic tissues and throphoblastic cells. So, amniotic fluid is not one of them.

So the most probably explanation for the presence of metaphases in direct amniotic fluid examination comes from some viable residual trophoblastic cells from an unsuspected and undetected degenerating twin. The exact time of the disappearance of twin cannot be determined but it may be expected before 6-8 weeks as early sonography around 6-8 weeks of gestation can reveal the majority of vanishing twin as echolucencies suggestive as a degenerating twin sac or sometimes an embryonic demise. At 11 weeks of pregnancy the sac may no longer visible. This patient had her first ultrasound at 12 weeks of pregnancy, no echolucencies and no visible sac has been observed. Another point emphazing the time of demise is the normal maternal serum level of PAPP-A and βhCG. Spontaneous reduction in twin pregnancies, within 4 weeks of biochemical measurement, is associated with higher levels of PAPP-A and βhCG. Increased nuchal translucency of the viable fetus may be probably explained by the fact of a higher prevalence of increased nuchal translucency in dizygotic twins pregnancies compared to singleton pregnancies (5.4 % vs 5.2%).

We report, for the first time, the presence of XY metaphase cells in an uncultured amniotic fluid and we propose an undetected degenerative dizygotic twin for explaining this finding.

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