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Application of Interphase Fluorescence in Situ Hybridization (Fish) In the Study of the Spontaneous Abortions Products

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ABSTRACT

Spontaneous Abortion is defined as the loss of a fetus weighing less than 500 grams before 20 weeks gestation counted from the first day of the last menstrual period. It is one of the most common complications of pregnancy; it represents 10 to 15% of all recognized pregnancies. The genetic causes explain 2/3 of these pregnancies failures. Knowing the karyotype of the miscarried pregnancy frequently helps a patient grieve and pursue further treatment. Our study was based on the molecular cytogenetic analysis of 22 products of spontaneous abortions by application directly on chorionic Villi of a specific interphase FISH: The AneuVysion. This kit includes five different chromosomal probes capable of detecting most commonly aneuploidies found in products of miscarriages, which are: 13, 18, 21, X and Y. This analysis allowed us to overcome the vagaries of cell culture, that remains the main challenge of conventional cytogenetics, but also allowed us to have an accurate and reliable results in less than 24 hours.

Keywords: Aneuploidies, AneuVysion, Chorionic Villi, FISH, Spontaneous abortion

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INTRODUCTION

The genetic cause explains more than two thirds of Spontaneous Abortions. Chromosomal abnormalities, particularly autosomal aneuploidies represent alone 70% of the fetal losses of the first trimester [1]. Cytogenetic analysis of spontaneous abortions products provides valuable information on the frequency of chromosomal abnormalities in various ethnic groups, their etiology, the risk of recurrence and the suspicion of mutagenic activity in a population [2, 3, 4]. However, cytogenetic studies have been severely hampered by classic tissue culture, partly because of the many failures of cell culture material collected postmortem and often contaminated during removal), and secondly because of the existence of cryptic rearrangements undetectable by standard karyotype. The molecular cytogenetic techniques have demonstrated that the chromosomes can be effectively studied from chorionic villi obtained from spontaneous abortions products without using tissue culture. In our study, we tried to overcome the difficulties of conventional cytogenetic testing the value of a molecular technique namely the FISH on Interphase nuclei. It is well known that numerical chromosomal abnormalities are the most frequent genetic mutations associated with spontaneous abortions and fetal deaths in utero [5, 6, 7]. For this reason, we used the FISH kit Aneuvysion able to detect aneuploidies of chromosomes 13, 18, 21, X and Y, the most common aneuploidies in products of spontaneous abortions.

MATERIAL AND METHODS

Biological samples and tissue preparation

Our study was carried jointly by the laboratories of Biotoxicology of Djillali Liabes University -Sidi Bel Abbes- and Medical Cytogenetic laboratory of the University Hospital Estaing -Clermont-Ferrand-. It was conducted on 22 women whose ranging in age from 19 to 45 years. 22 products of spontaneous abortions with gestational ages ranged from 7th to 20th weeks were provided by the Maternity Hospital of Sidi Bel Hassani Abdelkader Abbèset the CHU Estaing and Gabriel Montpied Clermont-Ferrand. This study is based on direct analysis by FISH using the AneuVysion kit on interphase nuclei obtained directly from chorionic villi extracted from the different products of spontaneous abortions. At the Biotoxicology laboratory, the placental chorionic villi extracted from spontaneous abortions products were dissected and about 50 mg have been treated (incubated in hypotonic solution at 37°C for 20 min, then fixed three times in 3:2 ethanol/acetic acid at 4°C for 15 min each time). The cells suspensions were then spread on dry slides. After that, they have been sent for molecular cytogenetic analysis using FISH on interphase nuclei technique at the Medical cytogenetic laboratory of Clermont-Ferrand, France.

Molecular cytogenetic analysis: Interphase FISH

Fluorescence in situ hybridization (FISH) analysis was applied on uncultured chorionic villi as rapid screening technique for the main aneuploidies namely 21, 18, 13,X and Y involving in spontaneous abortions [5, 8]. For this purpose, we used the AneuVysion assay kit for rapid

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Prenatal Test (35-161075- 50 Assays; Vysis ISO 9001) Multi-color probe, with sets of DNA probes (CEP 18,X,Y alpha satellite, LSI 13and 21; Multi-color probe): Alpha-satellite probes of the centromeric regions (CEP probes) for chromosomes 18, X and Y, Locus specific probes (LSI probes to detect the 13q14 region and the 21q22.13 to 21q22.2 region) for chromosomes 13 and 21.

The slides preparation with cells suspension were pretreated with pepsin at 37°C for 15 min then rinsed in PBS for 5min, dehydrated in increasing ethanol baths (70, 90, 100%) 2 min each time and air dried. The AneuVysion probes were prepared as mentioned in the manufacturer instructions then add to the slides which were covered with glass slide and sealed with rubber cement. Slides were placed in ThermoHybaid (denaturation at 73°C for 2min followed by another hybridization at 37°C overnight), then counterstained with DAPI/Vectashield. Finally slides were observed under epifuorescence microscope (Axioplane 2 imaging), fitted with camera (CoolCube 1) and analyzed using Metafer (Metafer Metasystems).

RESULTS

A total of 22 cases of spontaneous abortions were analyzed using AneuVysion on uncultured chorionic villi, but 5/22 cases (22,7%) were excluded from the study because of the absence or insufficient chorionic villi tissue or insufficient interphase nuclei on slides. Molecular cytogenetic analysis was successfully performed on 17 (77,3%) cases with conclusive result within 24H. Aneuploidies were detected in 6/17 cases (35.3%) **(Table 1)** including three cases of trisomies 21 (Down syndrome) 50% (Fig.1.A), two cases of monosomies X (Turner syndrome) 33,33% (Fig.1.B) and one case of mosaicism (16,67%) (Fig.1.C). The remains cases (11/17) (64, 7%) showed a normal karyotype.

Table 1: The abnormal result of uncultured chorionic villi cytogenetic analysis by FISH

Cases	*Maternal age (years)*	*Gestational age (weeks)*	*Previous obstetric history*	*Katyotype (FISH)*
1	43	12	G6P5	47,XX,+21
2	27	18	G3P2	45,X0
3	45	11	G6P13MTOPt2	47,XX,+21
4	42	18	G1P0	47,XY,+21
5	40	16	G2P1	45,X0
6	35	20	G4P3	46,XX/47,XX,+21

G: gravid; P: para; M: Miscarriage; TOP: termination of pregnancy; t21: trisomy 21

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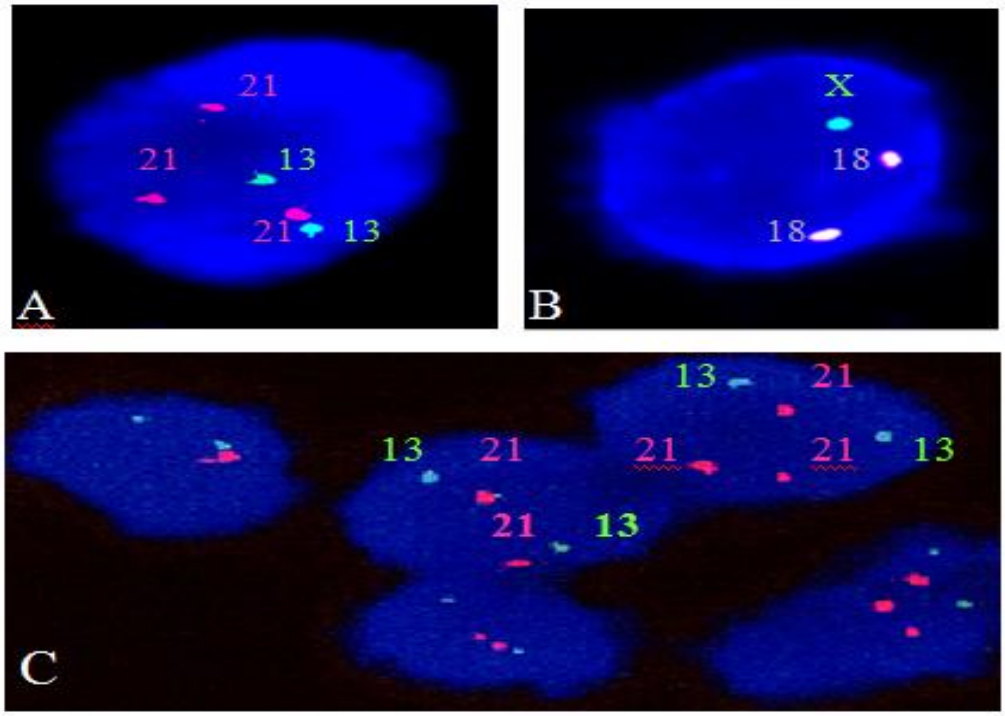

Fig.1 Interphase FISH on chorionic tissue obtained from missed abortion using CEP probes for chromosomes 18 (violet), X(green), Y(red) and LSI probes for chromosomes 13 (green), 21 (red). A: case 1 Interphase nuclei exhibiting trisomy 21(three red signals of chromosomes12).B: case 2 cell nuclei showing monosomy X (one X green signal). C: case 6 mosaicism form with two sets of cell nuclei 46,XX/47,XX,+21.

DISCUSSION

The cytogenetic analysis of aborted fetuses provides not only an important tool for reproductive and parental genetic counseling but is also essential to determine the possible etiological factors. It's well known that the numerical chromosomal aberration is the most common cytogenetic abnormalities found in spontaneous abortions [6, 7, 8].

In this study we tried to overcome difficulties of conventional cytogenetic, especially culture failure, by Fluorescent in situ hybridization (FISH) applied on interphase nuclei (uncultured chorionic villi) for rapid screening of the most frequent numerical abnormalities that could be at the origin of this spontaneous abortions using AneuVysion assay kit with sets of DNA probes for chromosomes 13, 18, 21, X and Y.

AneuVysion was successfully performed on 17/22 (77,33%) samples of curettage products outcome with a conclusive result within 24H. However 5/22 cases (22,7%) were excluded from the study since we did not get any interphases cells because of the absent or insufficient chorionic villi tissue. In our study, an abnormal karyotype was observed in 6/17 (**35,3%)** cases of spontaneous abortions: three cases of trisomy 21 (50%), two cases of monosomy X (33,33%) and one mosaic case (16,67%) **(Table 1).**This data was in agreement with results of earlier interphase FISH and molecular cytogenetic studies, which indicated that

approximately **30% to 40%** of spontaneous abortions are associated with chromosome abnormalities [6, 7, 8]. However, our data of chromosomal abnormality was much lower than the most result founded using conventional cytogenetic analysis **(50-60 %) [**2, 9, 10]. This rate may be low because we have not tested all chromosomes involved in spontaneous abortion, including chromosome 16 [7], in addition, the law number of cases studied (22 cases) limited our results. Moreover, this data supported the hypothesis raised by the previous researchers that the most fetuses with abnormal karyptype including autosomal trisomy, monosomy X and mosaic are spontaneously aborted in early gestational age [11].

The chromosomal abnormalities detected in our study were totally represented by numerical chromosomal defect with an extra or missing chromosome as result of non-disjunction during meiosis in aneuploidies cases. Furthermore the mosaicism form can be the result of post zygotic or mitotic errors [12, 13]. In agreement with literature our data revealed that aneuploidies namely autosomal trisomy and monosomy X were the most common abnormalities founded in the spontaneous aborted fetuses with a rate of 83,33 % , indeed trisomy 21(50%) was the predominant chromosomal aberration followed by monosmy X (33,33%) [5-8].

In the other hand, 11/17 (64,7%) cases was balanced for the chromosomes 13, 18, 21, X and Y **; t**his result suggest that this spontaneous abortions cases was not caused by these chromosomal abnormalities, but we cannot exclude definitely the genetic reason as origin of these pregnancies losses, since the chromosomal abnormalities are targeted, therefore it can be likely to find other abnormalities while using other DNA probes, or with other technical approaches dominating a more global vision of the whole genome.

In this serie, 80% of the aneuploidies cases detected by FISH corresponded to women with advanced maternal age (≥35 years old), actually the trisomy 21 cases founded 50% (n=3) was obviously associated with higher maternal age, suggesting that increased maternal age was the main predisposing factor (Table 1). In fact it is well known that the advanced maternal age increases significantly the risk of aneuploidies, mainly trisomies [6].

We have detected one case of trisomy 21 with already previous history of chromosomally abnormal miscarriage (TOP with trisomy 21), beside the advance maternal age (45 years), this recurrent aneuploidy (trisomy 21 in particular) increased risk of chromosomal abnormality in future pregnancy, which could be a result of gonadal mosaicism and required more investigation. The remains cases reported in this series were clearly in agreement with published hypothesis indicating that the spontaneous abortions associated with numerical chromosomal aberration are most likely a result of apparently sporadic segregation errors that increased significantly with increased maternal age [13].

In accordance with previously published data, at least 95 % of known monsomy X (Turner syndrome) fetus fail as embryo and appeared commonly in late miscarriage [14].

The cytogenetic analysis using AneuVysion assay kit had identified one mosaic case with two cell lines 46,XX/47,XX,+21 which could be result of maternal cell contamination, due to the fact that this specimen was an ovular fragment and was probably contaminated with maternal deciduas. Similar reports indicated that FISH is very efficient not only in the detection of mosaic form, but also so practical in identifying the existence of maternal cell contamination, a common problem during the conventional karyotype [5, 8].

CONCLUSION

The AneuVysion had afford us a very interesting alternative to cell culture, allowing us to perform tests with small quantities of material, not requiring living cells or sterile handling, a prerequisite for cell culture, sparing us the hazards of conventional cytogenetics. Indeed, and in many ways, FISH analysis was useful, offering the advantage of being accurate and reliable. This technique has a specificity and a sensitivity integrals, with relates to targeted screening for aneuploidy, but as it is the case for all other molecular techniques, it is recommended to combine with FISH another analytical method to confirm the results, but also to exclude the presence of other anomalies, because the major concern of this technique is that it can only detect targeted anomalies.

REFERENCES

[1] Rai R & Regan L. Recurrent miscarriage. Lancet 2006; 68:601–11.
[2] Hassold T, Chen N, Funkhouser J, Jooss T, Manuel B, Matsuura J, Matsuyama A, Wilson C, Yamane JA, Jacobs PA. Ann Hum Genet 1980; 44:151-164.
[3] Warburton D, Stein Z, Kline J, Susser M. Chromosome abnormalities in spontaneous abortion: data from the New York City study. In: Porter IH, Hook EB (eds) Human embryonic and fetal death. Academic Press, New York London 1980; 261-287.
[4] Boué A, Boué J, and Gropp A. Adv Hum Genet 1985; 14: 1–57.
[5] Dória S, Lima V, Carvalho B & *al*. J Assist Reprod Genet 2010; 27:657–662.
[6] Carvalho B, Doria S, Ramalho C & *al*. Eur J Obstet Gynecol Reprod Biol 2010.
[7] Azmanov DN, Milachich TV, Zaharieva BM & *al.* Eur J Obst Gynecol Reprod Biol 2007; 131: 127–131.
[8] Doria S, Carvalho F, Ramalho C & *al*. Eur J Obstet Gynecol Reprod Biol 2009.
[9] Dewald GW & Michels VV. Clin Obstet Gynecol 1986; 29: 865–885.
[10] Ford JH, Wilkin HZ, Thomas P. Aust N Z J Obstet Gynecol 1996; 36:314–8.
[11] Simpson JL. *Genetics.* CREOG Basic Science Monograph in Obstetrics and Gynecology. Council on Resident Education in Obstetrics and Fynecology, Washington, DC 1986.
[12] Frumkin T, Malcov M, Yaron Y & *al*. Mol Cell Endocrinol 2008; 282: 112–119.
[13] Robinson WP, McFadden DE, and Stephenson MD. Am J Hum Genet 2001; 69:1245–1254.
[14] Kelly TE, Ferguson JE, Golden W. Am J Med Genet 1992; 4 825-6.