Molecular analysis of Products of Conception (POC) by CGH ARRAYS

1.- Introduction. ¿What are CGH arrays?

CGH arrays allow the study of the 24 chromosomes to identify the presence of aneuploidies in fetal tissue from spontaneous miscarriages. Aneuploidies are the gain or loss of a copy of a chromosome that can result in spontaneous miscarriages or affected offspring. In ART treatments the presence of aneuploidies in the embryos can result in reproductive failure.

2.- Objective. When should be recommended?

In couples with spontaneous miscarriages it is crucial to know the origin of the gestational loss. Aneuploidy is one of the most important causes of spontaneous miscarriages. It is well-known that chromosomal abnormalities are present in more than 50% of first trimester fetal losses, in both spontaneous pregnancies and ART pregnancies (Martínez et al., 2010; Campos-Galindo et al., 2012).

For these reasons, the genetic analysis of fetal tissue from pregnancy losses is advised to investigate the etiology of the miscarriage and to counsel for the proper ART technique to improve reproductive outcome in a future pregnancy. One of the possibilities includes preimplantation genetic screening of the IVF embryos, before the transfer into the uterus.

Array CGH allow identifying chromosome copy number abnormalities for all 24 chromosomes in fetal tissue. However, after conventional curettage the possibility exist of contamination of the fetal tissue with maternal cells, with the risk of over-diagnosis for 46,XX fetuses. For this reason, a complementary analysis with polymorphic satellite markers if DNA extracted from maternal blood and fetus increases the accuracy of the test (Lathi et al., 2014).

3.- Indications. To whom?

Chromosomal analysis of fetal tissues would be indicated in any women suffering of a first trimester fetal loss. It is particularly relevant in couples with recurrent miscarriages and couples with a miscarriage after an ART cycle (Hodes-Wertz et al., 2012).

4.- Advantages. Strengths among other test?

Tissue culture is not required. Until recently, the classical cytogenetic study of fetal tissue was performed by G-bandning of chromosomes after tissue culture. However, with this approach growth failure rates could reach to 42% of the samples, with the consequence of inability to obtain results from these samples. Because of this, molecular approaches have been recently applied to the study of fetal tissue. Molecular techniques consist on DNA extraction and its direct analysis, without the need of tissue culture. With these techniques, such as array CGH, it is possible to estimate chromosome copy numbers without tissue culture and the risk of growth failure. Successful results in more than 98% of the analyzed samples. With array CGH plus the analysis to discard maternal cell contamination, an informative result can be obtained in close to 100% of the samples.

Analysis to rule out maternal cell contamination. A complementary study of polymorphic markers is performed in DNA extracted from maternal blood and from the fetal tissue in all samples in which a normal female result is obtained after array CGH analysis. The accuracy is increased and the risk of false negatives is considerable diminished with this complementary study. Maternal cell contamination can be present in more 33% of the samples.

5.- Sampling. How to collect the sample?

The fetal tissue sample can be collected in a sterile tube with a saline solution (≈ 10mL), by direct biopsy with hysteroembryoscopy. In cases of conventional curettage, the sample can be collected under similar conditions, but with special care to avoid bloody samples or collection maternal issue. For the complementary analysis it is important to include 5 mL of maternal blood collected in an EDTA tube.

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6.- Shipping. How, when, where, to whom?

The samples should be placed and transported in sterile tubes perfectly sealed. The samples can be shipped at room temperature and properly protected to avoid damaging during transport. The samples should be shipped in no more than one week after collection. Before shipping, the samples can be stored at 4°C at origin.

Delivery addres in Europe: IGENOMIX, Molecular Cytogenetics Laboratory, Parc Cientific Universitat de Valencia, C/ Catedrático Agustín Escardino, nº9, 46980 Paterna (Valencia), SPAIN.

7.- Sample processing and results reports. When?

The laboratory protocol requires between 24-48 hours, and results will be reported in one week time frame after sample reception at IGENOMIX.

8.- Methodology

The steps of the CGH array and maternal cell contamination protocol are as follows:

1) Genomic DNA extraction
2) Labelling of the simple DNA and DNA from female and male controls using Cy3 and Cy5 fluorophores.
3) DNA combination and precipitation
4) Hybridization in the CGH array platform (BlueGnome, Illumina)
5) Post-hybridization washing and scanning
6) Image analysis using specific software
7) Result interpretation and elaboration of the corresponding report

8) In all samples with a 46,XX result a complementary analysis is performed in the extracted DNA from maternal blood and fetal tissue. The analysis consists on the amplification of 16 polymorphic markers using the AmpFISTR Identifier Plus kit (Applied Biosystems) and the analysis in capillary electrophoresis. Genetic profiles from maternal and fetal DNA are compared to identify profile differences.
9.- Limitations of the technique

This technique does not allow detection of balanced structural abnormalities and unbalances of chromosome fragment size below 6Mb. Low mosaicism aneuploidies, triploidies/tetraploidies and uniparental disomies are not detected.

10.- How to start?

Contact to: info@igenomix.com

11.- FAQ’s

Can I collect the tissue in culture media?
Yes, it is possible to collect the tissue in any specific fetal tissue culture media, under sterile conditions.

Can I use a urine flask to collect the tissue?
Yes, it can be, but properly seal with parafilm and with the tissue completely immerged in the saline solution. A 10mL sterile tube is recommended, when possible.

Is it possible to perform the study in fetal tissue collected in a different manner?
Yes, if we can discriminate between fetal and maternal tissue. Assuming the high risk of maternal contamination and no-conclusive results is the tissue is not collected in proper conditions.

Is it possible to analyse fetal tissue from multiple pregnancies?
Yes, only when clinicians can collect the fetal tissue from the different fetus separately and placing them in different sterile tubes

REFERENCES:


- Ferro, Jaime; Martínez, Ma Carmen; Lara, Coral; Pellicer, Antonio; Remohí, José; Serra, Vicente. Improved accuracy of hysteroembryoscopic biopsies for karyotyping early missed abortions. Fertility and Sterility vol. 80 issue 5 November, 2003. p. 1260-1264

