

# REI Pearls: Pitfalls of Genetic Testing in Miscarriage



**The Skinny:** Genetic testing of miscarriage tissue is controversial and some people question if testing is helpful or not. This summary will: 1) outline the arguments for and against genetic testing; 2) describe the available genetic tests; and 3) discuss what we do with the information we obtain.

**To Test or Not To Test:** There is a lot of debate about whether women with miscarriage should have the products of conception tested for aneuploidy.

Opponents of testing say the tests are not useful because: 1) we already know that most miscarriages are due to genetic abnormalities, 2) a normal test does not exclude the possibility that an embryo was genetically abnormal, and 3) patients with recurrent pregnancy loss are less likely to have genetically abnormal embryos than people with a single, spontaneous loss.

Proponents of testing say that women may be reassured to learn that there was nothing they could have done to prevent the loss. Also, women who have recurrent losses of genetically normal embryos may be more likely to carry anti-phospholipid antibodies, and may benefit from repeated anti-phospholipid antibody testing or testing using a lab specializing in these assays. Finally, if a woman has recurrent losses and multiple embryos are found to be genetically abnormal, this person may benefit from IVF with preimplantation genetic screening.

## Available Genetic Testing of the products of conception:

**Karyotype:** The typical test on products of conception is a karyotype. Embryonic tissue is identified and cultured. Metaphase cells (cells that contain condensed chromosomes) are then examined with special stains to look for normal banding patterns.

**Advantages:** This test can accurately assess chromosome numbers, translocations, and some deletions and inversions. Karyotype testing is fairly inexpensive.

**Disadvantages:** Maternal contamination can be a problem. Endometrial cells may be more viable than embryonic tissue and may grow in culture better than embryonic tissue. Studies estimate that maternal contamination may affect up to 16% of karyotype analyses on products of conception. Therefore, when a test result reports 46XX, we don't know if this represents the genetic composition of the baby or maternal contamination. If the karyotype result shows a Y chromosome, then we know there was not maternal contamination. Karyotypes also do not detect single gene disorders, small gene mutations and small chromosome deletions, and do not tell you if an abnormality was of maternal or paternal origin.

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Bottom line: Karyotype is useful if it identifies an abnormal karyotype or a normal male karyotype because that is the only way you know for sure that the analysis reflects the genetic makeup of the fetus and that there was no maternal contamination.

**Karyotype with reflex to FISH:** This test is done identically to the karyotype test described above, but if the result is 46XX (normal female) additional testing is performed. FISH (fluorescent in-situ hybridization) is a technique which uses genetic probes, which attach to selected chromosomes, to assess for mosaicism, or aneuploidy not detected on the initial karyotype due to maternal contamination.

Advantages: FISH can be performed on a field of cells in culture, not just metaphase cells. So if a karyotype is normal (46XX) but the FISH study shows that some of the cells have a Y chromosome, then you know that the initial 46XX result was a misdiagnosis. Probes for other chromosomes can also be performed. These are usually done for chromosomes X, Y, 13, 18 and 21. If there are extra copies or missing copies of any of these chromosomes in the sample, then you know the baby was not genetically normal.

Disadvantages: The reflex testing typically involves additional probes for a set number of chromosomes, usually the chromosomes that are highly associated with miscarriage but can still be compatible with live birth (X, Y, 13, 18 and 21). The most common cause of loss is thought to be Turner's syndrome (45XO). However, there are other chromosome abnormalities which cause miscarriage but which are not tested for in the reflex FISH analyses. For example, some studies report Trisomy 16 (16 (47XX+16 or 47XY+16) as another common cause of miscarriage; chromosome 16 is not routinely tested in reflex FISH tests. In addition, due to the inherent error rate associated with any assay, the use of increasing numbers of FISH probes increases the assay error, thus leading to the possibility of misdiagnosis. Currently, the maximum number of probes used is typically 11; given that we have 23 pairs of chromosomes, this results in fewer than half of our chromosomes being tested for numerical abnormalities.

Bottom line: This test is an improvement from simple karyotype and has a better chance of detecting an abnormality. However, a 46XX result still does not exclude maternal contamination. If you use this testing, keep track of the percentage of male karyotypes and female karyotypes you obtain in your reports. If your results show mostly normal female karyotypes (XX), then there may something wrong with the method of analysis because nearly half of embryos should be XY.

**Microarray Chromosome Analysis:** Microarray technology allows for all chromosomes to be tested with a high degree of accuracy. The test samples sections of each chromosome to determine the total chromosome number. The results are

compared to the mother's genetics to ensure that the sample is indeed fetal tissue, and not maternal cell contamination.

Advantages: Microarray can reliably tell you if the sample is fetal in origin, or if it is purely maternal (contaminant). It can even determine if an abnormality is maternal or paternal in origin. It detects uniparental disomy (when a chromosome pair is from one parent), complete molar pregnancies (complete uniparental disomy), partial molar pregnancies, and certain deletions. Therefore a normal 46XX result can reliably be concluded as a genetically normal embryo. This can be extremely helpful in patient counseling.

Disadvantages: Testing requires maternal blood samples (or paternal blood samples if the loss occurred after an egg donation cycle, due to the lack of maternal genetic contribution to the embryo), to be sent with the specimen. Kits must be ordered independently by MD offices or by the hospital, and be taken to the operating room. Finally, the test may not detect all deletions or chromosomal rearrangements that can lead to miscarriage.

Bottom line: This is the best single test.

## How TRM uses these technologies:

### ***In patients without recurrent pregnancy loss:***

We don't routinely do any testing on these patients' products of conception. We counsel our patients that the vast majority of miscarriages are caused by a random abnormality with the embryo. A study published in Human Reproduction showed that most missed abortions were likely the result of a genetic abnormality, even when the total chromosome number was normal. In that study, embryoscopy was performed on missed abortions. Photographs of the embryos were taken and the embryo was sampled under direct visualization. In 91% of the cases, there was either a karyotype abnormality or the embryo was grossly malformed. Grossly malformed embryos could have mosaic abnormalities (meaning they have more than one cell line) and, as the embryo developed, some of the cells became abnormal and affected development. Grossly malformed embryos could also indicate a gene deletion or mutation.

If a woman with a single spontaneous loss wants genetic testing, and if we obtain abnormal results, this tends to provide comfort in knowing there was nothing she could have done to prevent the loss.

### ***In patients with recurrent pregnancy loss:***

In couple who have suffered repeated losses and had normal maternal and paternal karyotypes, embryo genetic testing can be helpful in a number of ways. We always counsel that normal parental karyotypes do not exclude genetic abnormalities as the

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cause of prior losses; the normal parental karyotype results simply eliminate the possibility of a parental translocation raising the risk of recurrent aneuploidy in offspring. Spontaneous aneuploidy events after fertilization are far more common than inherited translocation related abnormalities. Therefore, even with normal parental genetic testing, repeated embryo aneuploidy could be occurring.

Another study published last year in Human Reproduction evaluated the likelihood of fetal aneuploidy in more than 400 couples with recurrent pregnancy loss. In this population, 30% of couples had a known cause of recurrent loss. When they conceived again and miscarried, karyotype of the POCs found that these couples had a 55% chance of having an embryo with an abnormal karyotype. Furthermore, in all the patients, an abnormal pregnancy karyotype was the only abnormality found in 41% of couples.

These researchers also discovered that recurrent aneuploidy was the cause of 25% of recurrent miscarriage. A major limitation of this study was that it was prone to underestimate the actual rate of aneuploidy because it did not test for maternal contamination, nor did it perform embryoscopy. We might speculate if maternal contamination had been searched for, recurrent aneuploidy rates would only be higher.

If there is no other obvious cause for recurrent loss, **abnormal embryos** may tell us who is more likely to benefit from IVF with preimplantation genetic diagnosis testing. Despite having normal personal genetics, some couples produce very few genetically normal embryos, even when they have morphologically normal embryos. If these couples proceed with IVF, we can sample the embryos and perform genetic testing to select the embryos without detectable abnormalities, thus improving their chances of a live birth.

If there is no other obvious cause for recurrent loss, **normal embryos** can tell us which patients need more careful scrutiny for hard to detect abnormalities. Recurrent miscarriage of normal embryos has led us to send the mother's blood to reference labs which perform more sophisticated testing for anticardiolipin and antiphospholipid antibodies. Most commercial laboratories use kits to detect these antibodies. These kits can be falsely negative in cases of very high antibody titers. In some cases we have found that patients have high levels of these antibodies when we submit the specimens to reference laboratories. This can tell us who is more likely to benefit from heparin in future pregnancies.

If a patient has a known treatable cause of RPL, like antiphospholipid antibody syndrome, a genetic test of the embryo can tell you if this was possibly an anticoagulation treatment failure (for example, if the embryo is normal and maternal cell contamination has been excluded, we cannot exclude the possibility of gene mutations causing the loss, but it raises the suspicion of insufficient anticoagulation as

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a possible cause). In these cases, especially in heavier patients, we might increase our heparin dose during the next pregnancy.

**The Tests We Order:** Like most physicians, we may change what we do depending on the clinical situation and what the patient would do with the information. When we order genetic studies, we believe that the best possible way to handle the tissues is as follows: 1.) Unless you have the means to identify villi in the operating room, take the specimen to pathology and have the pathologist, or yourself, isolate segments of the embryonic tissue to be sent for genetic testing. 2.) Arrange with the genetic lab to have testing performed. Basic karyotypes are performed locally. If the karyotype is abnormal, you have your answer. To get a more comprehensive analysis, consider a microarray analysis, and send your specimen with a microarray kit, the completed requisition and specimen of the mother's blood to the lab with the sample. Microarray kits can be ordered from Natera at [www.natera.com](http://www.natera.com) and be delivered to your hospital or to your office. For our convenience, we keep these kits in our lockers at the hospital. Natera will dissect the issue for you, and even review the test results with the patient. The costs of these tests will vary according to a patient's insurance coverage.

**Summary:** Genetic testing of embryo tissue is not warranted in all patients, but can be helpful to give peace of mind, determine who might benefit from IVF with PGD, and decide if a more extensive workup is needed or to assess if treatment plans need to be augmented. If you have any additional questions about genetic testing and treatment, please don't hesitate to call us.

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