Miscarriage chromosome testing: utility of comparative genomic hybridization with reflex microsatellite analysis in preserved miscarriage tissue

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Objective: To assess the utility of comparative genomic hybridization (CGH) and reflex microsatellite analysis (MSA) for chromosome analysis of preserved miscarriage tissue.
Design: Observational study.
Setting: Academic recurrent pregnancy loss program.
Patient(s): Patients with recurrent early pregnancy loss, defined as two or more miscarriages at <10 weeks, and at least one preserved miscarriage specimen sent for CGH.
Intervention(s): Preserved miscarriage specimens were sent for CGH. If results were euploid female (46,XX), reflex MSA was performed to assess if the result was of miscarriage or maternal origin.
Main Outcome Measure(s): Results were recorded as either informative or uninformative. Uninformative results were classified as "CGH failed" or "maternal contamination."
Result(s): Fifty-eight women with 77 miscarriage specimens met the criteria. CGH failed in nine of the preserved miscarriage specimens owing to minimal pregnancy tissue, and two owing to poor-quality DNA. Twenty-two of the 33 specimens reported as 46,XX by CGH were sent for MSA; maternal contamination was confirmed in 23% (5/22). CGH was therefore informative in 79% (61/77) of the specimens; 64% (39/61) were euploid, and 36% (22/61) were noneuploid, with a 46,XX/46,XY ratio of 2.5.
Conclusion(s): CGH with reflex MSA is useful for obtaining chromosome results in preserved miscarriage specimens, although informative results were achieved in only 79% of specimens. Maternal contamination should be assessed after an initial diploid female result. (Fertil Steril® 2014;101:1349–52. ©2014 by American Society for Reproductive Medicine.)
Key Words: Comparative genomic hybridization, microsatellite analysis, cytogenetic analysis, recurrent pregnancy loss, miscarriage

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Recurrent pregnancy loss (RPL), defined by two or more failed clinical pregnancies, is a disease distinct from infertility (1). This physically and emotionally difficult reproductive problem affects ~5% of couples trying to establish a family (2). Patients increasingly ask why their miscarriages occur, but, unfortunately, miscarriage chromosome testing is not routinely performed and/or results are inconclusive. This leaves patients without answers and the physician without information to decide whether an RPL evaluation is warranted.

Based on the decision-analytic modeling study by Bernardi et al., performing chromosome testing of the second miscarriage is a cost-saving strategy to determine whether to proceed with an RPL evaluation (3). If the chromosome result of the second

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miscarriage is diploid female (46,XX, confirmed with microsatellite analysis [MSA]) or diploid male (46,XY), the miscarriage is classified as "unexplained," and an RPL evaluation of both partners is warranted. Presently, a standard RPL evaluation includes testing for a translocation in each partner and maternal testing for autoimmune, endocrine, infectious, and anatomic factors (1, 4).

Conversely, if the chromosome results of the second miscarriage are abnormal, termed "noneuploid," the miscarriage is classified as being "explained" and no further evaluation is warranted (3). Noneuploid results are most commonly numeric, specifically trisomy, monosomy, or polyploidy. These are usually random events, meaning the risk of a subsequent miscarriage is not increased (5), although the overall risk of a trisomic pregnancy increases with advancing maternal age (6). Numeric chromosome errors account for up to 70% of miscarriages at $<$6 weeks (7), and $\sim$50% of clinical miscarriages at 6 weeks to $<$10 weeks (8).

Miscarriage chromosome testing has classically been performed by cytogenetic analysis, which consists of culturing the cells, arresting the cells in metaphase, followed by Giemsa staining; banding patterns are assessed for both numeric and structural errors by experienced cytogenetic technicians. Although conventional cytogenetic analysis is widely accepted, there are limitations associated with the technology, including maternal cell contamination and culture failure. Additionally, if the miscarriage tissue is placed in formalin, the cells can not be cultured and cytogenetic analysis can not be performed.

Maternal cell contamination occurs when maternal decidua cells predominantly grow when admixed with miscarriage cells. The rate of maternal contamination varies considerably in the literature (9, 10). Most commonly, maternal contamination is due to lack of separating and washing the miscarriage tissue from the maternal decidua before culturing (11). Additionally, noneuploid miscarriage cells may grow poorly in culture media, allowing maternal cells to overgrow.

Comparative genomic hybridization (CGH) is increasingly used as an alternate methodology to obtain miscarriage chromosome results. CGH involves extraction of DNA, fluorescent labeling of miscarriage and reference DNA, followed by hybridization to a diploid target metaphase platform. Results are determined by comparison of relative fluorescence intensities with the use of an admixture of control DNA. Despite its great potential, CGH had its limitations as well; balanced structural chromosome rearrangements and some polyploidies are not identified. Preliminary studies suggest that CGH can be used when conventional cytogenetic analysis fails (12–14), when there is significant maternal contamination (15) or when the miscarriage tissue is formalin preserved.

Bell et al. proposed that CGH could be used with paraffin-embedded miscarriage tissue (16). Lomax et al. reported on the use of CGH when conventional cytogenetic analysis was not performed, when maternal contamination was suspected or when cell culture failed (17). Although several studies have discussed its promising use, its utility in clinical practice has not been adequately addressed.

Another DNA technology, MSA, has recently been introduced to assess maternal cell contamination (18). With a 46,XX result, DNA is extracted and compared with the woman's DNA at several highly polymorphic loci; if results are identical, maternal contamination is confirmed.

The objective of the present study was to determine the clinical utility of CGH and reflex MSA for chromosome analysis of preserved miscarriage tissue.

MATERIALS AND METHODS

University of Chicago Institutional Review Board approval was obtained, with written consents from all subjects. A query was performed with the use of the University of Chicago RPL Database (Microsoft Access 2007). The search included all subjects with a history of recurrent early pregnancy loss, defined as two or more ultrasound-documented miscarriages at $<$10 weeks, with at least one miscarriage specimen sent for CGH analysis. All subjects had been seen in consultation by one of the authors (M.D.S.) from July 2004 to December 2011 in the University of Chicago Recurrent Pregnancy Loss Program.

The RPL Database contains detailed obstetrical histories, including gestational age of miscarriages based on ultrasound findings at time of demise. Miscarriage chromosome results are recorded, based on review of records or subsequent testing of preserved miscarriage tissue.

If the patient had one or more preserved miscarriage specimens stored at an outside hospital or laboratory, and chromosome testing had not been performed, or the result was 46,XX, CGH testing of the preserved tissue was discussed. With the patient's consent, the paraffin-embedded tissue was requested and sent to an outside laboratory for CGH analysis (CMDX). If cryopreserved miscarriage specimens were stored at the University of Chicago, DNA was extracted and then sent to CMDX.

With a diploid female (46,XX) CGH result, the DNA was sent from CMDX and a tube of blood from the patient was requested. MSA testing was performed at the University of Chicago DNA Diagnostic Laboratory. If MSA was consistent with 46,XX of miscarriage origin, the miscarriage chromosome result was recorded as "46,XX, confirmed by MSA" in the RPL Database. Conversely, if MSA was consistent with maternal contamination, the miscarriage chromosome result was recorded as "maternal contamination."

If no chromosome result was obtained, either because of minimal pregnancy tissue for analysis or poor-quality DNA, the miscarriage chromosome result was recorded as "failed CGH." Both "maternal contamination" and "failed CGH" were classified as uninformative CGH results.

Data Analysis

Discrepancies and omissions in the data for each subject were corrected by chart review. The data were transferred to Microsoft Excel 2007 for analyses. Means, with standard deviations and ranges, are reported for demographic results. Differences between groups were analyzed with the use of the two-tailed *t* test for continuous variables.

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