A mathematical model for evaluation of maternal cell contamination in cultured cells from spontaneous abortions: significance for cytogenetic analysis of prenatal selection factors

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Objective: To develop a mathematical model for more precise estimation of the incidence of chromosomal abnormalities and the sex ratio among spontaneous abortions masked by maternal cell contamination.
Design: Retrospective analysis.
Setting: Academic medical center.
Patient(s): One hundred twelve samples of spontaneous abortion with a "46,XX" karyotype and 97 parents with aborted embryos.
Intervention(s): The presence of Y chromosome DNA in native tissues of "46,XX" spontaneous abortions was detected by amelogenin locus analysis. Detection of aneuploidies in noncultured tissues of "46,XX" abortions was performed by microsatellite DNA analysis and confirmed by fluorescence in situ hybridization.
Main Outcome Measure(s): Accuracy of cytogenetic evaluation of spontaneous abortions.
Result(s): Y chromosome DNA was revealed in 16% of the embryos with a "46,XX" karyotype. According to the mathematical model proposed, the frequency of chromosomal abnormalities in a sample of 478 abortions increased from 54.6% to 60.3%, and the sex ratio in embryos with normal karyotype changed from 0.66 to 1.02. The experimental validation of the model has shown that the observed and expected incidences of chromosomal abnormalities in "46,XX" abortions were in good agreement.
Conclusion(s): Maternal cell contamination clearly affects the incidence of registered chromosomal abnormalities and the sex ratio in spontaneous abortions. Correction for maternal cell contamination should be taken into account before invoking biological explanations of sex ratio bias and might be useful to include in diagnostic reporting. (Fertil Steril® 2005;83:964–72. ©2005 by American Society for Reproductive Medicine.)

Key Words: Chromosomal abnormalities, long-term cell cultures, maternal cell contamination, mathematical model, sex ratio, spontaneous abortions

Numerous studies have demonstrated a chromosomal abnormality rate of approximately 50%–60% among first-trimester spontaneous abortions (1–3). However, it seems that some portion of chromosomal aberrations remains undetected by conventional cytogenetics, owing to cryptic low-level mosaicism or cell culture failures (4, 5). Moreover, some results of cytogenetic analysis of "46,XX" embryos are prone to error, owing to possible contamination of long-term cultures by maternal cells. Maternal cell contamination (MCC) is a phenomenon arising from preferential maternal cell growth in vitro in comparison with low proliferative capacity of spontaneous abortions cells. When MCC is present, conventional cytogenetic analysis reveals 46,XX karyotype corresponding to the maternal tissue rather than to the embryo itself. Therefore, a true embryo karyotype remains unrecognized. Recent evidence suggests that MCC can affect 30%–90% of the embryonic cultures with 46,XX karyotypes (3, 6, 7). A high rate of MCC can not only distort the real incidence of chromosomal abnormalities but also have an influence on the estimated sex ratio among spontaneous abortions.

Despite a relatively wide distribution of the MCC phenomenon, there are no approaches for evaluating its effects and consequences for understanding cytogenetic factors of prenatal selection in man. The present report describes the outcomes of a retrospective analysis of the results of a conventional cytogenetic study of spontaneous abortions by molecular methods. We propose a mathematical model for estimating MCC effects. The differences between observed and predicted karyotype frequencies and sex ratio values, as well as the diagnostic power of conventional cytogenetic analysis of reproductive wastage, are discussed.

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MATERIALS AND METHODS

Description of the Mathematical Model

Let N be the number of all studied samples of spontaneous abortions, with four major types of chromosomal constitution: A: 46,XX; B: female spontaneous abortions with chromosomal abnormalities; C: 46,XY; D: male spontaneous abortions with chromosomal abnormalities.

$$N = A + B + C + D \quad (1)$$

The karyotypes of B, C, and D groups are beyond question. Conversely, some cases with 46,XX in group A might represent maternal cells, owing to MCC. Therefore, one can expect that embryos in this group might have any type of chromosomal complement, including 46,XX: A_{fn}: 46,XX (female normal); A_{fa}: female spontaneous abortions with chromosomal abnormalities (female abnormal); A_{mn}: 46,XY (male normal); A_{ma}: male spontaneous abortions with chromosomal abnormalities (male abnormal).

$$A = A_{fn} + A_{fa} + A_{mn} + A_{ma} \quad (2)$$

If N is a representative sample, then the relative proportions of B, C, D in equation (1) and A_{fa}, A_{mn}, A_{ma} in equation (2) should be identical.

Let k be a factor of MCC that is equal to the probability of male embryo detection in the A group:

$$k = \frac{A_{mn} + A_{ma}}{A}$$

then:

$$A = A_{fn} + A_{fa} + Ak.$$

The value of sex ratio in embryos with abnormal karyotype (SR_a) is MCC-independent:

$$SR_a = \frac{D}{B} = \frac{A_{ma}}{A_{fa}},$$

therefore:

$$A_{fa} = \frac{A_{ma}}{SR_a} = \frac{A_{ma} \cdot B}{D}.$$

The recorded frequency of chromosomal abnormalities in male spontaneous abortions (f_m) is also MCC-independent:

$$f_m = \frac{D}{C + D} = \frac{A_{ma}}{A_{mn} + A_{ma}} = \frac{A_{ma}}{A \cdot k}$$

Therefore:

$$A_{ma} = A \cdot k \cdot f_m = \frac{A \cdot D \cdot k}{C + D}; \quad A_{fa} = \frac{A \cdot B \cdot k}{C + D};$$

$$A_{mn} = \frac{A_{ma}(1 - f_m)}{f_m} = \frac{A \cdot C \cdot k}{C + D},$$

and

$$A_{fn} = A - A \cdot k - A_{fa} = A(1 - k) - \frac{A \cdot B \cdot k}{C + D}.$$

Obviously, if $k \to 0$ then $A_{fn} \to A$. On the other side, the maximum of k (when $A_{fn} = 0$) is:

$$k_{\max} = \frac{C + D}{C + D + B}$$

Maternal cell contamination participates in masking the real frequency of spontaneous abortions karyotypes. As shown in Table 1, the frequency of "46,XY" embryos, male or female spontaneous abortions with chromosomal abnormalities, and the frequency of chromosomal abnormalities in the total sample must be increased in $1 + Ak/(C + D)$ times. Otherwise, the frequency of "46,XX" karyotype must be decreased. Finally, the expected value of the sex ratio among spontaneous abortions with normal karyotype should be increased. The statistical significance of these changes in a given sample N is connected with the value of the factor k and must be estimated with the χ^2 test.

Because MCC masks the real karyotypes proportions among spontaneous abortions, it hampers the accuracy of classical cytogenetic analysis of reproductive wastage. This effect can be expressed in different ways. First, the MCC might be estimated as a part of any type of the chromosomal complement (including 45,X and "XX/XY" karyotypes) overlooked by conventional analysis (Q). If the probability of MCC is equal for any karyotype, then:

$$Q = \frac{A_{mn}}{A_{mn} + C} = \frac{A_{fa}}{A_{fa} + B} = \frac{A_{ma}}{A_{ma} + D} = \frac{A \cdot k}{A \cdot k + C + D}$$

It is necessary to note that karyotypes of some "46,XX" embryos were obtained by analysis of maternal cells also. The portion of results obtained in this way is $A_{fn} \cdot Q$, owing to independence of the MCC probability from any spontaneous abortions karyotype. Therefore, MCC might be evaluated either as a part of "46,XX" karyotypes obtained by analysis of maternal cells:

$$M = \frac{A - A_{fn}(1 - Q)}{A}$$

or as a frequency of spontaneous abortions with another karyotype in the studied group N (i.e., the probability of error):

$$E = \frac{A \cdot k(B + C + D)}{N(C + D)}.$$

Thus, the diagnostic accuracy (or power) of conventional cytogenetic analysis of spontaneous abortions can be determined as:

$$W = 1 - E = 1 - \frac{A \cdot k(B + C + D)}{N(C + D)}.$$