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Cytogenetic testing of anembryonic pregnancies compared to embryonic missed abortions

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Abstract
Purpose The objective of this study is to determine the rate of abnormalities detected by cytogenetic testing of first trimester miscarriages, in patients with and without an embryonic pole seen on ultrasound.
Materials and methods A retrospective study of 272 D&Cs for missed abortions in an academic infertility practice from 1999 to 2006. Karyotype results were compared with transvaginal ultrasound findings. Chi-squared analysis was used with a $P<0.05$ for significance.
Results There was a high rate of abnormal karyotypes in all miscarriages (65%). Rates of abnormal karyotypes were 58% and 68% in cases with anembryonic gestations and those with a fetal pole seen, respectively ($P>0.05$).
Conclusion The high rate of abnormalities detected in both groups suggests that useful results can be obtained from chromosomal testing of the POC regardless of ultrasound findings. Further studies on the prognostic value and cost effectiveness of chromosomal testing are needed.

Keywords Aneuploidy · Blighted ovum · Karyotype · Miscarriage · Anembryonic gestation

Introduction

First trimester loss occurs in approximately 15% of all clinically recognized pregnancies. When a miscarriage occurs, patients are often concerned not only with the cause of the miscarriage but also with the risk of recurrence. The majority (50–76%) of these losses is due to embryonic aneuploidy [1–9]. Although not uniformly shown in all studies, some authors have found that women who miscarry genetically normal embryos have higher recurrence rates than those who miscarry aneuploid embryos [4, 10, 11]. In our practice, we have found that knowing the karyotype of the miscarried pregnancy frequently helps a patient grieve and pursue further treatment. In addition, some authors have proposed that genetic analysis of POC may be more cost effective than a standard algorithm for the evaluation or recurrent pregnancy loss [12]. For these reasons, physicians may seek to perform cytogenetic evaluation on the products of conception to aid in counseling patients after a pregnancy loss.

Pregnancies in patients with a history of infertility or recurrent pregnancy loss are typically monitored by early ultrasound. Accordingly, miscarriages in these patients are often diagnosed by ultrasound between 6 and 10 weeks, and frequently an embryonic pole and cardiac activity are never seen. Given that there may be less viable tissue available for testing at this early stage, it is not clear whether cytogenetic testing of very early miscarriages is likely to produce meaningful results. The purpose of the current study was to determine the rate of genetic abnormalities and maternal contamination in the setting of a blighted ovum compared to missed abortions with the presence of fetal pole.

Materials and methods

In this retrospective study, data was collected from patients who underwent dilation and curettage for missed abortion in an academic reproductive endocrinology and infertility practice from 1999 to 2006. All patients had missed abortions diagnosed by ultrasound between the 6th and 10th weeks of gestation, and underwent dilation and curettage. Missed abortions were diagnosed by transvaginal

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ultrasound and included both anembryonic gestations, defined by no embryonic pole seen on ultrasound at 7 weeks gestation, and embryonic demises, where an embryonic pole was identified without cardiac activity. Typically, the D&C was performed within 2 weeks of diagnosis. Given the frequency of ultrasounds, the diagnosis was made within 2 weeks of the demise, making the maximum time from embryonic demise to D&C 4 weeks. The procedures were performed in an office setting with the use of paracervical block (1% lidocaine) and IV conscious sedation. Suction curettage under abdominal ultrasound guidance was performed. Placental villi were separated from the products of conception (POC) using a standardized technique, where the physician carefully washed and separated the villi from maternal decidua, as previously described [5]. The karyotype analysis was performed by the laboratory at our university using a standard tissue culture and GTW banding method. Ultrasound findings were recorded and compared to the karyotype results. Patient characteristics, such as age at time of miscarriage and pregnancy history were also collected. Student's *t*-test and chi-squared analysis were used. *P* values <0.05 were considered significant. Power analysis was performed which showed that a sample size of 75 subjects per group was needed to detect a 20% difference in aneuploidy rates (β=0.8, $\alpha \leq 0.05$). This study received Institutional Review Board approval.

Results

The characteristics of the patients are listed in Table 1. There were no significant differences in ages, prior miscarriages, natural conceptions, or use of IVF. A total of 272 miscarriages were included of which 91 were anembryonic (33%). As this was an infertility population, the average age of our patients was 36.8 years. Table 2 shows the types of abnormalities seen and their relative frequency in anembryonic pregnancies compared to those with a visible fetal pole. Autosomal trisomy was the most common abnormality seen, with trisomy 16 being the most common in both groups. Structural abnormalities, primarily chromosomal deletions and additions, were only seen in the anembryonic gestations. Other than that, the relative frequency of different types of abnormalities did not appear to differ significantly. In addition to the 272 miscarriages where the tissue was successfully cultured for cytogenetic analysis, there were 3 cases where analysis was attempted but the tissue failed to grow in culture.

In Table 3, the karyotype results and ultrasound findings of the 272 spontaneous abortions are reviewed. There was a high rate of abnormal karyotypes in all groups. Rates of abnormal karyotypes were not significantly different in miscarriages with embryonic poles compared to those that were anembryonic, at 68% and 58% respectively. The average and standard deviation for crown-rump length (CRL) in the cases where a fetal pole was seen was 7.4± 5.5 mm, with a range from 2 to 26 mm. When comparing miscarriages with and without a history of documented cardiac activity, there was no statistically significant difference in the rate of abnormal karyotypes or 46,XX results. The average age did not differ significantly between the groups.

Table 1 Patient characteristics (n=272)

	Anembryonic gestations n=82	Missed abortions with fetal pole n=190
Mean age ± SD in years	36.7±5.0	36.9±4.2
Patients≥35 years old	53 (63%)	137 (72%)
History of≥1 live birth	14 (17%)	42 (22%)
History of≥2 prior SAB	19 (23%)	33 (17%)
IVF conceptions	45 (55%)	106 (56%)
COH conception	17 (20%)	49 (25%)
Natural cycle conceptions	20 (24%)	49 (26%)
Number of cases with CRL>1.0 cm		49 (26%)

SAB: spontaneous abortions, *COH*: controlled ovarian hyperstimulation with clomiphene citrate, letrozole or FSH/HMG.

Table 2 Chromosomal abnormalities seen in early miscarriages with anembryonic and embryonic arrest where a fetal pole is seen

	Anembryonic pregnancies 91 total 53 cases with anomalies	Missed abortions with fetal pole 181 total 123 cases with anomalies
Monosomy X	2 (4%)	7 (6%)
Autosomal trisomy—single	28 (53%)	94 (76%)
Autosomal trisomy—multiple	8 (15%)	9 (7%)
Polyploidy–triploidy	1 (9%)	11 (9%)
Polyploidy–tetraploidy	5 (2%)	2 (2%)
Structural abnormalities (deletions/additions)	9 (17%)	0 (0%)

Discussion

In this study, there was a high rate of abnormalities detected in the karyotypes of miscarriages regardless of ultrasound findings prior to D&C. The abnormality rate in anembryonic gestations was 58%, which was not statistically different from the 68% rate seen in pregnancies with embryonic poles. This high rate of detected abnormalities

Table 3 The rate of cytogenetic abnormalities in relation to ultrasound findings

	Abnormal karyotype	Normal karyotype	46,XX	46,XY	Age (years) Mean±SD
Total n=272	176 (65%)	96 (35%)	61 (22%)	35 (13%)	36.8±4.5
No embryonic pole n=91	53 (58%)	38 (42%)	25 (27%)	13 (14%)	36.7±5.0
Embryonic pole seen n=181	123 (68%)	58 (32%)	36 (20%)	22 (12%)	36.9±4.2
History of cardiac activity n=138	97 (70%)	41 (30%)	24 (17%)	17 (12%)	37±4.3
No cardiac activity n=134	79 (59%)	55 (41%)	37 (28%)	18 (13%)	36.7±4.7

Abnormality rate, 46,XY or 46,XX results were compared. All P values were >0.05.

should encourage the physician who desires the information to perform the cytogenetic testing on POC.

Performing a karyotype analysis on POC after a miscarriage may be affected by maternal contamination. When a 46,XX result is obtained, it is difficult to know for sure if the result is from maternal contamination or a genetically normal embryo [13]. In the setting of a 46,XX result, the precise karyotype cannot be easily determined as there are no simple tests to distinguish maternal from fetal. We found a non-significant trend toward more 46,XX results in anembryonic gestations. However, the incidence of 46,XX results was higher than the incidence of 46,XY results in both groups suggesting that maternal contamination is present in anembryonic gestations as well as missed abortions with a fetal pole.

It is well known that the most common cause of first trimester loss is embryonic aneuploidy [1, 3, 7, 14, 15]. However, the benefit of performing a karyotype on the POC after miscarriage is debated. We believe that knowing the karyotype of the miscarriage can aid in counseling infertility and recurrent miscarriage patients regarding further testing and the prognosis of future pregnancies. In addition, this information may aid in the grieving process and may alleviate some of the emotional anguish often associated with the loss of a desired pregnancy. The finding of aneuploidy in the POC may also reduce the need for testing for thrombophilia and sometimes for immune disorders, often performed in this population. Conversely, for those with documented euploid miscarriages, testing may be indicated after two losses, depending on the clinical circumstances.

From a financial standpoint, testing the embryonic karyotype could lower costs if it would reduce the number of evaluations for recurrent pregnancy loss (RPL). The cost of a karyotype at our institution is $700 compared to the cost of a standard RPL work up being approximately $3,500 (parental karyotypes, anticardiolipin antibodies, lupus anticoagulant and thrombophilia testing). These estimates exclude evaluations for uterine cavity and thyroid function, which are routinely done in an infertility population. Including these additional tests would further increase the cost of the RPL evaluation. A formal cost analysis is beyond the scope of this paper but should be considered given the increasing number of tests being performed for repeated miscarriages and the trend to order them after only two losses in certain populations.

Despite the potential benefits of analyzing POC for karyotype, physicians may be hesitant to perform the testing when the ultrasound findings show minimal fetal growth because of concern for a low yield or high maternal contamination rate. We found a similar yield for abnormalities in these patients, which should encourage physicians to attempt to obtain embryonic karyotypes, if they feel it will aid in their future management or counseling of their patients.

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