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The frequencies of the presence of embryonic pole and cardiac activity in early miscarriages with abnormal karyotypes

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Summary

Aims: The objective of this study was to compare the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with normal and abnormal embryonic karyotypes. *Materials and Methods:* From January 2008 to December 2012, 405 patients with early miscarriage were evaluated during pregnancy by regular ultrasound, and karyotyping was performed on chorionic villus tissue after curettage. The frequencies of the presence of an embryonic pole and cardiac activity were compared between patients with a normal embryonic karyotype and patients with an abnormal embryonic karyotype. *Results:* Of the 405 samples, 224 cases (55.3%) had an abnormal karyotype, and 181 cases (44.7%) had a normal karyotype. The frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with normal embryonic chromosomes (71.8% and 57.5%, respectively) were similar to those of miscarriages with abnormal embryonic chromosomes (74.1% and 62.1%, respectively). The frequencies of the presence of an embryonic pole and cardiac activity were higher in miscarriages with viable autosomal trisomies (trisomies 21, 13, and 18), monosomy X, and triploidy than in miscarriages with a normal karyotype or other abnormal karyotypes. *Conclusions:* The frequencies of the presence of an embryonic pole and cardiac activity are higher in miscarriages with viable autosomal trisomies, monosomy X, and triploidy than in miscarriages with a normal karyotype or other abnormal karyotypes.

Key words: Miscarriage; Chorionic villus; Karyotype analysis; Ultrasound.

Introduction

Miscarriage affects approximately 10-15% of clinically recognized pregnancies and is the most common complication of pregnancy [1]. Eighty percent of miscarriages occur in the first 12 weeks of pregnancy [2]. Anatomic, genetic, endocrinological, immunological, and thrombophilic factors are associated with miscarriage [3]. Miscarriage due to various underlying pathologies may occur at different stages of the pregnancy [4]. More than half of miscarriages are related to chromosome abnormalities [5]. Accordingly, there is a question of whether miscarriages with an abnormal embryonic karyotype occur at different gestational stages compared with normal embryonic karyotype miscarriages. The presence of an embryonic pole and cardiac activity are the primary landmarks of embryo development. The rate of miscarriage decreases significantly after embryonic cardiac activity is demonstrated [6]. Thus, the differences in the frequencies of the presence of an embryonic pole and cardiac activity are important to investigate in miscarriages with normal and abnormal karyotypes. Reports indicate that chromosomal abnormalities are associated with up to 90% of losses before the development of an embryo. Between eight and 11 weeks of gestation, approximately 50% of losses are related to chromosome abnormalities. Between 16 and 19 weeks of gestation, this proportion decreases to 30% of losses. After 20 weeks of gestation, the rate of aneuploidy in stillbirths is less than 15% [7]. Previous studies have indicated that compared to losses occurring later in gestation, preembryonic losses are more likely to be associated with fetal aneuploidy [8,9]. However, there are some conflicting reports. Munoz *et al.* reported that the chromosomal abnormality rate is similar in cases of miscarriage with an absent or present embryo [10]. In a report by Coulam *et al.*, the frequency of an empty gestational sac and the presence of cardiac activity prior to pregnancy loss was the same in chromosomally abnormal and normal conceptuses [11]. Given the conflicting results of existing studies, the purpose of the current study of 405 patients with early miscarriage was to explore possible associations between an abnormal embryonic karyotype, the frequency of the presence of an embryonic pole, and cardiac activity at the time of diagnosis.

Materials and Methods

Population

In this retrospective study, data were collected from January 2008 to December 2012 among 405 patients who were admitted to Sun Yat-Sen Memorial Hospital and received dilation and curettage for a miscarriage in the first trimester. According to our protocol, patients who are admitted for threatened abortion or who have a history of recurrent miscarriage receive serial human chorionic gonadotropin (HCG) level measurements and transvaginal ultrasound examinations at six to seven weeks of gestation. If no fetal cardiac activity is observed, ultrasound is repeated one week later. If

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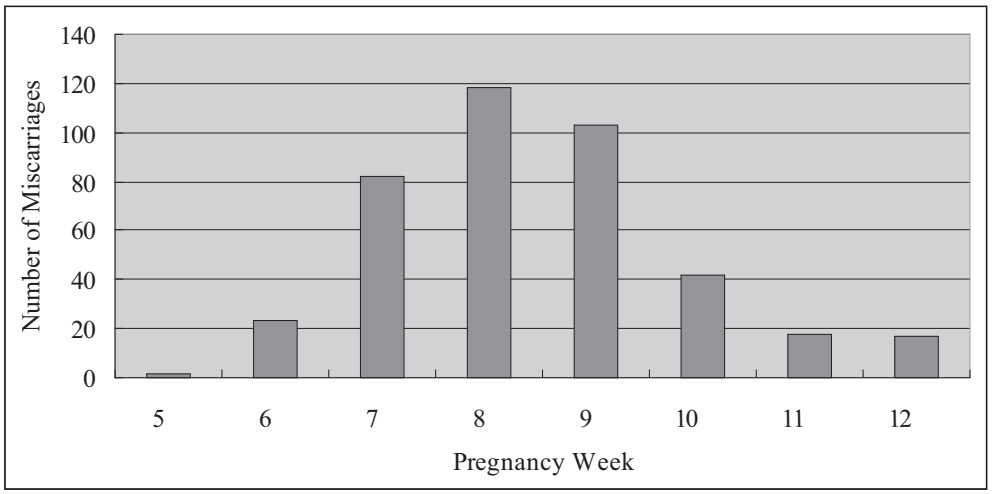

Figure 1. — Distribution of gestational age at the time of miscarriage.

fetal cardiac activity is observed, ultrasound is repeated every two weeks until ten to 12 weeks of gestation.

Miscarriage was diagnosed based on the following criteria: (1) gestational sac diameter of greater than 20 mm without a yolk sac, (2) an embryo with a crown-rump length (CRL) of greater than six mm without cardiac activity and (3) loss of cardiac activity that was previously identified. If there was doubt, a scan was repeated after one week. The gestational age was calculated from the last menstrual period, records of basal body temperatures, ultrasound measurements or the date of embryo transfer. If miscarriage was diagnosed, dilation and curettage were performed, and karyotyping of chorionic villi was performed after obtaining the patient's consent.

The patient records noted observations of an embryonic pole or cardiac activity prior to miscarriage. Patient characteristics were also collected, including maternal age at the time of miscarriage and pregnancy history. Recurrent miscarriage was defined as three or more clinical pregnancies that ended in a first-trimester loss (including the current miscarriage). Patients were excluded for the following reasons: (1) a serial ultrasound examination was not performed before the miscarriage, (2) karyotyping of chorionic villi either failed or was not performed, (3) the patient had multiple pregnancies, (4) miscarriage occurred at more than 12 weeks of gestation or (5) biochemical pregnancy or ectopic pregnancy was present.

Karyotype analysis

The production of conception was obtained by dilation and curettage under sterile conditions after obtaining the patient's informed consent. Chorionic villi were transported in RPMI culture medium and subsequently inspected under a dissecting microscope to release the villi from the maternal deciduas and blood clots. The chorionic villi were then minced and digested in dispase and cultured in the medium. The laboratory at the present hospital performed the karyotype analysis using standard tissue culture techniques and the G-banding technique.

Statistical Analysis

The statistical analysis was performed using the software SPSS 16.0. Continuous data were reported as means and standard deviations and were analyzed with *t*-tests. Categorical data were reported as percentages and were analyzed with chi-square tests and *p*-values of less than 0.05 were considered to be statistically significant.

The ethics review board at Sun Yat-Sen Memorial Hospital at Sun Yat-Sen University approved this study, and all of the patients provided written informed consent.

Results

A total of 405 subjects were included in the analysis. The mean maternal age was 32.2 ± 4.9 years (20-45), and the mean gestational age was 8.8 ± 1.4 weeks (5.9 - 12). In total, 129 (31.9%) of the subjects were 35 years or older. The number of previous spontaneous abortions ranged from 0 to 10. A total of 237 patients presented with recurrent miscarriage (including the current miscarriage). The distribution of gestational age at the time of miscarriage is displayed in Figure 1.

The karyotype results of the 405 subjects are shown in Table 1. In total, 181 (44.7%) of the 405 specimens exhibited a normal karyotype. Of the normal karyotypes, 106 were 46, XX and 75 were 46, XY. The ratio of 46, XX to 46, XY was 1.4 : 1. In total, 224 (55.3%) specimens exhibited abnormal karyotypes. A total of 132 of the abnormal karyotypes were trisomic, including 21 viable autosomal trisomies (trisomies 21, 18, and 13) and 111 other autosomal trisomies. The most frequent trisomy was 16 (40 cases), followed by trisomies 22, 15, and 21. Other abnormal karyotypes included monosomies (5.4%), triploidies (5.4%), tetraploidies (0.7%), structural abnormalities (3.7%), double abnormalities (4.0%), and mosaicisms (3.5%).

The clinical characteristics of the abnormal and normal embryonic karyotype groups are shown in Table 2. Maternal age, history of recurrent miscarriage, and gestational age were significantly different between the miscarriages with abnormal and normal karyotypes (all, $p < 0.05$). The distribution of gestational age at the time of miscarriage by abnormal and normal karyotype is shown in Figure 2.

The frequencies of the presence of an embryonic pole and cardiac activity in the abnormal karyotype group were 74.1% and 62.1%, respectively, which was similar to the normal karyotype group (71.8% and 57.5%, respectively, $p > 0.05$, Table 3).

The frequencies of the presence of an embryonic pole and cardiac activity were higher in miscarriages with viable au-

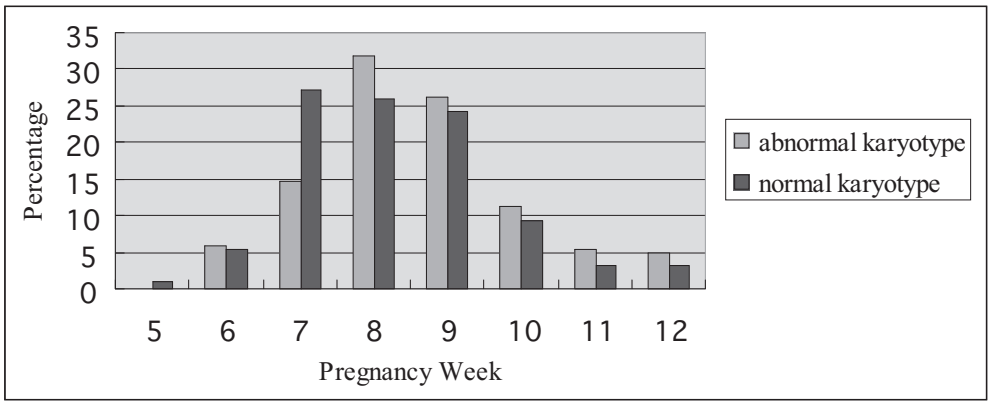

Figure 2. — Distribution of gestational age at the time of miscarriage with abnormal karyotype and normal karyotype.

Table 1. — *Karyotype results in 405 miscarriages.*

	Number	Percentage (%)
Normal karyotype	181	44.7
46, XX	106	26.2
46, XY	75	18.5
Viable autosomal trisomies	21	5.2
+21	8	2.0
+18	6	1.5
+13	7	1.7
Other autosomal trisomies	111	27.4
+2	7	1.7
+3	6	1.5
+4	3	0.7
+5	1	0.2
+7	5	1.2
+8	2	0.5
+9	2	0.5
+10	4	1.0
+11	4	1.0
+12	3	0.7
+14	5	1.2
+15	9	2.2
+16	40	10
+17	1	0.2
+20	2	0.5
+22	16	4.0
Monosomies	22	5.4
Monosomy X	17	4.2
-21	4	1.0
-18	1	0.2
Triploidies	22	5.4
Tetraploidies	3	0.7
Structural abnormalities	15	3.7
Double abnormalities	16	4.0
Mosaicisms	14	3.5
Total	405	100%

Table 2. — *Clinical characteristics of the abnormal and normal karyotype groups.*

	Normal karyotype group (n=181)	Abnormal karyotype group (n=224)	*p*
Maternal age, y	30.9 ± 4.5	33.3 ± 4.9	<0.01
Gestational age, wk	8.6 ± 1.4	8.9 ± 1.4	0.02
Gravidity	3.8 ± 1.9	3.4 ± 1.8	0.09
History of live birth	18 (34.8%)	32 (14.3%)	0.187
Maternal age ≥ 35 y	36 (19.9%)	93 (41.5%)	<0.01
Recurrent miscarriage	117 (64.6%)	120 (53.6%)	0.025

Table 3. — *The frequencies of the presence of an embryonic pole and cardiac activity in the abnormal karyotype and normal karyotype groups.*

Embryonic karyotype	N	Presence of an embryonic pole n (%)	*p*	Presence of cardiac activity n (%)	*p*
Normal	181	130 (71.8%)	0.606	104 (57.5%)	0.348
Abnormal	224	166 (74.1%)		139 (62.1%)	

Table 4. — *The frequencies of the presence of an embryonic pole and cardiac activity and the gestational age among different abnormal karyotypes.*

Karyotype	N	Presence of an embryonic pole n (%)	Presence of cardiac activity n (%)	Gestational age (weeks)
Normal karyotype	181	130 (71.8%)	104 (57.5%)	8.6±1.4
Viable autosomal trisomies	21	20 (95.2%)[a]	18 (85.7%)[a]	9.5±1.6[a]
Monosomy X	17	17 (100%)[b]	17 (100%)[b]	10.3±1.1[b]
Triploidy	22	22 (100%)[c]	21 (95.5%)[c]	9.3±1.1[c]
Other abnormal karyotype	164	107 (65.2%)[d]	83 (50.6%)[d]	8.6±1.3[d]

a: viable autosomal trisomies vs. normal karyotype ($p < 0.05$), vs. other abnormal karyotypes ($p < 0.05$). b: monosomy X vs. normal karyotype ($p < 0.05$), vs. other abnormal karyotypes ($p < 0.05$). c: triploidy vs. normal karyotype ($p < 0.05$), vs. other abnormal karyotype ($p < 0.05$). d: other abnormal karyotype vs. normal karyotype ($p > 0.05$).

tosomal trisomies (95.2% and 85.7%, respectively), monosomy X (100% and 100%, respectively), and triploidy (100% and 95.5%, respectively) than in miscarriages with a normal karyotype (71.8% and 57.5%, respectively, all $p < 0.05$) and other abnormal karyotypes (65.2% and 50.6%, respectively, all $p < 0.05$). There was no difference in the frequency of miscarriage between the normal karyotype and other abnormal karyotype groups (Table 4). The gestational ages of the viable autosomal trisomies, monosomy X, and triploidy groups were higher than those of the normal karyotype and other abnormal karyotype groups (all, $p < 0.05$).

Discussion

Approximately 10-15% of clinically recognized pregnancies result in miscarriage, and the majority (60-70%) of these miscarriages can be attributed to detectable chromosomal abnormalities. Cytogenetic analysis of the production of conception contributes to the discovery of the causes of miscarriage, and patients can be counseled about further testing and the prognosis for future pregnancies. Aneuploidy in miscarriage may also reduce the need for testing for other causes, such as immunological issues and thrombophilia, which can lower the financial cost [12]. The present data revealed that 55.3% of miscarriages had an abnormal karyotype, which is consistent with previous reports [13]. However, chromosomal analysis is unavailable at some hospitals and cannot always be performed. An exploration of the clinical and ultrasound characteristics of miscarriages with abnormal and normal karyotypes would aid in the counseling of these patients.

The present data revealed that the percentage of recurrent miscarriage in the abnormal karyotype group (53.6%) was lower than the percentage in the normal karyotype group (64.6%), which is consistent with previous studies [14,15]. Stephenson *et al.* revealed that the rate of abnormal karyotypes in recurrent miscarriage was 46%, which was lower than the 63% in the control group [16]. These findings suggest that a recurring cause other than abnormal karyotype likely leads to recurrent pregnancy loss [14]. The risk of miscarriage increases as the woman's age increases. The risk of miscarriage between six and 12 weeks of gestation in women less than 35 years of age is 9-12%. The risk increases to 40-50% in women older than 40 years of age, as there is a markedly increased incidence of trisomic embryos [17-19]. The present data indicated that in the abnormal karyotype group, the mean age, and percentage of women with advanced age was higher than in the normal karyotype group. Previous studies have also demonstrated that advanced age was the greatest risk factor for an abnormal embryonic karyotype in miscarriage [3,20].

Miscarriage is a heterogeneous condition that is associated with several underlying causes. Miscarriage due to various underlying pathologies may occur at different pregnancy stages. Previous studies have indicated that pre-embryonic losses are more likely to be associated with fetal aneuploidy than losses occurring later in gestation [8,9]. However, in the present study, the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with an abnormal karyotype were 74.1% and 62.1%, respectively, which were not significantly different from the normal karyotype frequencies (71.8% and 57.5%, respectively). The present findings are consistent with a number of chorionic villi sampling studies. A study by Munoz *et al.* revealed similar abnormal karyotype rates in anembryonic and embryonic groups (61% vs. 68%, respectively) [10]. Lathi *et al.* reported that the abnormality rate in anembryonic gestations was 58%, which was not significantly different from the 68% rate that was observed in pregnancies with embryonic poles. In a comparison of miscarriages with and without a history of documented cardiac activity, a previous study found no significant difference in the rate of abnormal karyotype [21]. Studies in patients presenting with miscarriage have not reached the same conclusions. Therefore, the risk of an abnormal embryonic karyotype cannot be predicted by whether an embryonic pole or cardiac activity is present prior to miscarriage.

Most embryos with chromosomal numerical abnormalities will miscarry, but a few can live until term, such as those with trisomies 21, 18, and 13 (called viable autosomal trisomy), monosomy X and triploidy [20,22]. Miscarriage with these viable abnormal karyotypes may occur in different gestational stages compared with other abnormal karyotypes. The present data indicate that the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with monosomy X, triploidy, and viable autosomal trisomies are higher than the frequencies in miscarriages with other abnormal and normal embryonic karyotypes. Moreover, the gestational ages of miscarriage with these viable aneuploidy karyotypes are older than the gestational ages of the other groups. The Munoz *et al.* study found that viable aneuploidy in miscarriage with an embryonic pole was more common than viable aneuploidy in miscarriage without an embryonic pole (19% vs. 2%) [10]. Bessho *et al.* found that the CRLs of monosomy X, trisomy 21, and triploidy were longer than the CRLs of normal karyotype or other trisomies [9]. This indicates that when miscarriage occurs, there is a greater degree of embryonic development in conceptuses with triploidy, monosomy X and trisomy 21 compared to other karyotypes. The chronological differences of embryo lifespans with abnormal karyotypes may be associated with programmed death that is activated by a particular lethal gene.

The present study has several limitations. The authors compared the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with normal and abnormal karyotypes. Other ultrasound-observable miscarriage-related appearance findings, such as small gestational

sacs, small fetus size, and enlarged yolk sacs, were not included in this study. Other abnormal ultrasound findings have been found to be associated with abnormal karyotypes. Compared with cases with normal ultrasounds, the frequency of chromosomal anomaly is significantly higher in the presence of abnormal morphological features (33.8% vs. 85.2%, respectively), such as early symmetrical arrested growth, small gestational sac, small fetus, enlarged yolk sac, and empty sac [23]. Ljunger *et al.* found that small embryonic poles were significantly more likely to be associated with aneuploidy than normal-sized embryonic poles (70.1% vs. 50.0%, respectively) [24]. An investigation of ultrasounds in miscarriages with different abnormal embryonic karyotypes would contribute to identifying the association between abnormal karyotypes and specific morphological types in early miscarriage.

In conclusion, embryonic karyotype anomaly is a major reason for early miscarriage. There are no differences in the frequencies of the presence of an embryonic pole and cardiac activity in miscarriages with normal and abnormal karyotypes. However, the presence of an embryonic pole and cardiac activity in miscarriages with monosomy X, triploidy and viable trisomy is more common than in miscarriages with a normal karyotype or other abnormal karyotypes.

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