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Is the absence of a yolk sac associated with chromosomal abnormality in early pregnancy loss?

K. Jiang[1], Z. Yang[1], W. Sun[1], Y. Ouyang[2]

[1] Department of Ultrasound, Daqing Oilfield General Hospital, Zhongkang Road, Daqing
[2] Reproductive and Genetic Hospital of Citic-Xiangya, Xiangya Road, Changsha (China)

Summary

Objective: To compare the abnormal karyotype rate between missed miscarriages with and without yolk sac (YS) and among groups with different YS intervals. *Materials and Methods:* Data of 214 patients who underwent dilation and curettage (D&C) for singleton pregnancy loss prior to 12th week of gestation were retrospectively analyzed. According to presence or absence of a YS on ultrasound, EPLs were divided into two groups. Ultrasound findings were correlated with karyotype analysis, which was performed by comparative genomic hybridization (CGH) plus fluorescence in situ hybridization (FISH) technology. *Results:* The chromosomal abnormality rate was significantly higher in EPLs with YS than without, either in all cases (50.0% *vs*. 28.0%, $p \leq 0.001$) or in only male cases (57.4% *vs*. 31.8%, $p \leq 0.001$). The yolk sac diameter (YSD) of EPLs with abnormal karyotypes was significantly larger compared to EPLs with normal karyotypes (4.7 ± 2.2 *vs*. 4.1 ± 2.3 mm, $p \leq 0.001$). When classified into different intervals according to the YSD, there were statistical differences in abnormal karyotype rate among different intervals ($p = 0.034$). The abnormality rate increased with the YSD. When the YSD was 9.1-12 mm, the EPLs had the highest risk of chromosomal abnormality (75.0%); when the YSD was 0-3 mm, the risk was the lowest (41.9%). When compared to group A, the abnormality rates were significantly higher in B ($p = 0.023$), C ($p = 0.033$), and D ($p = 0.048$) groups. *Conclusion:* EPLs with YS had a higher risk of chromosomal abnormalities than without and the abnormality rate increased with the YSD.

Key words: Yolk sac; Chromosomal abnormality; Transvaginal sonography; Karyotype analysis.

Introduction

The secondary yolk sac (YS) is the first visible structure within a gestational sac, which is thought to have nutritive, metabolic, immunologic, endocrine, and hematopoietic function for the primary exchange between the embryo and mother before the placental circulation is established [1, 2]. From its first appearance at about the 5th week of gestation when the gestational sac diameter over 11 mm until the 8th week of gestation, the YS shows an almost linear tendency; afterwards it flattens until the end of the first trimester [3-5].

The rapid development of transvaginal sonography (TVS) enables the observation of early pregnancy structure much clearer and earlier. The YS has an anechoic center and a regular well-defined echogenic rim on ultrasound, which is the most prominent structure in the gestational sac during the first eight weeks [6]. The range of YS diameter in normal pregnancies based on first-trimester gestational age has been established [7] and previous studies have well studied the effects of its size, shape, and absence on pregnancy outcomes. Too large or too small YS, deformed, and absent YS are all demonstrated to be correlative with adverse pregnancy outcomes [2, 5, 7-9]. However, few researches have focused on the correlation of YS and chromosomal abnormality, and we have limited information. Papaioannou *et al.* [10] reported an increased YS size in pregnancies with trisomy 21. Angiolucci *et al.* [11] found the gestational sac without a visible YS had a significantly higher abnormal karyotype rate than EPLs with normal ultrasound. While in the study of Romero *et al.*[12] found that the EPLs without YS had a markedly lower karyotype rate. The present authors hypothesized that the prevalence of genetic abnormalities in EPLs without YS was higher than EPLs with YS and the YS size in EPLs with abnormal karyotype was larger than with normal karyotypes.

The aim of this study is to compare the abnormal chromosomal rate between missed miscarriages, with and without YS, and among groups with different YS intervals.

Materials and Methods

This study was approved by the Ethics Committee of the hospital. Data of 214 patients who underwent dilation and curettage (D&C) for singleton pregnancy loss prior to 12th week of gestation between January 2006 and June 2009 were retrospectively analyzed.

TVS scans were performed equipped with a 5-9 MHz vaginal color Doppler probe to examine the location and viability of early

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pregnancy. All patients received at least two TVS scans in the initial 12 weeks of gestation to confirm EPL. The gestational age was determined according to the last menstrual period if the woman had a regular menstrual cycle or the crown-rump length (CRL) / gestational sac (GS) measurements. The diagnostic criteria for EPL were: a second scan which was performed seven days after the first scan demonstrating the cessation of previously detected cardiac activity or continued absence of embryo-fetal structures inside the gestational sac in serial scans (empty sac) [11]. The mean diameter of yolk sac was obtained by placing the calipers inside the yolk sac wall and averaging the diameters of three perpendicular measurements [10]. Women with twin or multiple pregnancies or with a proven etiology of abortions were excluded.

According to presence or absence of YS on ultrasound, EPLs were divided into two groups. YS, the size of GS, and CRL (once observed) were also measured. Each ultrasound marker was measured twice. The size of YS was subject to the final measurement before D&C. Ultrasound findings were correlated with karyotype analysis, which was performed by comparative genomic hybridization (CGH) plus fluorescence in situ hybridization (FISH) technology. Informed consents for D&C and cytogenetic analysis were obtained from each patient.

Data were analyzed using SPSS version 18.0 software. Measurement data were expressed as the mean ± standard deviation (SD). Differences of means between two groups were analyzed using Student's *t*-test. Enumeration data were expressed as rate/percentage. Comparisons of rates/percentage were using the chi-square analysis / Fisher's exact test. $P < 0.05$ was considered significant.

Results

In the 214 cases of EPLs, there were 164 cases with YS (76.6%) and 50 cases without (23.4%). Except the diagnostic time was significantly later for EPLs with YS than those without (7.9 ± 3.6 *vs*. 7.0 ± 3.5 weeks, $p = 0.001$), other characteristic parameters all did not show differences between these two groups. The chromosomal abnormality rate was significantly higher in EPLs with YS than without (50.0% *vs*. 28.0%, $p = 0.006$) (Table 1).

The proportion of female losses was larger than male in both groups (with YS: 57.9% *vs*. 42.1%; without YS: 58.0% *vs*. 42.0%). An additional analysis including only male cases was also carried out to exclude the effect of maternal cell contamination (MCC) and the chromosomal abnormality rate was still significantly higher in EPLs with YS (57.4% *vs*. 31.8%, $p \leq 0.001$) (Table 1).

There were no differences in the rates of viable autosomal trisomy ($p = 0.932$), monosomy ($p = 0.459$), and complex abnormalities (more than one type of chromosomal abnormality, $p = 0.332$) between these two groups (Table 2). In EPLs with YS, the yolk sac diameter (YSD) of EPLs with abnormal karyotypes was significantly larger compared to EPLs with normal karyotypes (4.7 ± 2.2 *vs*. 4.1 ± 2.3 mm, $p \leq 0.001$). When classifying EPLs into different intervals according to the YSD, there were statistical differences in abnormal karyotype rate among different intervals ($p = 0.034$). The abnormality rate increased with the YSD. When the YSD was 9.1-12 mm, the EPLs had the highest risk of chromosomal abnormality (75.0%); when the YSD was 0-3 mm, the risk was the lowest (41.9%). When compared to group A, the abnormality rates were significantly higher in B ($p = 0.023$), C ($p = 0.033$), and D ($p = 0.048$) groups. There were no significant differences between B and C ($p = 0.707$), C and D ($p = 0.683$), and B and D ($p = 0.457$) groups (Table 3).

Table 1. — *Comparison of demographic data and chromosomal abnormality rate between missed miscarriages with or without YS.*

	With YS n=164	Without YS n=50	*p*
Maternal age (years)	32.4 ± 4.9	32.1 ± 5.0	0.073[#]
BMI (kg/m^2)	21.8 ± 3.1	21.2 ± 2.9	0.174[#]
Previous miscarriage	35 (21.3%)	11 (22.0%)	0.921[&]
Gestational age at diagnosis (weeks)	7.9 ± 3.6	7.0 ± 3.5	0.001[#]
Chromosomal abnormality rate of all losses	50.0% (82/164)	28.0% (14/50)	0.006[&]
Chromosomal abnormality rate of male losses	57.4% (39/68)	31.8% (7/22)	0.037[&]

[#] *Student's t-test,* [&]*Chi-square test, YS: yolk sac.*

Table 2. — *Chromosomal distribution in missed miscarriages with or without YS.*

	With YS n=164	Without YS n=50	*p*
Normal karyotype	82 (50.0%)	36 (72.0%)	0.006[&]
Abnormal karyotype	82 (50.0%)	14 (28.0%)	
Viable autosomal trisomy	12 (7.3%)	2 (4.0%)	0.932[&]
Other autosomal trisomy	58 (35.4%)	9(18.0%)	
Monosomy	9 (5.5%)	1 (2.0%)	0.459[&]
Complex abnormality	3 (1.8%)	2 (4.0%)	0.332[*]

[&]*Chi-square test,* [*]*Fisher's exact test.*

Table 3. — *Comparison of chromosomal abnormality rate of different YSD intervals*

YSD (mm)	Normal karyotype	Abnormal karyotype	Total	Abnormal karyotype rate	*p*
0-3 (A)	33	17	50	34.0%	0.034[&]
3.1-6 (B)	35	42	77	54.6%	
6.1-9 (C)	12	17	29	58.6%	
9.1-12 (D)	2	6	8	75.0%	

[&]*Chi-square test.*

Discussion

In this study, the total abnormality rate was 44.9%, which was similar with the results of previous studies [11, 13]. It was not surprising that the diagnostic time of EPLs with YS was later than without and for YS there was an embryonic structure with later appearance than the gestational sac [3, 14].

MCC is a common problem in cytogenetic analysis. In this study, the proportion of female losses was larger than male losses, thus, an additional analysis excluding female cases was conducted, which demonstrated the results of all cases that the chromosomal abnormality rate was significantly higher in EPLs with YS than without. It seems quite different from the present authors' intuitive thinking that the more abnormal the conceptus, the more likely it with genetic abnormalities [15]. The authors speculate that the genetic factor plays a more important role after the appearance of a YS with a high risk of chromosomal abnormality.

The association between a small, large or absent YS and miscarriage have been reported in previous studies [5, 8, 16, 17], but there is very few information available concerning its size with genetic abnormality. The study of Angiolucci *et al.*[11] found a high prevalence of chromosomal abnormalities in EPLs with enlarged YS (28/30, 93.3%), and trisomy 22, and trisomy 2 were the most common two kinds of abnormalities. Papaioannou *et al.* [10] reported that trisomy 21 was relative with increased YS size. In the present study, the authors found the YS size of EPLs with abnormal karyotypes was significantly larger than normal ones (4.7 ± 2.2 *vs*. 4.1 ± 2.3 mm). The largest YS size was found in EPLs with trisomy 2, trisomy 22, trisomy 16 (5.8, 4.6, and 4.5 mm in median, respectively) and trisomy 16 and trisomy 22 were found to be the most common two abnormalities: slightly different from the findings of Angiolucci *et al.*, where different study sample size and laboratorial technology may be the cause.

The absence or small YS in the first trimester had been demonstrated to be predictors of poor pregnancy outcomes [5, 8, 17]. However, in the present study, the authors found that the abnormality rate increased with the YS size, which indicates that EPLs with a smaller YSD or absent YS have a relative low risk of genetic abnormalities. In parallel, the appearance of a YS or with larger size are more possible to be relative with chromosomal abnormalities. From the present data, we can see only a few of EPLs end with an extremely large YS (4.9%, over 9 mm), and most cases end with 0-6 mm (77.4%). As we know, the YSD is usually 3-4 mm and it increases up to the 10^{th} or 11^{th} week of gestation [18]. Most researches regard 5 or 6 mm as the upper limit for the size of a normal yolk sac in pregnancies from the 5^{th} to the 10^{th} gestational weeks [3, 5]. There were no statistical differences found in the abnormality rate among B, C, and D groups (YSD from 3.1-12 mm) in this study. Why did EPLs with an extremely large YSD have a similar abnormality rate as EPLs with normal YSD? The YS is the primary hematopoietic organ in the embryo and the increased YSD may reflect the defective hematopoiesis [10, 19], which may cause the early demise of pregnancy other than chromosomal abnormality. Further study with a larger sample size is needed to elucidate this question.

In this study, the authors used CGH+FISH to carry out genetic analysis. Most chromosomal abnormalities in pregnancy failure are numerical abnormalities [20, 21], and CGH is a good technique to quickly screen the entire genome for chromosomal changes. The limitation of conventional G-banding, such as long-duration cell culture, external contamination, culture failure, and selective growth of maternal cells could also be overcome by CGH [22, 23]. Using FISH to screen polyploidy made the genetic analysis more comprehensive and accurate. However, there were still some limitations in this study. First, the sample size was not large enough. Second, CGH was unable to detect mosaicism, which may cause an underestimation of the abnormality rate. Finally, the authors did not analyze detailed types of genetic abnormalities, which may carry out in our future work.

In conclusion, EPLs with YS had a higher risk of chromosomal abnormalities than without and the abnormality rate increased with the YSD.

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Corresponding Author:
KAIHUI JIANG, M.D.
Department of Ultrasound, Daqing Oilfield General Hospital
Zhongkang Road, Daqing
Heilongjiang 163001 (China)
e-mail: Jiangkhh@163.com