

Correlation analysis between ultrasound findings and abnormal karyotypes in the embryos from early pregnancy loss after in vitro fertilization-embryo transfer

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Received: 28 June 2016 / Accepted: 22 September 2016 / Published online: 28 October 2016
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Abstract

Purpose The purpose of the study is to evaluate the correlation between ultrasound findings and abnormal karyotypes in early pregnancy losses (EPLs) after in vitro fertilization-embryo transfer (IVF-ET).

Methods This retrospective analysis assessed 2172 cases of EPL after IVF-ET occurring between January 2008 and December 2013. The cases were examined via transvaginal ultrasonography (TVS). Embryonic tissue karyotyping following miscarriage was performed using a comparative genomic hybridization (CGH) analysis with fluorescence in situ hybridization (FISH). The correlations between the ultrasound findings and the karyotypes were evaluated.

Results Six categories of ultrasound findings were observed: normal ultrasound, empty sac, yolk sac only, small gestational sac, small embryonic pole, and early symmetrical arrested growth. The overall rate of abnormal karyotypes was 44.9 %

(976/2172), and the rate of abnormal karyotypes associated with a normal ultrasound, empty sac, yolk sac only, small gestational sac, small embryonic pole, and early symmetrical arrested growth was 49.5 % (218/440), 28.1 % (138/491), 43.4 % (197/454), 50.0 % (43/86), 49.8 % (155/311), and 57.7 % (225/390), respectively. Compared with the other groups, the prevalence of chromosomal abnormalities was significantly higher in the early symmetrical arrested growth group but was markedly lower in the empty sac group in all cases and when cases of 46,XX were excluded ($p < 0.05$). Trisomy 16 was the most common chromosomal abnormality in the yolk sac only, small embryonic pole and early symmetrical arrested growth groups. In the empty sac, small gestational sac and normal ultrasound groups, monosomy X was the most frequent abnormality.

Conclusions Chromosomal anomalies may be associated with specific types of ultrasound findings in EPLs after IVF-ET.

Capsule This study aimed to analyze the correlation between ultrasound findings and abnormal karyotypes in EPLs after IVF. The results suggest that chromosomal anomalies may be associated with specific types of ultrasound findings.

Electronic supplementary material The online version of this article (doi:10.1007/s10815-016-0821-2) contains supplementary material, which is available to authorized users.

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Keywords In vitro fertilization-embryo transfer · Transvaginal ultrasonography · Early pregnancy loss · Karyotype · Ultrasound findings

Introduction

Approximately 10–15 % of natural pregnancies end with an early miscarriage, which traditionally refers to an intrauterine pregnancy loss at <12 weeks of gestation [1]. Over 95 % of miscarriages occur in the first trimester, and more than half of spontaneous miscarriages result from chromosomal abnormalities, mostly aneuploidies [2–4]. Of these, the most common abnormalities are autosomal trisomies (60 %), followed by monosomy X (20 %) and polyploidy (20 %) [5, 6]. Advanced maternal age (MA) is associated with an increased

risk of miscarriage and chromosomal abnormalities [4, 7]. The spontaneous miscarriage rate is only 3 % in women younger than 30 years old but as high as 29 % in women older than 40 years old [8], and the incidence of chromosomal abnormalities increases from 48 to 65 to 92 % over the same age range [9, 10].

Transvaginal ultrasonography (TVS) provides high-resolution imaging, which makes it possible to identify pregnancies at an early stage and to predict pregnancy outcomes by determining the gestational sac size, embryonic pole length, embryonic heart rate, existence of a yolk sac, and yolk sac size. Abnormal ultrasound findings are highly predictive of early pregnancy loss (EPL) [11–15]; however, whether the ultrasound findings for embryos from EPLs are related to specific chromosomal abnormalities remains unclear.

Few studies have evaluated the correlation between ultrasound findings and the karyotypes of embryos from EPLs, and the results that are available are inconsistent. Goldstein et al. and Coulam et al. reported that ultrasound findings should not be used to predict the karyotypes associated with spontaneous miscarriages [16, 17]. However, recent studies have demonstrated correlations between ultrasound findings and chromosomal anomalies [18, 19]. Therefore, the objective of this study was to investigate the correlation between ultrasound findings and abnormal karyotypes associated with EPL after in vitro fertilization-embryo transfer (IVF-ET) based on the largest sample from a single reproductive center to date. A comparative genomic hybridization (CGH) analysis and fluorescence in situ hybridization (FISH) technology were used for the cytogenetic analysis.

Materials and methods

Study design and setting

This study was a retrospective cohort study conducted at the Reproductive and Genetic Hospital of CITIC-Xiangya (Changsha, China). Ethics approval was obtained from the Ethics Committee of the Reproductive and Genetic Hospital of CITIC-Xiangya.

Study population

Between January 2008 and December 2013, 5860 IVF patients who experienced singleton EPL were diagnosed at our hospital. At our hospital, serum β -hCG levels were checked 2 weeks after embryo transfer (ET), and patients with increased serum β -hCG levels were referred for TVS to clinically confirm the pregnancy and assess embryo viability 4–5 weeks after ET. TVS scans were performed by experienced sonographers using a 5–9 MHz vaginal probe (GE Voluson E8/730, GE Tech Co., Ltd., New York, America). All of the

patients received at least two TVS examinations at our hospital between the sixth and 12th weeks of gestation to diagnose EPL. After patient exclusion, the data from 2172 included patients were analyzed. Dilatation and curettage (D&C) were performed under ultrasound guidance in all 2172 patients after the confirmation of EPL.

Ultrasound measurements

Criteria for the diagnosis of EPL were as follows: (a) the absence of an embryo with a heartbeat ≥ 2 weeks after a scan that showed a gestational sac without a yolk sac; (b) a non-viable embryonic pregnancy confirmed on the first scan in which the embryonic pole length was ≥ 7 mm; or if the embryonic pole length was < 7 mm on the first scan and non-viability was confirmed based on reproducible evidence of the absence of fetal heart activity; or if the embryonic pole failed to lengthen over 1 week; and (c) an embryonic pregnancy that was viable at the initial scan but had no observable fetal heart activity at the 12th week [20].

The size of the gestational sac was calculated based on the average of three measurements of its perpendicular diameter using calipers placed at the inner edges of the trophoblast [21]. The size of the yolk sac was calculated based on the average of three measurements of its perpendicular diameter relative to the center of the yolk sac wall [22]. The length of the embryonic pole was measured along the anterior to posterior axis [21, 23]. The embryonic heart rate was measured from frozen M-mode images using electronic calipers [24]. The ultrasound parameters were compared with the biometric reference data presented in Papaioannou et al.'s [25] report, which provides reference values for embryonic pole length, embryonic heart rate, gestational sac diameter, and yolk sac diameter relative to gestational age (GA)/embryonic pole length in normal pregnancies. GAs were accurately recorded in this study, which was helpful for assessing the ultrasound findings. The EPLs were classified into different morphological groups according to different ultrasound findings. Clinical characteristics such as MA, duration of infertility, and the transfer cycle of each patient were also recorded.

Cytogenetic analysis

To conduct the genetic analyses, we used CGH, which is an ideal technique for quickly screening the entire genome for chromosomal changes [26, 27]. The maternal decidua, mucus, and blood clots were dissected away from all miscarried embryos under a stereomicroscope to collect the chorionic villi. Genomic DNA was then extracted from these carefully separated chorionic villi samples. FISH technology was used to screen for polyploidies. A digital image analysis system that included a Zeiss Axioplan 2 microscope equipped with a Metachrome II cooled-charged device camera (Zeiss,

Oberkochen, Germany) was used for the karyotype analyses. Informed consent for the cytogenetic analysis was obtained from all of the patients.

Main outcome measures

The frequency of abnormal karyotypes associated with each type of abnormal ultrasound finding was compared with the frequency in the normal ultrasound group.

Statistical analysis

Statistical analyses were conducted using SPSS version 17.0 (SPSS, Inc., Chicago, IL). Measurements are expressed as the mean \pm standard deviation (SD), and an analysis of variance (ANOVA) was used to analyze the differences among the groups. Enumerated data are expressed as the rate (percentage), and two sets of rates were compared using the chi-square test. Differences with $p < 0.05$ were considered significant.

Results

Between January 2008 and December 2013, 5860 IVF patients who experienced singleton EPL were diagnosed at our hospital. A total of 3688 patients were excluded for the following reasons: the pregnancies were complete miscarriages; parental chromosomal abnormalities were present; the patient received donor eggs, underwent a preimplantation genetic diagnosis (PGD) or preimplantation genetic screening (PGS); ultrasound results were not available or the patient had received only one TVS examination between the sixth and 12th weeks of gestation; the patient refused to consent to the cytogenetic tests; or the patient had conditions proven to be associated with miscarriages, such as uterine anomalies and positive anti-cardiolipin antibody tests. Finally, a total of 2172 patients were included for analysis.

Ultrasound findings

Six different categories of ultrasound findings were identified from repeated TVS scans of 2172 embryos associated with EPL after IVF-ET: (1) normal ultrasound, 440 cases; the measurements of the gestational sac, yolk sac and embryonic pole were consistent with the GA of the embryo (all were between the fifth and 95th percentile for the expected size) [25]; (2) empty sac, 491 cases with an absence of fetal structures inside the gestational sac [18]; (3) yolk sac only, 454 cases; only a yolk sac was present in the gestational sac, and no embryonic pole or cardiac activity were present [4]; (4) small gestational sac, 86 cases; the diameter of the gestational sac was <5 th percentile for its expected size, but the embryonic pole length >5 th percentile for its expected size [25]; (5) small embryonic

pole, 311 cases; the diameter of the gestational sac was consistent with or larger than predicted based on GA (≥ 5 th percentile for the expected size), but the embryonic pole length was shorter than expected (<5 th percentile for the expected size) [25]; and (6) early symmetrical arrested growth, 390 cases; simultaneous growth arrest of the gestational sac and the embryonic pole occurred (both <5 th percentile for their expected size) [25].

No significant differences were found in the clinical parameters, including MA, duration of infertility, transfer cycle, day-3 FISH levels, infertility type, and embryo grade, among the patients in each of these six groups (Table 1).

Comparisons of the chromosomal abnormality rate

The rate of abnormal karyotypes across all 2172 cases was 44.9 % (976/2172). The frequencies of abnormal karyotypes among the different ultrasound finding groups were statistically different (overall comparison: $p < 0.001$). Compared with the other groups, the chromosomal abnormality rate was significantly higher in the groups with a normal ultrasound ($p = 0.029$) and early symmetrical arrested growth ($p < 0.001$) but was markedly lower in the empty sac group ($p < 0.001$). No significant differences were found in the rates of chromosomal abnormalities between the yolk sac only, small gestational sac, and small embryonic pole groups and the remaining groups ($p > 0.05$; Table 2).

Association between ultrasound findings and chromosomal abnormalities

Of the 976 cases with abnormal karyotypes, trisomy 16, trisomy 22, monosomy X, trisomy 21, trisomy 15, and trisomy 13 were the most frequently observed abnormalities, accounting for 62.2 % (607/976; Fig. 1). The [supplementary table](#) shows the distribution of karyotypes associated with each category of ultrasound findings. In the yolk sac only, small embryonic pole, and early symmetrical arrested growth groups, trisomy 16 was the most common abnormality; in the empty sac, small gestational sac and normal ultrasound groups, monosomy X was the most common abnormality.

Additional analysis

Among the 1196 (55.1 %) cases with a normal karyotype, there were 697 cases with 46,XX and 499 with 46,XY (58.3 and 41.7 %, respectively, [supplementary table](#)). To avoid the effect of maternal cell contamination (MCC), an additional analysis was performed excluding the 697 cases with 46,XX, and the results were consistent with the initial analysis: the frequencies of abnormal karyotypes among the different ultrasound finding groups were statistically different (overall comparison: $p < 0.001$). Compared with the other

Table 1 Comparison of clinical parameters in the 6 ultrasound finding groups

Ultrasound findings	n	Maternal age (years)	Infertility duration (years)	Transfer cycle (n)	Day-3 FSH (IU/L)	Infertility		Transferred no.
						Primary	Secondary	
Normal	440	32.1 ± 5.0	5.1 ± 3.9	1.5 ± 0.9	6.7 ± 2.0	181	259	2.0 ± 0.1
Empty sac	491	31.9 ± 4.7	5.4 ± 3.9	1.4 ± 0.6	7.0 ± 2.6	215	276	2.0 ± 0.2
Yolk sac only	454	32.0 ± 4.9	5.8 ± 4.1	1.5 ± 1.0	7.0 ± 2.8	177	277	2.0 ± 0.2
Small gestational sac	86	32.4 ± 2.8	5.5 ± 3.9	1.6 ± 0.8	6.3 ± 1.4	36	50	2.0 ± 0.2
Small embryonic pole	311	32.5 ± 4.8	5.4 ± 3.6	1.3 ± 0.6	7.2 ± 2.8	104	207	2.0 ± 0.0
Early symmetrical arrested growth	390	32.8 ± 4.9	5.8 ± 4.1	1.4 ± 0.7	7.1 ± 1.9	159	231	2.0 ± 0.2
p value		0.077 ^a	0.216 ^a	0.909 ^a	0.474 ^a	0.117 ^b		0.452 ^a

^a Comparisons were made via an analysis of variance

^b Comparison was made via a chi-square test

groups, the rate of chromosomal abnormalities was significantly higher in the early symmetrical arrested growth group (76.9 vs. 59.9 %, $p < 0.001$) and was significantly lower in the empty sac group (47.6 vs. 70.6 %, $p < 0.001$), while no differences were found between the groups with normal ultrasound findings (69.6 vs. 64.4 %, $p = 0.092$) or the yolk sac only (67.2 vs. 65.0 %, $p = 0.459$), small gestational sac (65.5 vs. 65.4 %, $p = 0.998$), small embryonic pole (70.6 vs. 64.6 %, $p = 0.087$), and the other groups.

Discussion

In this study, we retrospectively assessed the frequency of chromosomal abnormalities in EPLs with different ultrasound findings. The chromosomal anomalies showed a stronger correlation with early symmetrical arrested growth and a weaker correlation with an empty sac than other findings did. Therefore, correlations between some ultrasound findings and chromosomal anomalies may exist.

Among the 2172 cases, 44.9 % were associated with chromosomal anomalies, which is similar to the rates reported in previous studies [4, 16, 28]. The mean MA of the women with embryos that had abnormal karyotypes was significantly higher than that of the women with embryos that had normal karyotypes (32.5 ± 4.6 vs. 31.5 ± 4.6 years, $p < 0.01$), supporting the suggestion that MA is a potential risk factor for fetal chromosomal abnormalities [4, 7].

Ultrasound findings

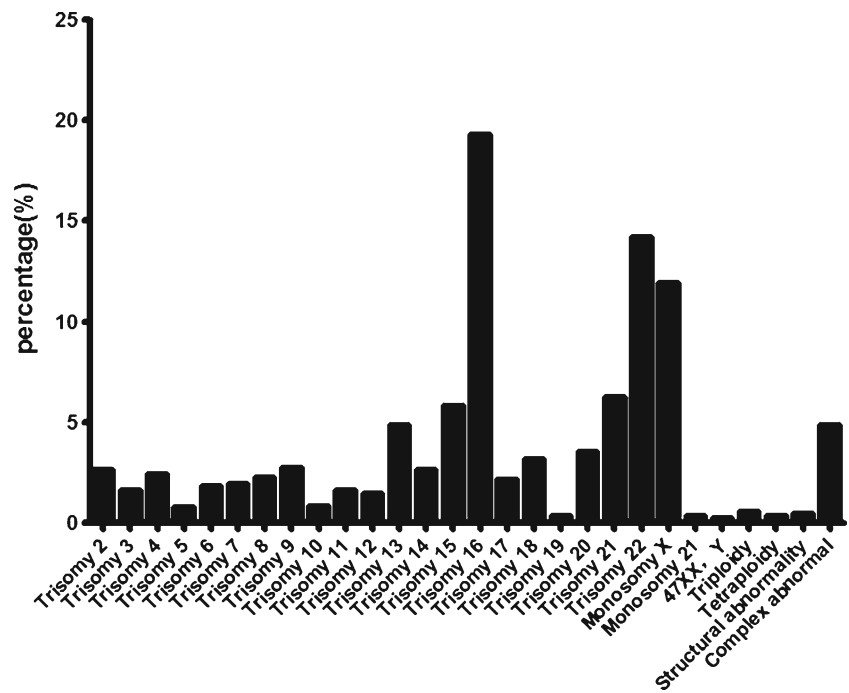
In our study, an empty sac was the most common ultrasound finding (22.6 %, 490/2172). Empty gestational sacs have been studied previously, and the results describing their correlation with abnormal karyotypes are inconsistent [5, 16–18] (Table 3). Many previous studies are prone to correlate empty gestational sacs with a high prevalence of abnormal karyotypes [16–18]. However, Hsin-Hsin et al. [29] and Romero et al. [5] found a relatively low prevalence of genetic abnormalities associated with pre-embryonic losses. In the study by

Table 2 Comparison of abnormal karyotype rates in the 6 ultrasound finding groups

Ultrasound findings	Abnormal	Total	%	Subsamples			p value
				Abnormal	Total	%	
Normal ultrasound	218	440	49.5	758	1732	43.8	0.029
Empty sac	138	491	28.1	838	1681	49.9	<0.001
Yolk sac only	197	454	43.4	779	1718	45.3	0.457
Small gestational sac	43	86	50.0	933	2086	44.7	0.335
Small embryonic pole	155	311	49.8	821	1861	44.1	0.060
Early symmetrical arrested growth	225	390	57.7	751	1782	42.1	<0.001
Total	976	2172	44.9				N/A

Groups were compared using the chi-square test, p value: each ultrasound finding group was compared with the other groups

Fig. 1 Karyotype distribution in the 976 embryos with abnormal karyotypes identified after IVF-ET



Angiolucci et al. [18], although the abnormality rate was high, the risk of chromosomal abnormalities in EPLs with an empty sac is the lowest among different types of abnormal ultrasound findings, which agrees with our findings. In our study, the frequency of abnormal karyotypes in this group was even lower than that in the normal group (28.1 vs. 49.5 %). This indicates that non-genetic factors may play an important role in early pregnancy failures associated with an empty gestational sac.

Early symmetrical arrested growth features the simultaneous arrest of growth of both the gestational sac and the embryonic pole. We found that this condition was correlated with the highest risk of chromosomal abnormalities, which is consistent with the results of a study by Angiolucci et al. [18] (Table 3). However, the rate of chromosomal abnormalities in the early symmetrical arrested growth group in our study was much lower than that reported in this previous study (57.7 vs. 100 %). It should be noted that there were only 12 cases with this morphological type in the study by Angiolucci et al. compared with 162 cases in ours, which is one possible cause for this discrepancy.

The abnormality rate in the group with normal ultrasound findings differed from the abnormality rates of other groups in the main analysis, while an opposite result was got in the additional analysis. Consequently, we were unable to draw a confident conclusion about the correlation between normal ultrasound findings and chromosomal abnormalities in this study.

Previous studies have shown different results for the correlation between a small embryonic pole and abnormal

karyotypes [17, 18] (Table 3). In this study, the abnormality rate in the small embryonic pole group was similar to that of the other groups, including the group with normal ultrasound findings, which is inconsistent with the results of Angiolucci et al. [18]. Different laboratory methods (CGH vs. G-banding) and study populations and the larger sample size in our study may be the possible causes of this inconsistency. Further studies will be needed to fully explain the factors responsible for this inconsistent result.

A gestational sac with only a yolk sac, also referred to an anembryonic sac, is caused by the very early demise of the embryonic pole [30]. Few articles have evaluated the correlation between an anembryonic sac and chromosomal abnormalities, and their results were inconsistent [2, 3, 5, 29] (Table 3). Our study found that the risk of chromosomal abnormality with this type of ultrasound finding was not significantly higher than the risk associated with other abnormal ultrasound findings. In addition, we found that the small gestational sac group also had a rate of abnormal karyotypes similar to that of the other groups, supporting the previously reported results [18].

Association between ultrasound findings and chromosomal abnormalities

We found that the presence of only a yolk sac was the most frequent condition identified via ultrasound in the embryos with trisomy 16, which is a lethal chromosomal abnormality. We speculate that the genes located in chromosome 16 might be involved in the early development of the embryo and that

Table 3 Relationship between different ultrasound findings and abnormal karyotypes in previous studies and the present study

Author	n	Study design	Study population	Cytogenetic methods	Abnormal karyotype rates in relation to different ultrasound findings							
					Normal ultrasound	Small gestational sac	Small embryonic pole	Early symmetrical arrested growth	Enlarged yolk sac	Yolk sac only	Empty sac	
Goldstein et al. 1996 [16]	102	Retrospective	General population	G banding	–	–	–	–	–	9/21(42.9 %)	12/25(48.0 %)	–
Coulam et al. 1997 [17]	137	Retrospective	ART population	G banding	21/32(65.6 %)	–	42/66(63.6 %)	–	–	–	–	23/39(59.0 %)
Angiolucci et al. 2011 [18]	156	Prospective	General population	G banding	23/68(33.8 %)	6/68(8.8 %)	16/17(94.1 %)	12/12(100 %)	28/30(93.3 %)	–	–	13/18(72.2 %)
Hsin-Hsin et al. 2014 [29]	223	Retrospective	General population	G banding	–	–	–	–	–	–	–	^b Anembryonic losses 37/80(46.3 %)
Romero et al. 2015 [5]	73	Prospective	General population	CMA	–	–	–	–	–	–	–	^a Pre-embryonic losses 1/11(9.1 %)
Our study 2016	2172	Retrospective	IVF population	CGH + FISH	49.5 % (218/440)	50.0 % (43/86)	49.8 % (155/311)	57.7 % (225/390)	–	43.4 % (197/454)	28.1 % (138/491)	–

CMA Chromosomal microarray analysis, CGH comparative genomic hybridization, FISH fluorescence in situ hybridization

^a Pre-embryonic losses include yolk sacs only and empty sacs

^b Anembryonic losses include yolk sacs only and empty sacs

aberrations in this chromosome may cause the demise of the embryo. Normal ultrasound findings were the most frequent results for embryos with monosomy X and trisomy 21, which are both viable chromosomal defects. Our results indicate that monosomy X and trisomy 21 have no visible effect on the early growth of the embryo. In the 47 cases with complex chromosomal abnormalities, those involving more than one type of chromosomal abnormality, early symmetrical arrested growth, an empty gestational sac, and the presence of only a yolk sac were the most common findings; however, determining whether complex chromosomal abnormalities are correlated with these three ultrasound findings will require further studies.

The associations between a low fetal heart rate, yolk sac size, and chromosomal abnormalities have been investigated in previous studies [31–33]. We did not examine these factors in detail in this study because they overlap with (or are included in) the six categories of ultrasound findings examined here. Their correlations with chromosomal abnormalities will be analyzed in our future work.

Limitation

MCC is a common problem in studying embryonic tissue samples from early pregnancy failures, and our research was confined to samples from infertility patients. Another limitation is that CGH is unable to detect mosaicism. In addition, the hybridization proportion of probe cannot reach 100 %. The strength of the probe signal and subjectivity may affect the FISH results. Furthermore, the biometric reference data for classifying ultrasound findings in this study are from the general population rather than the IVF population.

Final considerations and conclusion

In this study, we found that the risk of chromosomal abnormality was highest in the EPLs with early symmetrical arrested growth and lowest in those with an empty sac. Identifying whether a pregnancy loss is caused by a genetic abnormality is clinically important. Our findings may help clinicians determine the causes of miscarriages and provide guidance for subsequent pregnancies.

In summary, chromosomal anomalies in embryos from EPLs after IVF-ET may show a stronger correlation with some ultrasound findings associated with early pregnancy failure than others.

Acknowledgments The authors thank Lizhi Huang, Yueer Li, Qingqing Wu, and Kailan Xiong for assistance in collecting and sorting raw data. The authors wish to thank Genetic Center of Reproductive and Genetic Hospital of CITIC-Xiangya for providing professional consulting and help in collecting and analyzing genetic data.

Compliance with ethical standards

Funding This study was supported by the National Natural Science (No. 81471432), State Key Development Program for Basic Research of China (No.2012CB944901) and the Scientific Research Foundation of Reproductive and Genetic Hospital of Citic-Xiangya.

Conflict of interest The authors declare that they have no conflict of interest.

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