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ORIGINAL ARTICLE *Early pregnancy*

Correlation between chromosomal distribution and embryonic findings on ultrasound in early pregnancy loss after IVF-embryo transfer

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STUDY QUESTION: Do early pregnancy losses (EPLs) with and without embryos differ in chromosomal distributions?

SUMMARY ANSWER: The chromosomal abnormality rate is significantly higher in miscarriages with embryos than without after *in vitro* fertilization (IVF)-embryo transfer.

WHAT IS KNOWN ALREADY: Chromosomal abnormalities are the main causes of EPLs, the rate of which is up to 24–30% in the IVF population. Little research has been conducted on the correlations between the chromosomal distributions of EPL and the existence of an embryo or with the postmortem embryonic pole length, and the existing results have been inconsistent.

STUDY DESIGN, SIZE, DURATION: The data of 2172 women who underwent dilation and curettage (D&C) from January 2008 to December 2013 for missed abortion were analyzed retrospectively. The existence of an embryonic pole and the length of the postmortem embryonic pole of the EPL were evaluated by transvaginal sonography (TVS). Ultrasound findings were compared with karyotype results.

PARTICIPANTS/MATERIALS, SETTING, METHOD: This analysis included 2172 infertility patients who had singleton pregnancies and experienced EPLs after IVF-embryo transfer. The EPLs were divided into embryonic and anembryonic groups based on TVS diagnosis. The crown–rump length of the fetal pole (observed once) was measured twice for each fetus after confirmation of fetal death, subject to the final measurement before D&C. The karyotype analysis was performed using comparative genomic hybridization (CGH) plus fluorescence *in situ* hybridization technology.

MAIN RESULTS AND THE ROLE OF CHANCE: The chromosomal abnormality rate was significantly higher in male miscarriages with an embryo than in those without an embryo (54.14% versus 37.50%, $P \leq 0.001$). In the anembryonic group, the abnormal karyotype rate was significantly higher in the yolk sac only than that in the empty sac group (46.11% versus 29.77%, $P = 0.001$); in the embryonic group, the abnormal karyotype rate in miscarriages with postmortem embryonic pole length >20 mm was significantly lower than that in miscarriages with pole length <10 mm ($P = 0.006$) and 10–20 mm ($P = 0.036$). There were significant differences in abnormal karyotype rates among miscarriages of different developmental stages ($P \leq 0.001$). The cases with embryonic stages had the highest risk (54.89%) of an abnormal karyotype and those with fetal stages had the lowest risk (18.18%). There were significant differences in the length of postmortem embryonic poles among groups with different karyotypes ($P \leq 0.001$). In addition, trisomy 21, monosomy X and triploidy had the longest lengths of postmortem embryonic poles (16, 15.3 and 11.6 mm, respectively).

LIMITATIONS, REASONS FOR CAUTION: Although the efficacy of a non-parametric test is less than that of a parametric test, non-parametric testing was used to compare the embryonic pole lengths in this study, owing to the non-normal distribution and non-homogeneous variances caused by limited cases of some rare chromosomal abnormalities. Another limitation was that CGH was unable to detect mosaicism. Furthermore, the results were not compared with a non-IVF population. Finally, maternal cell contamination is a major problem in studying miscarriage tissue, even using molecular techniques.

WIDER IMPLICATIONS OF THE FINDINGS: Although TVS findings clearly cannot replace karyotype information, our results are important because they call attention to the fact that EPLs that occur after implantation but prior to embryo formation frequently have normal karyotypes. This finding might direct future research towards studies of DNA sequences, or of epigenetic correlations with pregnancy failure.

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Key words: chromosomal abnormalities / early pregnancy loss / anembryonic pregnancy / postmortem fetal pole length / IVF-embryo transfer

Introduction

In natural conception, ~15% (Goldhaber and Fireman, 1991; Gindler *et al.*, 2001) of clinical pregnancies end in miscarriage, a majority of which occur in the first trimester (Jurkovic *et al.*, 2013), especially during the initial 8 weeks of gestation (Simpson, 1990). Chromosomal abnormalities cause more than half of early pregnancy losses (EPLs), and aneuploidy is the most frequently observed abnormality (Lathi and Milki, 2002; Hogge *et al.*, 2003; Zhang *et al.*, 2011). In the *in vitro* fertilization (IVF) population, the miscarriage rate is ~24–30% (Ezra and Schenker, 1995; Orvieto *et al.*, 2000), which is much higher than that in the ordinary population. Furthermore, there have been only a few studies (Plachot, 1989; Causio *et al.*, 2002) that have focused on cytogenetic analysis of EPL after IVF; thus, we have only limited information.

Transvaginal sonography (TVS) features high-resolution images, enabling the observation of embryonic structures and their viability. On TVS examination, EPLs show a range of developmental arrest stages: some might show a completely empty sac, while others might have evidence of an embryo, ranging from little evidence to a properly formed embryo. Miscarriages with different karyotypes can have disparate developmental potentials (Andrews *et al.*, 1984; Byrne *et al.*, 1985; Minelli *et al.*, 1993); therefore, there might exist some correlation between the degree of fetal development and types of chromosomal abnormalities (Angiolucci *et al.*, 2011; Ljunger *et al.*, 2011). Under such a premise, the EPLs with and without embryos on ultrasound examination might differ in karyotypes, and the different lengths of postmortem fetal poles could indicate different types of chromosomal abnormalities. What then would be the situation in the IVF population?

Three previous studies have compared the chromosomal distribution in association with the presence or absence of embryos in EPLs, and the results were inconsistent. The earlier two studies (Lathi *et al.*, 2007; Munoz *et al.*, 2010) showed no significant differences, but the more recent study (Cheng *et al.*, 2014) found that the abnormality rates differed significantly. Furthermore, little research has been conducted into the correlations between postmortem fetal pole length and chromosomal karyotypes. The aim of this study was therefore to compare the chromosomal distribution in embryos from EPLs with or without embryos and to determine the correlations between postmortem fetal pole length and different karyotypes after IVF treatment.

Materials and methods

Data from 2172 patients who underwent dilation and curettage (D&C) for missed abortion in the Reproductive and Genetic Hospital of Citic-Xiangya, Hunan, China from January 2008 to December 2013 were retrospectively analyzed. Written informed consent was obtained before D&C was implemented. All of the patients were infertile and experienced singleton pregnancy loss after IVF treatment. The flow of patients and the inclusion criteria are shown in Fig. 1.

A routine blood test for serum β-hCG was performed on Day 14 after IVF-embryo transfer. If β-hCG on Day 14 was >200 mIU/ml, routine TVS examinations were performed on Days 28, 45 and 70 by experienced sonographers to confirm the location, viability and growth of pregnancies; if β-hCG on Day 14 was ≤200 mIU/ml, a TVS scan was scheduled on Days 21–22, and re-examinations were performed every 7–10 days in the first trimester until the measurements of pregnancies were consistent with gestation or EPL was diagnosed. In addition, if patients presented with clinical symptoms, a TVS scan was scheduled sooner.

All of the patients received at least two TVS examinations between the 6th and 12th gestational weeks to confirm EPL. A GE VOLUSON E8/730 (General Electric Tech Co., Ltd., New York, USA) equipped with a 5–9 MHz vaginal color Doppler probe was used. EPL was diagnosed after the cessation of previously detected cardiac activity or continued absence of embryo-fetal structures inside the gestational sac on serial scans (Angiolucci *et al.*, 2011). EPLs were divided into embryonic and anembryonic groups, based on the presence or absence of an embryonic pole during diagnosis. The size of the gestational sac and the crown–rump length of the fetal pole (observed once) were measured during ultrasound examination. The length of the postmortem embryonic pole was measured twice for each fetus after confirmation of fetal death, subject to the final measurement before D&C, which was performed within 2 weeks of the diagnosed miscarriage. Patients' characteristics, such as maternal age (MA), infertility duration and grade of the transferred embryo were also documented. The embryo grading criteria in our hospital were as follows: grade I, blastomere sizes are regular, even division, fragmentation <5%, no multinucleation; grade II, most blastomere sizes are regular, fragmentation <20%, no multinucleation; grade III, blastomere sizes are irregular, fragmentation 20–50%, evidence of multinucleation; and grade IV, blastomere sizes are irregular, fragmentation >50%, evidence of multinucleation (Yi *et al.*, 2016).

In our center, cytogenetic tests for products of conception (POC) were routinely offered for patients to help to determine the causes of miscarriage. Karyotype analysis was performed by comparative genomic hybridization (CGH) plus fluorescence *in situ* hybridization (FISH) technology. The process of CGH detection was described in our previous study (Tan *et al.*, 2004). FISH was used to screen for polyploidy. Karyotype analysis was conducted using a digital image analysis system containing a Zeiss Axioplan 2 microscope equipped with a Metachrome II cooled-charged device camera (Zeiss, Oberkochen, Germany). Informed consent for cytogenetic tests of POC was obtained from all of the included patients. This study was approved by the institutional review board of the Reproductive and Genetic Hospital of Citic-Xiangya.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 19.0 (SPSS Inc., Chicago, IL, USA).

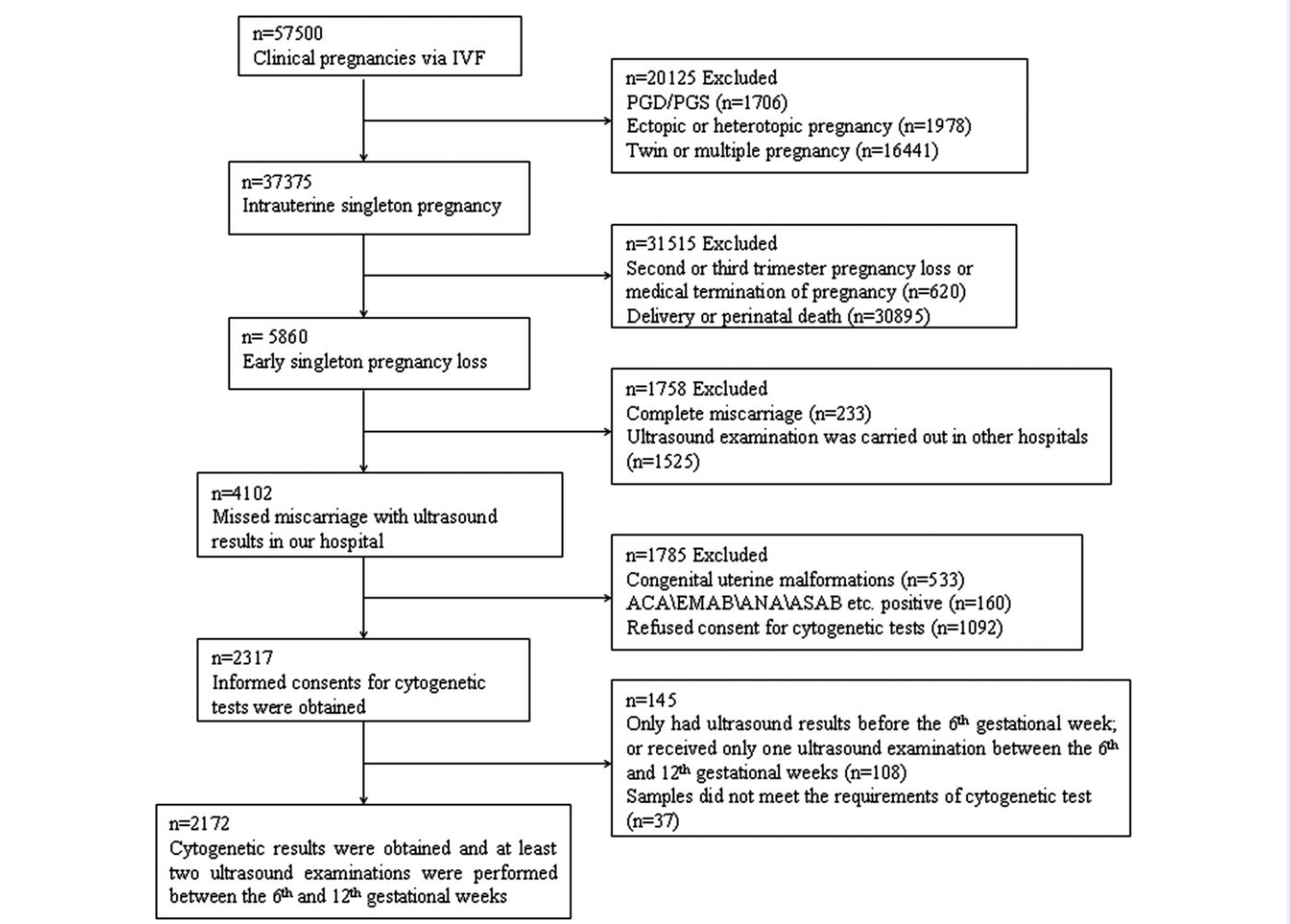

Figure 1 A flow chart of the cases included in this study of chromosomal distribution and embryonic findings on ultrasound in early pregnancy loss after IVF-embryo transfer. PGD, preimplantation genetic diagnosis; PGS, preimplantation genetic screening; ACA, anti-cardiolipin antibody; EMAB, endometrial antibody; ANA, antinuclear antibody; ASAB, anti-sperm antibody.

Student's *t*-test, Chi-square analysis or Fisher's exact test was used to compare the differences between the two groups. The overall differences in postmortem embryonic pole length with different chromosomal karyotypes were compared with the Kruskal–Wallis test. The differences among these groups were analyzed by Dunnett T3 after ranking. A value of $P < 0.05$ was considered as significant.

Results

Karyotype analyses were successful in all 2172 cases, 1227 of which were found to be embryonic (56.5%), while 945 were anembryonic miscarriages (43.5%). The characteristics and chromosomal abnormality rates are listed in Table I. Except for the days after embryo transfer at diagnosis, which was significantly higher in the embryonic group than that in the anembryonic group ($P \leq 0.001$), there were no differences found in other characteristics between these two groups.

When comparing miscarriages with or without embryos ($P = 0.998$) and with normal or abnormal karyotypes ($P = 0.105$), there were no statistically significant differences in the sex distribution. The proportion of female embryos was higher than that of male embryos in each group: embryonic group (56.72% versus 43.28%), anembryonic group (56.72% versus 43.28%), normal karyotype group (58.28% versus 41.72%) and abnormal karyotype group (54.81% versus 45.18%). To avoid the influence of maternal cell contamination (MCC), which is a major problem with studies of miscarriage tissue, only male losses were included to compare the chromosomal abnormality rate between the embryonic and anembryonic groups. The chromosomal abnormality rate was significantly higher in the embryonic group than in the anembryonic group (54.14% versus 37.50%, $P \leq 0.001$; Table I).

Table II shows the chromosomal distribution of male losses in these two groups. Autosomal trisomy was the most common abnormality in both groups. Trisomy 16 (10.00%) and trisomy 22 (8.30%) were the two most common abnormalities in both groups. Among the viable autosomal trisomies, trisomy 21 and trisomy 13 were more frequently found than trisomy 18. There were no differences in the rates of viable autosomal trisomy ($P = 1.000$), monosomy 21 ($P = 0.582$), triploidy ($P = 0.073$), tetraploidy ($P = 0.188$) or structural ($P = 1.000$) and multiple abnormalities ($P = 0.133$) between these two groups. Complex abnormality refers to more than one type of chromosomal abnormality, including double and multiple abnormalities (more than or equal to

Table I Comparison of characteristics and chromosomal abnormality rate between missed miscarriages with or without embryos.

	With embryo $n = 1227$	Without embryo $n = 945$	P
Maternal age (years)	32.33 ± 4.88	31.95 ± 4.85	0.07[b]
BMI (kg/m^2)	21.68 ± 2.99	21.11 ± 2.63	0.161[b]
Infertility duration (years)	5.40 ± 3.95	5.55 ± 3.96	0.49[b]
Infertility factor			0.14[c]
Male	680 (55.42%)	542 (57.35%)	
Female	171 (13.94%)	148 (15.66%)	
Male+female	376 (30.64%)	255 (26.99%)	
Infertility type			0.30[c]
Primary	482 (39.28%)	392 (41.48%)	
Secondary	745 (60.72%)	553 (58.52%)	
Previous miscarriage	278 (22.66%)	201 (21.27%)	0.440[c]
Transferred embryo no.	2.02 ± 0.18	2.00 ± 0.25	0.28[b]
Transferred embryo grade			0.29[a]
I	619 (50.45%)	458 (48.47%)	
II	604 (49.22%)	480 (50.79%)	
III	4 (0.33%)	7 (0.74%)	
IVF technique			0.20[c]
IVF	595 (48.49%)	440 (46.56%)	
ICSI	259 (21.11%)	184 (19.47%)	
FET	373 (30.40%)	321 (33.97%)	
Days after embryo transfer at diagnosis (days)	44.17 ± 10.82	36.79 ± 10.47	≤0.001[b]
Chromosomal abnormality rate of male losses	54.14% (288/532)	37.50% (153/408)	≤0.001[c]

[a]Fisher's exact test was used.
[b]Two-sample *t*-test was used.
[c]Chi-square test was used.
Measurement data are expressed as the mean ± SD. FET, frozen embryo transfer.

Table II Cytogenetic analysis in 940 male missed miscarriages with or without embryo via ultrasound examination.

Karyotype	With embryo	Without embryo	Total
Normal			
46, XY	244 (45.86%)	255 (62.50%)	499 (53.09%)
Abnormal			
Viable autosomal trisomy			
+21	25 (4.70%)	9 (2.21%)	34 (3.62%)
+13	19 (3.57%)	10 (2.45%)	29 (3.09%)
+18	6 (1.13%)	6 (1.47%)	12 (1.28%)
Other autosomal trisomy			
+2	2 (0.38%)	10 (2.45%)	12 (1.28%)
+3	3 (0.56%)	1 (0.25%)	4 (0.43%)
+4	7 (1.32%)	3 (0.74%)	10 (1.06%)
+5	3 (0.56%)	1 (0.25%)	4 (0.43%)
+6	5 (0.94%)	2 (0.49%)	7 (0.74%)
+7	3 (0.56%)	5 (1.23%)	8 (0.85%)
+8	10 (1.88%)	1 (0.25%)	11 (1.17%)
+9	13 (2.44%)	1 (0.25%)	14 (1.49%)
+10	2 (0.38%)	0	2 (0.21%)
+11	6 (1.13%)	4 (0.98%)	10 (1.06%)
+12	4 (0.75%)	3 (0.74%)	7 (0.74%)
+14	12 (2.26%)	2 (0.49%)	14 (1.49%)
+15	21 (3.95%)	4 (0.98%)	25 (2.66%)
+16	53 (9.96%)	41 (10.05%)	94 (10.00%)
+17	7 (1.32%)	1 (0.25%)	8 (0.85%)
+19	1 (0.19%)	1 (0.25%)	2 (0.21%)
+20	9 (1.69%)	11 (2.70%)	20 (2.13%)
+22	61 (11.47%)	17 (4.17%)	78 (8.30%)
Monosomy 21	1 (0.19%)	2 (0.49%)	3 (0.32%)
47, XXY	0	2 (0.49%)	2 (0.21%)
Triploidy	5 (0.94%)	0	5 (0.53%)
Tetraploidy	0	2 (0.49%)	2 (0.21%)
Structural abnormality	1 (0.19%)	1 (0.25%)	2 (0.21%)
[a]Complex abnormality			
Double abnormalities	9 (1.69%)	12 (2.94%)	21 (2.23%)
Multiple abnormalities	0	1 (0.25%)	1 (0.11%)
Total	532	408	940

[a]Complex abnormality refers to more than one type of chromosomal abnormality, including double and multiple abnormalities (more than or equal to three abnormalities) in this study.

three abnormalities) in this study. The cytogenetic results of female losses are shown in Supplementary Table SI.

The abnormal karyotype rate of male losses according to different ultrasound findings is listed in Table III. The anembryonic group could be further divided into an empty sac group and a yolk sac only group, based on TVS examination. The abnormal karyotype rate was significantly higher in the yolk sac only group than in the empty sac group (46.11% versus 29.77%, $P = 0.001$). In the embryonic group, the abnormal karyotype rate was the highest in miscarriages with post-mortem embryonic pole length <10 mm (56.39%), followed in order by 10–20 mm (52.48%) and >20 mm (31.25%) (overall comparison, $P = 0.021$). The abnormal karyotype rate in miscarriages with post-mortem embryonic pole length >20 mm was significantly lower than that with lengths of <10 mm ($P = 0.006$) and 10–20 mm ($P = 0.036$), while no significant difference was found between lengths of <10 mm and 10–20 mm ($P = 0.479$).

If miscarriages were classified into different developmental stages (Romero *et al.*, 2015), the abnormality rates were 37.50, 54.89 and 18.18% in cases of pre-embryonic ($n = 408$), embryonic ($n = 521$, an observed embryo less than the corresponding size for 10 weeks' gestation) and fetal stages ($n = 11$, a crown–rump length >30 mm or other biometry consistent with that at ≥10 weeks' gestation), respectively (overall comparison, $P \leq 0.001$). The difference persisted when comparing embryonic losses with pre-embryonic losses ($P \leq 0.001$)

and with fetal losses ($P = 0.016$) but not between pre-embryonic and fetal losses ($P = 0.225$).

The correlation between postmortem embryonic pole length and different karyotypes was also analyzed, excluding the 697 cases with 46,XX. There were statistically significant differences in the length of the postmortem embryonic pole with different karyotypes ($P \leq 0.001$). The postmortem embryonic pole length with each abnormal karyotype was significantly different from the length with a normal karyotype ($P \leq 0.001$). The median postmortem embryonic pole lengths relative to different cytogenetic findings are shown in Fig. 2. Trisomy 21, monosomy X and triploidy were found with the longest lengths of the postmortem embryonic pole (16, 15.3 and 11.6 mm, respectively).

Table III Abnormal karyotype rate in relation to different ultrasound findings in male losses.

Ultrasound findings	Normal karyotype	Abnormal karyotype	Total	Abnormal karyotype rate (%)	*P*
Without embryo	255	153	408	37.50	0.001[a]
Empty sac	151	64	215	29.77	
Yolk sac only	104	89	193	46.11	
With embryo	244	288	532	54.14	0.021[b]
<10 mm	174	225	399	56.39	
10–20 mm	48	53	101	52.48	
>20 mm	22	10	32	31.25	

[a]Comparison between empty sac and yolk sac only groups was using chi-square test.
[b]Comparisons in embryonic group were all using chi-square test (overall and between embryos with different length intervals).
Overall comparison ($P = 0.021$):
<10 mm versus 10–20 mm ($P = 0.479$);
<10 mm versus >20 mm ($P = 0.006$);
10–20 mm versus >20 mm ($P = 0.036$).

Discussion

As we know, MCC is a common problem in conventional tissue culture, and the problem also exists in molecular techniques, such as CGH. The proportion of female embryos was much higher than male embryos in this study. In addition to MCC, the more stable maternal X chromosome in XY embryos (Evdokimova *et al.*, 2000) and early selection *in utero* or during embryo development (Cheng *et al.*, 2014) might also play roles because the proportion of female losses was also higher than that of male losses in the abnormal karyotype group. Therefore, the analysis of the abnormal karyotype rate in this study was confined to male losses, and the abnormality rate of 46.91% (441/940) suggested that IVF treatment did not increase the risk of chromosomal abnormality, as demonstrated previously (Qin *et al.*, 2013).

The abnormal karyotype rate in the embryonic group was significantly higher than that in the anembryonic group; this finding was in agreement with a previous study (Cheng *et al.*, 2014) but was inconsistent with two other studies (Lathi *et al.*, 2007; Munoz *et al.*, 2010). Why then do EPLs with embryos have a higher abnormality rate than EPLs without embryos? One possible explanation might be that non-genetic factors, such as maternal causes, affect the early development of the embryonic pole and thus cause its very early demise. However, after the embryonic pole has developed, genetic factors might play a critical role in early miscarriage.

Interestingly, we found that the abnormality rate in miscarriages with a yolk sac only was markedly higher than in those with an empty sac in the anembryonic group, which suggested that the genetic factor had relatively more important effects on the very early demise of embryo structures than the appearance of the yolk sac. However, most previous studies (Goldstein *et al.*, 1996; Angiolucci *et al.*, 2011) have been prone to regarding an empty sac to be correlated with a

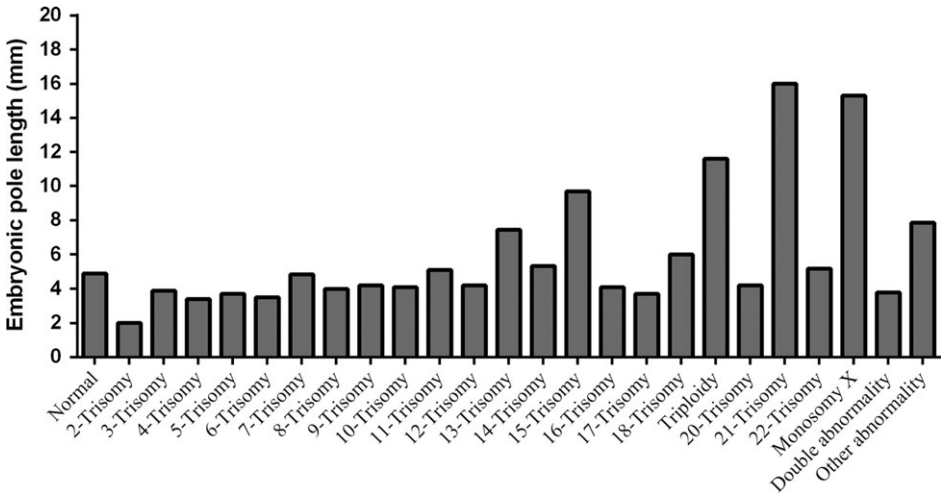

Figure 2 The median postmortem embryonic pole lengths in relation to different cytogenetic findings. Trisomy 21, monosomy X and triploidy were found with the longest embryonic pole lengths (16, 15.3 and 11.6 mm).

high prevalence of chromosomal abnormalities. Miscarriage is a complex process, caused by multi-etiological factors. Apart from chromosomal abnormality, other causes, including fetal anomaly, immunological factors and hormone factors, have also been identified (Regan, 1991). Although any maternal diseases have been treated, or women recovered from them before the IVF cycle, the influence of basic endocrine or other unknown factors still could not be entirely excluded. Because an empty sac is mainly correlated with normal karyotypes, when we observe this type of EPL, non-genetic factors should be considered.

The first miscarriage is always sporadic, so it does not increase the chromosomal abnormality rate in the next pregnancy (Cheng *et al.*, 2014). Therefore, it seems wasteful and unnecessary to perform cytogenetic tests for each patient in the first miscarriage, although if patients want to know the approximate causes, TVS results can be useful. However, for recurrent pregnancy loss (RPL), if an anembryonic miscarriage, especially with an empty sac, was found, it is necessary to refer patients to evaluation for RPL. These experiences are important in our clinical practice for providing clues to possible causes and guidance for future pregnancies.

In the embryonic group, the abnormal karyotype rate was lowest in miscarriages with postmortem embryonic pole length >20 mm, suggesting that longer postmortem embryonic pole length might be correlated with a lower risk of chromosomal abnormality. Correspondingly, the abnormal karyotype rate was low in the fetal stage, and most cases in this stage, as anticipated, had viable autosomal trisomy in this study. Few studies have analyzed the abnormal karyotype rate across different developmental stages. One recently published study by Romero *et al.* (2015) supported the difference in abnormal karyotype rate among different developmental stages and the embryonic stage having the highest risk of karyotype abnormality. However, one discrepancy was that, although there were no significant differences between pre-embryonic and fetal stages in either our study or that of Romero *et al.* (2015), the abnormality rate at the fetal stage was lower than that in the pre-embryonic stage in our study, which was the opposite of the results of Romero *et al.* (2015). The cases enrolled in the study by Romero *et al.* (2015) were prior to 20 weeks of gestation, while they were prior to 12 weeks of gestation in ours, which might have caused the relatively fewer cases and lower abnormality rate of the fetal stage in our study.

In this study, some karyotypes, such as trisomy 21, monosomy X and triploidy, were found to be associated with longer postmortem embryonic poles than others, which powerfully demonstrated the viewpoint that some karyotypes might have stronger potential to develop further before demise (Goldstein *et al.*, 1996). Compared to miscarriages with normal karyotypes, the pole lengths of some miscarriages with abnormal karyotypes were shorter, whereas some were longer, but all of them had significant differences from the normal group in our study. However, Dickey *et al.* (1994) claimed that the embryonic pole lengths of miscarriages with abnormal karyotypes were shorter than normal ones. Different study sample sizes and diagnostic skills might have resulted in this discrepancy. Although it is difficult to judge karyotypes directly from the embryonic pole lengths, due to the length overlapping with some karyotypes, some embryonic pole lengths might be correlated with specific karyotypes. For example, in the embryonic group, 33.33% of trisomy 21 cases and 37.80% of monosomy X cases had a postmortem embryonic pole length greater than 20 mm, whereas a greater than 20 mm postmortem embryonic pole length could only rarely be found in miscarriages with other karyotypes. Thus, a postmortem embryonic pole length greater than 20 mm seemed to be predictive of trisomy 21 or monosomy X, corroborating the findings of some previous studies (Bessho *et al.*, 1995; Ikegawa, 1995).

Notably, although the efficacy of a non-parametric test is less than that of a parametric test, we used non-parametric tests to analyze the embryonic pole length due to non-normal distribution and non-homogeneous variances caused by limited cases of some rare chromosomal abnormalities. Another limitation of our work was that CGH was unable to detect mosaicism; therefore, there were no cases of mosaicism included in our study. Furthermore, the results were not compared with those of a non-IVF population. Finally, MCC is a major problem in studying miscarriage tissue, even using molecular techniques.

In summary, in early pregnancy failure diagnosed by TVS, embryo presence was associated with an increased likelihood of chromosome abnormality, and some postmortem fetal pole lengths were correlated with specific karyotypes after IVF treatment.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

Y.O., X.L. and Y.Y. collected the data. Y.O. performed the statistical analysis and drafted the manuscript. Y.T. mainly responsible for collecting and analyzing laboratory data. F.G. and G.L. helped to collect relevant clinical data. G.L. and X.L. conceived the study, supervised the data collection and helped to draft the manuscript. All authors have been involved in revising this manuscript and approved the final version.

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Conflict of interest

None declared.

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