Ultrasound Obstet Gynecol 2011; **37**: 219–225
Published online 12 January 2011 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.7681

Association between different morphological types and abnormal karyotypes in early pregnancy loss

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KEYWORDS: abortion; chromosomal abnormalities; CRL; early pregnancy loss; gestational sac; karyotype; transvaginal ultrasound; yolk sac

ABSTRACT

Objectives *To identify the abnormal transvaginal ultrasound (TVS) findings typical of aneuploidic pregnancies that end with early pregnancy loss (EPL).*

Methods *This was a prospective clinical trial over a 2½-year period from January 2004 to June 2006 at the University Hospital of Cagliari, Italy. One hundred and fifty-six singleton pregnancies with EPL were evaluated by TVS, both before and at the moment of EPL diagnosis. Fetal karyotyping was performed on products of conception using microsatellite analysis to exclude maternal contamination in 46,XX cases. The proportions of abnormal karyotypes were compared among different groups of EPLs characterized by different morphological features.*

Results *Six morphological types were identified in EPLs, one normal and five abnormal (small gestational sac, small embryo/fetus, early symmetrical arrested growth, enlarged yolk sac and empty sac). The highest rate of chromosomal abnormalities was observed in the early symmetrical arrested growth group (100%), followed by small embryo/fetus (94.1%), enlarged yolk sac (93.3%) and empty sac (72.2%) groups. The majority of cases of trisomy 22 (92.3%) were in the enlarged yolk sac group and the majority of cases with a 45,X karyotype were in the small embryo/fetus group (77.8%).*

Conclusions *There is an association in EPLs between some abnormal karyotypes and some morphological types. The demonstration by TVS of abnormalities in the development of early pregnancy structures could be helpful for predicting aneuploidy in EPLs. Copyright © 2011 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

The efficiency of reproductive processes is low in humans. The conception rate is lower than 30% per cycle[1] and over 60% of conceptuses are lost prior to 12 weeks[2]. Preclinical loss rates range between 22% and 55%[1–5], whereas loss rates after early clinical recognition of pregnancy decrease with increasing gestational age; before 8 weeks of gestation the loss rate is 12–15%, after 8 weeks of gestation the likelihood of losing a viable pregnancy is only 3%, and after 16 weeks it is only 1%[6]. In contrast, fetal loss rate increases with increasing maternal age[7].

Transvaginal ultrasound (TVS) has made it possible to identify early pregnancy, to study early embryo-fetal structures, to recognize complications of early pregnancy and to establish criteria for diagnosis of non-viable pregnancies[8,9]. Moreover, TVS has rapidly replaced all other techniques used to study normal human development in the first trimester. Analysis of the sonographic features of the early gestational sac has shown that the first structures to appear are the celomic cavity and the secondary yolk sac[10]. Many studies on the prognostic significance in early gestation of the presence or absence, size and shape of the gestational sac, yolk sac and embryo-fetus as potential predictors of poor pregnancy outcome have been published[11–17]. The majority of these studies suggest an association between the abnormal sonographic appearance of early pregnancy structures and early pregnancy loss (EPL).

Cytogenetic studies performed on products of conception have demonstrated that chromosomal abnormalities are the most common cause of EPL, accounting for over 50% of fetal demise[18–22]. However, few of these studies investigated the relationship between chromosomal abnormalities and TVS findings in EPL. Moreover, only a few ultrasound findings were taken into account, such

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Accepted: 7 April 2010

as the presence or absence of an embryonic pole, variations from the expected crown–rump length (CRL) of the embryo-fetus, presence of trophoblastic disease or empty sac[23–25].

Some authors investigated specific abnormal ultrasound findings trying to correlate them with some abnormal karyotypes. Unfortunately, however, they were unable to exclude maternal contamination of the samples of products of conception, because molecular techniques were not applied. Moreover, the number of cases having both sonographic and cytogenetic data was very limited[15,16,26].

The aim of the present study was to investigate prospectively whether associations exist between abnormal morphological features and abnormal karyotypes in a large number of EPLs with both TVS and cytogenetic data. Microsatellite analysis was used in order to exclude maternal contamination in 46,XX cases[27].

METHODS

Between January 2004 and June 2006, we examined with TVS all early pregnancies presenting at the Prenatal Diagnosis Service of the Division of Gynecology, Obstetrics and Pathophysiology of Human Reproduction at the Department of Surgery, Maternal-Fetal Medicine and Imaging of the University of Cagliari. From among these we selected those with asymptomatic singleton pregnancy at the time of the first scan who then suffered an EPL. To be included in the study, the EPL cases had also to meet the following criteria: spontaneous conception, regular menstrual cycles (range, 26–32 days) in the last year, at least one TVS scan performed prior to documentation of EPL, and successful karyotyping from products of conception. Informed consent for cytogenetic analysis on products of conception was obtained.

TVS scans were performed using a Hitachi Astro scanner (Hitachi Medical Corporation, Tokyo, Japan), equipped with a 5–7.5-MHz transvaginal transducer. Gestational age at the time of EPL was based on the last menstrual period. The EPL was diagnosed during a second scan by demonstration of the cessation of previously detected cardiac activity or by the continued absence of embryo-fetal structures inside the gestational sac in serial scans (empty sac). The second scan was performed 3–30 days after the first scan: as soon as possible if an early pregnancy complication was suspected because of the commencement of bleeding or pain; at a short scheduled interval (7 days) in the case of an empty sac, abnormal morphological features or bradycardia observed during the first scan; or at the time of the nuchal scan in asymptomatic pregnancies with normal morphological features and normal heart beat at the first scan. Pregnancies were brought to an end by dilatation and curettage (D & C) after sonographic confirmation of EPL. Quantitative assessment of the gestational sac was performed by averaging the diameters obtained by three perpendicular measurements according to Lindsay *et al.*[11] and Blaas *et al.*[28]. Quantitative assessment of the yolk sac was obtained by averaging the diameters obtained by three perpendicular measurements, placing the calipers inside the yolk sac wall according to Lindsay *et al.*[11]. Abnormalities in yolk sac development were detected by comparing the diameters obtained in the cases of EPL with biometric reference data reported by Lindsay *et al.*[11], who provided specific graphs with reference ranges of yolk sac diameter vs. menstrual age, CRL and gestational sac diameter, with 95% CIs, for patients with normal pregnancy outcome. The embryo-fetus was measured by evaluating the CRL according to biometric reference data reported by Grisolia *et al.*[29].

The products of conception were obtained from the D & C procedure under sterile conditions. Once the maternal decidua had been eliminated, cytogenetic analysis was performed directly on chorionic villus cells before culture, on cultured chorionic villus cells and on cultured cells from other embryonic tissue. The procedures used for the purposes of this study differed slightly from standard protocols. For each sample, we used at least two methods (direct cytogenetic analysis and cell culture) or two different tissues, or several cell cultures from the same tissue prepared in parallel. When the cytogenetic analysis highlighted a normal female karyotype, maternal and embryo-fetal DNA (deoxyribonucleic acid) was compared using molecular analysis of inheritance of polymorphic DNA markers (microsatellite analysis), in order to exclude from the study any samples with maternal cell contamination[27]. Cytogenetic analysis was performed using the GTG banding technique (visualizing G-bands using trypsin and Giemsa staining), with a resolution of at least than 350 bands. For each sample we analyzed no fewer than eight clones for *in situ* cultures, or 15 metaphases in the case of direct analysis. When necessary, fluorescence *in-situ* hybridization was used.

The mean gestational age and mean maternal age among different groups of TVS morphological features found in EPLs were compared using single factor analysis of variance (ANOVA) and multiple range testing. The proportion of chromosomal abnormalities in the different groups of morphological features were compared using Fisher's exact test; the relative risk and 95% CI were calculated with Katz's approximation. Analysis was carried out using InStat version 3.0 (GraphPad Software Inc., San Diego, CA, USA, 1994).

RESULTS

From January 2004 to June 2006, there were 2479 early pregnancies examined by TVS in our unit. Of these, 483 were EPLs, of which 327 (67.7%) were excluded for the following reasons: pregnancy was not singleton ($n = 27$ (5.6%)), conception was not spontaneous ($n = 69$ (14.3%)), menstrual cycle in the last year was not regular ($n = 45$ (9.3%)), no TVS scan prior to documentation of EPL ($n = 92$ (19.0%)) and no embryo-fetal karyotype ($n = 94$ (19.5%)). Of the 327 EPLs excluded from the study, 231 were symptomatic at the time of the first scan.

The remaining 156 (32.3%) EPL cases met the criteria for inclusion in the study. Their mean (± SD) maternal age was 35.6 ± 4.7 years and the overall mean (± SD) gestational age at EPL diagnosis was 8 + 0 (± 1 + 3) weeks. In only four cases was there a history of recurrent miscarriage; three women had each suffered three previous miscarriages and one woman had suffered four, all for unexplained reasons (both the women and their partners had normal karyotype and negative family history, and the women were negative for uterine abnormalities immunological disorders and thrombophilic disorders). Karyotyping of products of conception had not been performed in the previous miscarriages. Karyotyping of these cases in the present study revealed three to have chromosomal abnormalities (47,XXY; 47,XX,+18; 69,XXY) and one had a normal female karyotype.

Comparison of ultrasound findings from the first scan (performed prior to documentation of EPL, when there was a heart beat) with biometric reference data[11,29] led to the identification of five different morphological types (Figures 1–6): (1) normal ultrasound, with gestational sac, yolk sac and embryo-fetus measurements all consistent with gestational age; (2) small gestational sac, with gestational sac < 5th centile for expected size and normal CRL (Figure 1), with a difference between gestational sac diameter and CRL of < 5 mm (Dickey *et al.*[14]); (3) small embryo-fetus, with gestational sac diameter consistent with gestational age or larger, but CRL < 5th centile for expected size (Figure 3); (4) early symmetrical arrested growth, with simultaneous arrest of growth of both gestational sac and CRL; and (5) enlarged yolk sac, with yolk sac diameter > 95th centile for expected size (Figure 5). A sixth morphological type, empty sac, was identified on observing the repeated absence of fetal structures inside the gestational sac in more than one TVS scan. There were 68 (43.6%) cases with normal ultrasound and the other 88 (56.4%) cases were each categorized into one of the five abnormal morphological types (Table 1). There were no significant differences in maternal age or gestational age at the time of EPL diagnosis among the six groups.

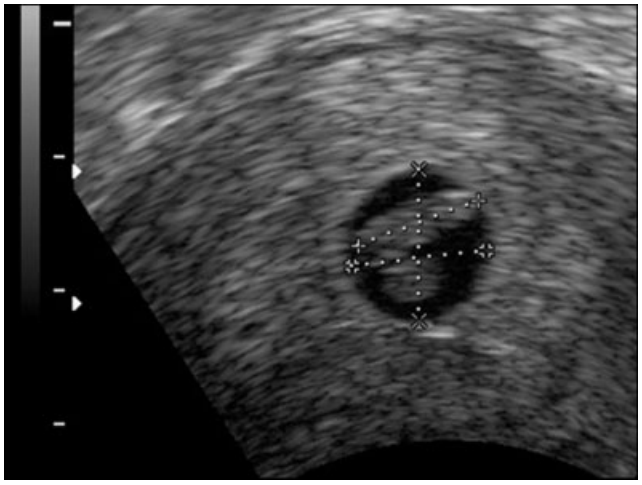

Figure 1 Sonographic appearance at 7 + 0 weeks of small gestational sac in a case which then suffered early pregnancy loss, showing small mean gestational sac diameter (11 mm, × and ⌗ calipers) and normal crown–rump length (9 mm, + calipers).

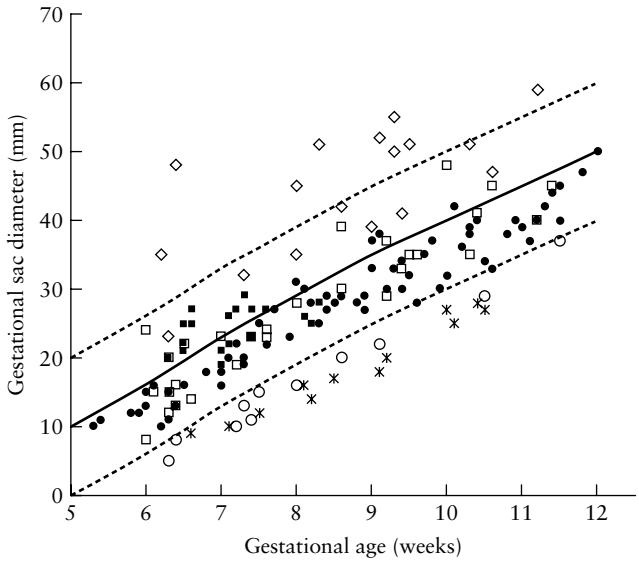

Figure 2 Gestational sac mean diameter in early pregnancy losses with different sonographic morphological features: normal ultrasound (●), enlarged yolk sac (□), small gestational sac (○), small embryo-fetus (◇), early symmetrical arrested growth (⁕) and empty sac (■), plotted on normal reference curves (5th, 50th and 95th centiles) from Grisolia *et al.*[29].

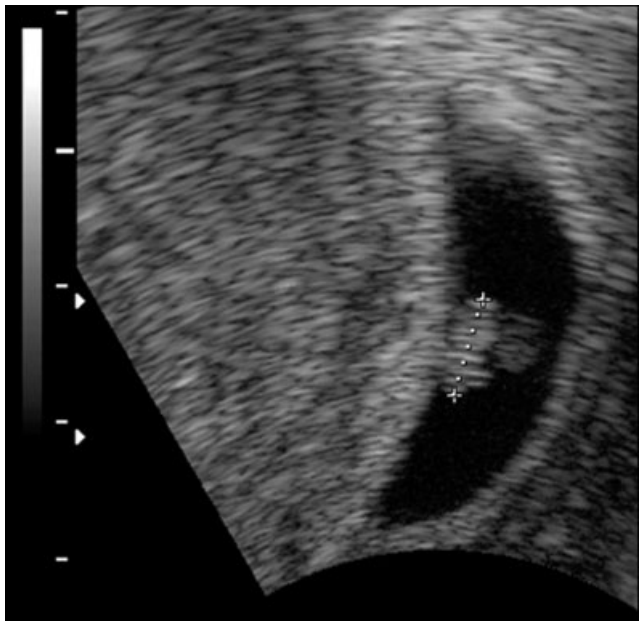

Figure 3 Sonographic appearance at 7 + 3 weeks of small embryo-fetus in a case which then suffered early pregnancy loss, showing small crown–rump length (5 mm, calipers) and normal/large mean gestational sac diameter (23 mm).

Cytogenetic analysis revealed 58 (37.2%) normal and 98 (62.8%) abnormal karyotypes. Trisomy 21 and trisomy 22 were the most frequent chromosomal abnormalities, each occurring in 13 cases. Trisomy 16 occurred in 10 cases, 45,X karyotype and triploidies in nine cases each, structural abnormalities in eight cases,

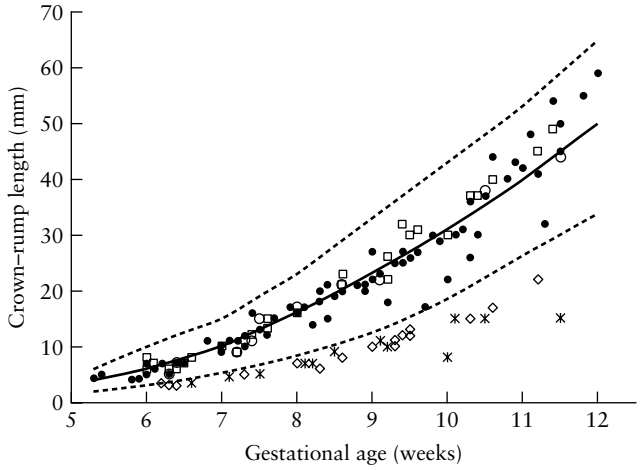

Figure 4 Crown–rump length in early pregnancy losses with different sonographic morphological features: normal ultrasound (●), enlarged yolk sac (□), small gestational sac (○), small embryo-fetus (⁎) and early symmetrical arrested growth (◇), plotted on normal reference curves (5th, 50th and 95th centiles) from Grisolia *et al.*[29].

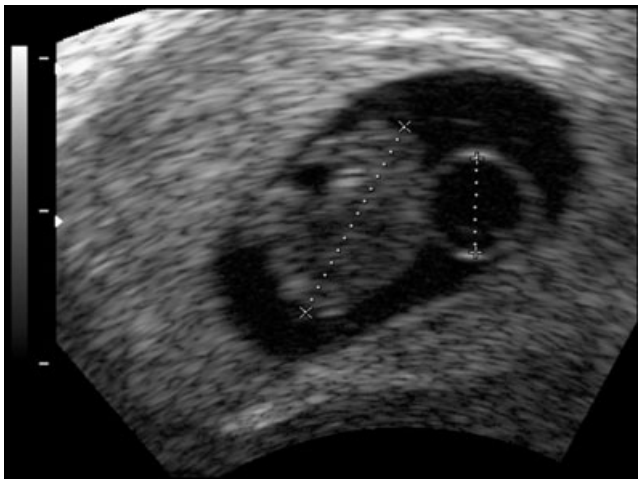

Figure 5 Sonographic appearance at 7 + 3 weeks of enlarged yolk sac in a case which then suffered early pregnancy loss, showing large yolk sac mean diameter (6.1 mm, + calipers) and normal crown–rump length (13.6 mm, × calipers).

trisomy 15 in six cases and trisomy 13 in five cases. Among the 58 cases with normal karyotype, the male to female sex ratio was 0.71 or 24 : 34 (Table 1).

The number of chromosomal abnormalities found in EPLs with normal ultrasound was compared with those found in EPLs of the different abnormal morphological types (Table 2). The prevalence of chromosomal abnormalities was significantly higher when there were abnormal morphological features compared with cases having normal ultrasound (75/88, 85.2% vs. 23/68, 33.8%, $P < 0.0001$). Specifically, the highest prevalence of chromosomal abnormalities was found in the early symmetrical arrested growth group (12/12, 100%), followed by the small embryo-fetus group (16/17, 94.1%), the enlarged yolk sac group (28/30, 93.3%) and the

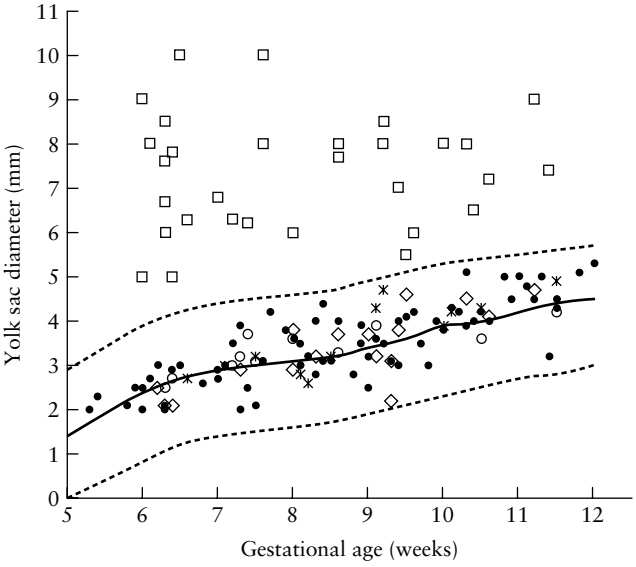

Figure 6 Yolk sac mean diameter in early pregnancy losses with different sonographic morphological features: normal ultrasound (●), enlarged yolk sac (□), small gestational sac (○), small embryo-fetus (⁎) and early symmetrical arrested growth (◇), plotted on normal reference curves (5th, 50th and 95th centiles) from Lindsay *et al.*[11].

empty sac group (13/18, 72.2%) (Table 1). The prevalence of chromosomal abnormalities in each of these four abnormal morphological types was significantly higher than that in EPLs with normal ultrasound (Table 2). In contrast, the prevalence of chromosomal abnormalities in the small gestational sac group (6/11, 54.6%) was no different from that in EPLs with normal ultrasound (Table 2).

Ten of the 13 trisomy 21 cases were EPLs with normal ultrasound. Twelve of the 13 trisomy 22 cases were in the enlarged yolk sac group ($P < 0.001$, relative risk (RR), 1.65; 95% CI, 1.23–2.22). Seven of the nine cases with 45,X karyotype were in the small embryo-fetus group ($P < 0.001$; RR, 1.68; 95% CI, 1.12–2.49) (Table 1).

DISCUSSION

The first 8 weeks of gestation are fundamental for human development because the major and vital anatomical structures begin to develop during this time. The rate of pregnancy loss before 8 weeks of gestation is five times higher than that after 8 weeks[6] and the majority of published studies suggest an association between abnormalities in the sonographic appearance of early anatomical structures and EPL[11–13,15,16,29].

Both normal and abnormal morphological features can be found in EPLs but the majority of EPLs have abnormal morphological features. The small gestational sac morphological type consisted of cases showing normal growth of the CRL together with arrested growth of the gestational sac. These morphological features have already been defined by other authors as 'small gestational sac syndrome'[14,15] and our study confirms that

Table 1 Karyotypes found in different morphological types of early pregnancy loss

Karyotype	*Normal ultrasound findings* (n)	*Abnormal ultrasound findings* (n)						*Total* (n)
		Small gestational sac	*Small embryo-fetus*	*Early symmetrical arrested growth*	*Enlarged yolk sac*	*Empty sac*	*Total*	
Abnormal								
Trisomies								
Trisomy 22	0	0	0	1	12	0	13	13
Trisomy 21	10	0	3	0	0	0	3	13
Trisomy 16	0	0	0	4	3	3	10	10
Trisomy 15	0	0	0	2	3	1	6	6
Trisomy 13	4	1	0	0	0	0	1	5
Trisomy 20	0	0	0	0	1	1	2	2
Trisomy 18	2	0	0	0	0	0	0	2
Trisomy 14	0	0	0	1	1	0	2	2
Trisomy 12	0	0	0	2	0	0	2	2
Trisomy 10	1	0	1	0	0	0	1	2
Trisomy 7	0	0	0	1	0	1	2	2
Trisomy 19	0	0	0	0	1	0	1	1
Trisomy 17	0	0	0	0	1	0	1	1
Trisomy 11	0	1	0	0	0	0	1	1
Trisomy 9	0	0	1	0	0	0	1	1
Trisomy 8	0	0	0	0	0	1	1	1
Trisomy 2	0	0	0	0	1	0	1	1
Total	17	2	5	11	23	7	48	65
45,X	2	0	7	0	0	0	7	9
47,XXY	0	0	1	0	1	0	2	2
69,XXX	1	1	2	0	0	0	3	4
69,XXY	2	3	0	0	0	0	3	5
92,XXXX	0	0	0	0	0	1	1	1
92,XXYY	0	0	0	0	0	1	1	1
Structural abnormality	1	0	1	1	2	3	7	8
Double trisomy	0	0	0	0	2	1	3	3
Total abnormal	23	6	16	12	28	13	75	98
Normal								
46,XX	27	3	0	0	1	3	7	34
46,XY	18	2	1	0	1	2	6	24
Total normal	45	5	1	0	2	5	13	58
Total	68	11	17	12	30	18	88	156

Table 2 Comparison of proportion of abnormal karyotypes found in early pregnancy losses (EPLs) with abnormal morphological features vs. proportion of abnormal karyotypes found in EPLs with normal ultrasound

Morphological type	*Abnormal karyotypes/ total karyotypes* (n)	*Fisher's exact test*	
		P	*RR (95% CI)*†
Normal ultrasound	23/68		
Abnormal ultrasound	75/88	< 0.0001	4.48 (2.64–7.61)
Small gestational sac	6/11	0.1990*	1.45 (0.74–1.46)
Small embryo-fetus	16/17	< 0.0001	11.25 (1.66–75.93)
Early symmetrical arrested growth	12/12	< 0.0001	NC
Enlarged yolk sac	28/30	< 0.0001	9.93 (2.57–38.29)
Empty sac	13/18	0.0061	2.38 (1.11–5.12)

*Not significant. †Relative risk (RR) and 95% CI calculated using the Katz's approximation. NC, Relative risk cannot be calculated.

this morphological type does not correlate with a specific chromosomal abnormality.

The small embryo-fetus type included EPLs showing normal or increased growth of the gestational sac together with arrested growth of the CRL. This morphological type is probably the same as that defined in 1997 by Coulam *et al.*[16] as 'fetus smaller than expected for gestational age', although these authors did not compare the CRL with the gestational sac size. In contrast with previous publications, we found the vast majority of EPLs with karyotype 45,X to be of this morphological type.

The early symmetrical arrested growth type included EPLs showing concomitant early arrested growth of both the CRL and the gestational sac. All cases that manifested

these features had an abnormal karyotype, although there was no association with a specific chromosomal abnormality.

The enlarged yolk sac morphological type has already been described[11,12] but an association between enlarged yolk sac and specific chromosomal abnormalities has never been described. In contrast, we found the enlarged yolk sac feature in the vast majority of cases of EPL that had a trisomy 22 karyotype. This association suggests that fundamental genes for embryonic development may be localized on chromosome 22, and their overexpression might determine potentially lethal abnormalities in the embryo-fetal circulation. The accumulation of fluid inside the yolk sac could be an early sign of such abnormalities in the embryo-fetal circulation. In all cases of EPL with enlarged yolk sac, the abnormal increase in yolk sac size was already present before miscarriage and therefore this cannot be attributed to postmortem events[30].

The empty sac morphological type has been described previously by many authors[11,12,17]. The incidence of chromosomal abnormalities was significantly higher in this group of EPLs than in those with normal ultrasound.

Finally, 10 of the 13 cases of trisomy 21 were EPLs with normal ultrasound. This finding confirms that abnormalities of anatomical structures are difficult to detect by TVS in an embryo-fetus with trisomy 21 before 11 weeks of gestation[31,32].

Overall, these results demonstrate that morphological features are often associated with specific chromosomal abnormalities in EPLs. The small embryo-fetus type was found to correlate with a 45,X karyotype, enlarged yolk sac correlated with trisomy 22 and normal ultrasound correlated with trisomy 21. These results are at variance with previous reports by other authors showing no association between morphological features and karyotypes in EPL[14–16,26]. However, the total percentage and distribution of the different chromosomal abnormalities as well as the male to female sex ratio found in the present study did not differ significantly from those reported in previous cytogenetic studies performed on EPLs[20–22,24,25], adding strength to our results.

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