Chorionic villous vascularization related to phenotype and genotype in first trimester miscarriages in a recurrent pregnancy loss cohort

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STUDY QUESTION: Is there an association between chorionic villous vascularization, ultrasound findings and corresponding chromosome results in early miscarriage specimens from a cohort of recurrent pregnancy loss patients?

SUMMARY ANSWER: We did not find a significant difference in vascularization scores of chorionic villi between embryonic, yolk sac or empty sac miscarriages, or between euploid and noneuploid miscarriages.

WHAT IS KNOWN ALREADY: At least half of first trimester miscarriages are due to embryopathogenesis associated with chromosome errors and/or major congenital anomalies, resulting in an empty sac, a yolk sac or an embryonic miscarriage. Absent and decreased chorionic villous vascularization is usually present in these pregnancies.

STUDY DESIGN, SIZE, DURATION: For this retrospective study, 60 hematoxylin and eosin slides of miscarriage tissue of less than 10 weeks gestational age were collected from an academic institution. All patients were seen in consultation between July 2004 and October 2009.

PARTICIPANTS, SETTING, METHODS: Chorionic villous vascularization was determined using a previously published classification. The results were validated and compared with the ultrasound findings and corresponding chromosome results.

MAIN RESULTS AND THE ROLE OF CHANCE: There were 53 embryonic miscarriages, 5 yolk sac miscarriages and 2 empty sac miscarriages. Chromosome results were obtained in 59 of the 60 miscarriages; 37.3% were euploid and 62.7% were noneuploid. Validation of the vascularization score between observers was reasonable to good (Kappa 0.47–0.76), and 59% of the cases were classified as avascular. The vascularization score did not differ between euploid or noneuploid miscarriages, or between embryonic, yolk sac or empty sac miscarriages. Avascular villi were seen more frequently in miscarriages trisomic for chromosome 16, when compared with miscarriages with other trisomies (6 out of 7 versus 8 out of 22, \(P = 0.04\)).

LIMITATIONS, REASONS FOR CAUTION: Unfortunately, the number of samples in the study was limited.

WIDER IMPLICATIONS OF THE FINDINGS: Avascular villi may indicate abnormal early placentation as a part of embryopathogenesis. Further study is warranted to determine whether a genetic cause can be found to explain these results.

Key words: recurrent miscarriage / vascularization / chromosome results / ultrasound / pregnancy loss
Introduction

At least half of first trimester miscarriages are associated with chromosome abnormalities (Hassold et al., 1980). In addition, there are miscarriages with embryonic developmental defects that have normal chromosome results (Philipp et al., 2003, 2004; van den Berg et al., 2012). Miscarriage implies an intrauterine pregnancy loss, whereas early miscarriage is defined as an intrauterine pregnancy loss of less than 10 weeks gestational age. These first trimester miscarriages can be classified as anembryonic (empty sac), yolk sac (gestational sac with yolk sac but no embryo) or embryonic miscarriage. We do not understand why miscarriages occur if embryopathogenesis is not associated with chromosome errors and/or major congenital anomalies. We hypothesize that a defective vascularization of the placenta may be involved in the underlying cause.

Vascularization starts from the hemangioblastic cell cords that are the precursors of both capillary endothelium and hematopoietic stem cells. These structures are easily identified in trophoblastic tissue of empty sac miscarriages and complete hydatidiform mole pregnancies (Lisman et al., 2004, 2005). Vasculogenesis of normal chorionic villi is then characterized by the maturation of these hemangioblastic cell cords into luminized vessels and their margination to peripherally located vessels at the end of organogenesis, to finally form the vasculosyncytial membrane (te Velde et al., 1997). Defective vasculascularization has been documented in embryonic miscarriages, but is more pronounced in empty sac miscarriages (Meegdes et al., 1988; te Velde et al., 1997; Lisman et al., 2005). Hypovascularization of chorionic villi appears to be associated with early miscarriage and is unaffected by a prolonged intrauterine retention (Meegdes et al., 1988). The changes in vascular parameters in the first trimester chorionic villi of miscarriage, such as the mean functional vascular area, the number of vessels with a lumen and whether hemangiogenic cords are peripherally or centrally located, are the result of defective development due to abnormal vasculogenesis, rather than to postmortem changes (Lisman et al., 2004).

In 1969, Philippe and Boué were the first to study the phenotype—karyotype relationship using microscopic investigation of chorionic villi from miscarriage specimens (Philippe and Boué, 1969; Bouie et al., 1976). They described a case of polyploidy in which the trophoblast showed hydropic degeneration of the chorionic villi with numerous vesicles. Microscopic examination revealed microcysts that are the result of invagination of the trophoblast into edematous, poorly vascularized villous stroma. Since then, several studies have been performed, and an association between placental histology and chromosome results has been found in triploid miscarriages (Mingullion et al., 1989; Fox, 1993; Roberts et al., 2000). For example, trisomy 16 and 22 are most often found in anembryonic (empty sac) miscarriages. Additionally, monosomy X (45, XO) is usually associated with an embryonic or fetal miscarriage (Byrne et al., 1985; Canki et al., 1988), and a smaller than expected embryo was found to be associated with aneuploidy (Canki et al., 1988; Munoz et al., 2010; Lijner et al., 2011). Others found that some types of chromosomal abnormalities, such as trisomy 22, seem to be miscarriage at a later stage than those with trisomy 16, multiple trisomies and unusual other variants (Goldstein et al., 1996; Munoz et al., 2010). Additionally, villous morphology in miscarriages has previously been studied, partly based on hypovascularization of peripheral villi. This was found to be an inaccurate indicator of chromosomal errors (Mingullion et al., 1989; Coulam et al., 1997).

In 2006, a histological classification for early pregnancy chorionic villous vascularization (Grade: I, normal; IIA, mild hypoplasia; IIB, severe hypoplasia and III, avascular) was developed and validated for clinical use (Hakvoort et al., 2006).

This scoring system may be helpful to determine whether miscarriages occur due to abnormal embryonic and/or chorionic development.

The aim of this study is to compare the results of this validated histological classification for early pregnancy chorionic villous vascularization with ultrasound findings and chromosome results of early miscarriages from a cohort of well-characterized recurrent pregnancy loss patients.

Materials and Methods

The study is an analysis of prospectively collected data and samples. These subjects were identified through the University of Chicago Recurrent Pregnancy Loss Database, created and managed by one of the authors (M.D.S.). All subjects signed a written consent for inclusion in the recurrent pregnancy loss database and use of excess miscarriage tissue for future research purposes. Institutional research board approval was obtained from the University of Chicago.

Patient selection

A patient was included, if she met the following criteria:

1. Seen in consultation by the author (M.D.S.) between July 2004 and October 2009.
2. A history of recurrent early pregnancy loss, defined as 2 or more miscarriages of less than 10 weeks gestation, based on ultrasound findings.
3. Miscarriage tissue was sent to University of Chicago Cytogenetics for chromosome testing and to Pathology for histological assessment.

Ultrasound diagnosis of miscarriage

An empty sac miscarriage was defined by a mean sac diameter of greater than 8 mm without visualization of a yolk sac; in these cases, an embryonic arrest at a gestational age of 28 days was assigned. A yolk sac miscarriage was defined by a mean sac diameter of greater than 16 mm with the presence of a yolk sac, but no embryo; for this study, an embryonic arrest at a gestational age of 35 days was assigned. An embryonic miscarriage was defined as an embryo of at least 5 mm without cardiac activity; the moment of embryonic demise was based on the crown rump length measurement after the cardiac activity had stopped.

Standard miscarriage chromosome testing

Chromosome testing was performed using conventional cytogenetic analysis (Bernardi et al., 2012). If conventional cytogenetic analysis failed, comparative genomic hybridization (CGH) was performed using cryopreserved
miscarriage tissue. In case of a 46, XX result, EDTA blood was obtained from the patient and microsatellite analysis was performed and the result would have been excluded, if the sample was of maternal origin.

**Standard histological testing**

Miscarriage tissue was sent to the University of Chicago Pathology Laboratory; chorionic villi were isolated and embedded in paraffin, and the slides were stained with hematoxylin and eosin (H&E) for interpretation.

**Chorionic villous vascularization studies**

For each miscarriage specimen in this cohort, unstained and H&E slides were prepared from the paraffin blocks stored in Pathology. The slides and the demographic data for all subjects were coded for anonymity.

One coded slide from each miscarriage was sent to four independent observers who were blinded to the subject’s clinical history and miscarriage results. Each observer classified the slides in one run. Two of the observers were experienced, and two were inexperienced, in assessing chorionic villous vascularization.

Each observer assessed the chorionic villous vascularization using the following scoring system from Hakvoort et al., 2006:

‘Grade 0: unknown. There are insufficient number of villi available for evaluation.

Grade I: normal. Vessels with nucleated blood cells are present in almost every (at least nine of 10) villus, have a very clear appearance and are located centrally as well as peripherally (in contact with the trophoblastic layer). In some villi, the number of vessels is even numerous (>5).

Grade IIA: mild hypoplasia. Vessels with nucleated blood cells are not present in all villi, less numerous and predominantly located centrally.

Grade IIB: severe hypoplasia. Villi are predominantly avascular; however, in a single villus, a vessel is present with one or more nucleated blood cells.

Grade III: avascular. All villi are avascular, although sporadically a very small vessel, with or without a nucleated blood cell, may be present.’

Features including fibrosis, hydropic degeneration, trophoblast inclusions and abnormal trophoblast proliferation were recorded, if present to the opinion of the observer, without using criteria other than usual in cases of routine microscopic examination of chorionic villi.

**Analysis of data**

The scores of the experienced and inexperienced observers were compared by calculating the Kappa value. The overall vascularization scores were determined by counting the scores of the two experienced observers twice and the scores of the two inexperienced observers once and dividing by six.

The vascularization scores were compared with the type of early miscarriage, the retention time (time between embryonic demise and collection of the tissue) and the chromosome results.

Data analysis was performed using the SPSS software (version 17.0, Chicago, Illinois). Chi-square test was used for categorical variables. Spearman’s rank correlation coefficient was used to determine the relationship between the chromosome results and the number of previous miscarriages. Mann–Whitney U-test was used to determine whether there was a relation between the vascularization score and gestational age at the time of miscarriage.

**Results**

A total of 60 miscarriages from 57 patients met the inclusion criteria and were used for microscopic evaluation of the vascularization score. In 1 of the 60 miscarriages, the chromosome testing was not successful; therefore, a total of 59 miscarriage specimens from 56 patients with recurrent early pregnancy loss could be analyzed.

The miscarriage tissue was obtained by dilatation and curettage using office manual vacuum aspiration (51 cases) or under general anesthesia in (7 cases), whereas 1 patient passed the tissue spontaneously. The demographics of the 56 women are shown in Table 1. This cohort had a total of 197 prior pregnancies.

Of the 59 miscarriages studied, 53 were conceived spontaneously and 6 of the pregnancies were conceived through IVF. Characteristics of the studied miscarriages are shown in Table 2.

### Table I Demographics of the recurrent early pregnancy loss subjects (*n* = 56 patients with a total of 197 prior pregnancies).

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravidity</td>
<td>4.5 (1.7)</td>
<td>1–8</td>
</tr>
<tr>
<td>Number of prior live births (n = 19)</td>
<td>0.3 (0.5)</td>
<td>0–2</td>
</tr>
<tr>
<td>Number of prior miscarriages &lt;10 weeks (n = 144)</td>
<td>2.6 (1.3)</td>
<td>0–6</td>
</tr>
<tr>
<td>Number of fetal demise ≥10 weeks (n = 13)</td>
<td>0.2 (0.6)</td>
<td>0–2</td>
</tr>
<tr>
<td>Preterm delivery (n = 7)</td>
<td>0.1 (0.3)</td>
<td>0–1</td>
</tr>
<tr>
<td>Number of neonatal deaths (n = 3)</td>
<td>0.05 (0.2)</td>
<td>0–1</td>
</tr>
<tr>
<td>Elective abortion (n = 9)</td>
<td>0.2 (0.4)</td>
<td>0–2</td>
</tr>
<tr>
<td>Genetic termination (n = 2)</td>
<td>0.04 (0.2)</td>
<td>0–1</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>African-American</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Alcohol use in pregnancy</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Recreational drug use</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>First born male</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>First born female</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

### Table II Patient characteristics at the time of the subsequent miscarriages (n = 59).

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at miscarriage (years)</td>
<td>36.0 (4.2)</td>
<td>25.0–46.0</td>
</tr>
<tr>
<td>Body mass index (kg/height²) at miscarriage</td>
<td>27.5 (6.4)</td>
<td>20.0–47.0</td>
</tr>
<tr>
<td>Gestational age (days) at demise</td>
<td>46.6 (9.0)</td>
<td>28.0–68.0</td>
</tr>
<tr>
<td>Retention time (days) between demise and tissue collection</td>
<td>14.4 (8.3)</td>
<td>1–34</td>
</tr>
</tbody>
</table>

*In one miscarriage, no dating ultrasound was performed, and in another case, no ultrasound was performed at demise so the gestational age at the time of demise and the retention time were not available.*
**Phenotype–genotype relation**

Embryonic miscarriage was more frequent than empty sac or yolk sac miscarriage (53 versus 2 versus 5). Chromosome testing was successful in 59 out of 60 miscarriages; 22 were euploid and 37 were noneuploid, as shown in Table 3. Among the euploid miscarriage, there were 15 male karyotypes and 7 female karyotypes (ratio 2.1; \( P = 0.19 \)). This difference is not significant probably as a result of our small sample size. Euploid and noneuploid chromosome results were observed in all types of miscarriages, and there were no significant differences. Trisomy 16 was the most frequent noneuploid result.

**Evaluation of the scoring system**

Validation of the vascularization score between the experienced and inexperienced observers turned out to be reasonable to good (Kappa 0.47–0.76), as shown in Table 4. In 10 of the 60 cases, there was an insufficient number of villi (<10) to evaluate, and they were all given a vascularization score 0 by all observers. Villi were mainly avascular (score III: 29 out of 50) when compared with the other grades (score IIB: 7 out of 50, score IIA: 11 out of 50 and score I: 3 out of 50).

In five patients, hydropic degeneration was found. In three of these cases, the villi were avascular, one case showed mild and one case showed severe hypoplasia. Four of them had an euploid chromosome result, and one of the avascular miscarriages was trisomic for chromosome 16. In only one case, fibrosis was seen; this case showed severe hypoplasia and had monosomy X. Trophoblast inclusion and abnormal trophoblast proliferation were not found in any of the cases.

No difference was found in the vascularization score between empty sac or yolk sac miscarriages and embryonic miscarriages: grade I (0 out of 6 versus 3 out of 43), grade IIA (1 out of 6 versus 9 out of 43), grade IIB (0 out of 6 versus 7 out of 43) or grade III (5 out of 6 versus 24 out of 43) (\( P = 0.67 \)).

The vascularization score did not significantly differ between miscarriages with an euploid or noneuploid chromosome result: grade I (1 out of 19 versus 2/30), grade IIA (2 out of 19 versus 8 out of 30), grade IIB (4 out of 19 versus 3 out of 30) or grade III (12 out of 19 versus 17 out of 30) (\( P = 0.51 \)). All the chromosome results of the miscarriages and their corresponding vascularization scores are shown in Table 5.

In the trisomy 16 miscarriages, all of which were embryonic, grade III vasculization was more frequent when compared with the other trisomies (6 out of 7 versus 8 out of 22, \( P = 0.04 \)). There was no correlation between the number of previous miscarriages and the chromosome results (\( r_s = -0.04, P = 0.76 \)).

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**Table III** Type of miscarriage according to chromosome results (\( n = 59 \)).

<table>
<thead>
<tr>
<th>Chromosome results</th>
<th>Total</th>
<th>Empty sac miscarriage</th>
<th>Yolk sac miscarriage</th>
<th>Embryonic miscarriage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euploid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46,XY (one with inversion)</td>
<td>15</td>
<td>1</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>46,XXa</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Monosomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45,X</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>−21</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Trisomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>+8</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>+9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>+13 (one with inversion)</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>+14 [one with der(13;14) (q10;q10)]</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>+15</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>+16</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>+20</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>+22</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Polyploidy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69,XXX</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>92,XXXX</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68,XXX,-11</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>68,XXY,-13</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>68,XXY,del(1)(q11),-4(4)/68,XXY,-4(3)/69,XXY(2)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td>2</td>
<td>5</td>
<td>52</td>
</tr>
</tbody>
</table>

\( ^a \text{Confirmed to be of chorionic origin by microsatellite analysis.} \)
Gestational age and vascularization

The gestational age at the time of miscarriage was known in 57 cases and ranged from 4^{0}\text{t}o 9^{+5} weeks of gestation (median: 6^{+2}, SD 1^{+2} weeks of gestation). There was no significant difference between the gestational age in days at time of miscarriage between the miscarriages with a euploid result and the miscarriages with a noneuploid chromosome result (euploid: median 46.0, SD 10.5, range 28.0–68.0, noneuploid: median 43.5, SD 8.1, range 28.0–68.0, \(P = 0.50\)).

Retention time and vascularization score

The miscarriages avascular villi did not show a longer retention time than miscarriages with more normal vascularization (difference between a normal vascularization score and an avascular score, \(P = 0.96\)), as shown in Table 6.

Discussion

Our study shows that the histological scoring system we used for the classification of chorionic villous vascularization is a reproducible tool, as reflected by the Kappa value \(\geq 0.47\). The villi were found to be mainly avascular in this study. In addition, we show that the chorionic villous vascularization of empty sac or yolk sac miscarriages does not differ from that of embryonic miscarriages. The high number of embryonic miscarriages in our study (52 out of 59; 88%) when compared with other studies (181 out of 272; 67%) (Lathi et al., 2007) may be due to ultrasound examination performed earlier in pregnancy. According to the studies of Byrne et al. (Byrne et al., 1985) and Canki et al. (Canki et al., 1988), who demonstrated that 45,X miscarriages are usually embryonic, the two 45,X cases in our study were also embryonic. Trisomy 16 was the most frequent noneuploid result that was expected, based on other published studies (Hassold et al., 1980; Stephenson et al., 2002; Group Eshre Capri Workshop, 2008). There was no difference in vascularization scores between euploid and noneuploid miscarriages; however, we saw high rates of avascular villi in trisomy 16 miscarriages. We also showed that the retention time has no significant influence on the vascularization score.

In our study, 63% of the miscarriages were aeuoploid. Stephenson et al. found chromosomal errors in about half of 420 recurrent miscarriage specimens (Stephenson et al., 2002). This difference may be explained by the fact that the mean age of the women in our study was above 35 years. It is known that the number of chromosomal errors rapidly increases after that age (Hassold and Chiu, 1985). It should also be stated that our numbers are too small to give a good representation of the frequency of chromosomal errors in recurrent miscarriage.

One of the possible causes of recurrent early pregnancy loss is lethal submicroscopic chromosomal changes (Philipp et al., 2003). These submicroscopic chromosomal changes, also termed DNA copy number variants (CNVs), can be detected by array CGH. With array-CGH, it is possible to evaluate the whole genome at a much higher resolution than with conventional cytogenetic analysis.
(Schaeffer et al., 2004; Bejiani and Shaffer, 2008; Menten et al., 2009; Robberecht et al., 2009). Previous studies of CNVs in miscarriages with the use of array-CGH indicate that small chromosomal changes are present in 1–13% of miscarriages (Schaeffer et al., 2004; Benkhalifa et al., 2005; Shimokawa et al., 2006; Menten et al., 2009; Robberecht et al., 2009; Zhang et al., 2009; Rajcan-Separovic et al., 2010a,b). These studies have the potential to identify CNVs that could lead to developmental failure and in this way find possible ‘miscarriage CNVs’. Rajcan-Separovic et al. (2010b) identified inherited CNVs that contain genes that impact early pregnancy, specifically TIMP2 (metalloproteinases-2 inhibitor) and CTNNAA3 (α-T-catenin), both of which act as inhibitors of trophoblast invasion and are only maternally expressed in the placenta (Okamoto et al., 2002; Li et al., 2003; Oudejans et al., 2004; Seval et al., 2004; van Dijk et al., 2004); thus, the authors proposed them as candidate miscarriage genes. The finding of avascular chorionic villi in miscarriages with euploid chromosome results in our study may yet indicate the presence of CNVs that interfere with trophoblast invasion and early trimester development and in this way cause miscarriage. We plan to investigate the presence of these and other possible miscarriage CNVs in euploid miscarriages with avascular chorionic villi.

We found a high male/female ratio in this study (2.1; 15 males and 7 females), probably due to our small numbers, but a possible explanation could be paternally expressed imprinted X chromosome CNVs (Lanas et al., 1999; Sangha et al., 1999; Robinson et al., 2001). However, others have not found an association between skewed X-chromosome inactivation and recurrent miscarriage (Hogge et al., 2007; Warburton et al., 2009) or a skewed sex ratio in recurrent miscarriages (Jobanputra et al., 2011). Another explanation found in the literature for a high male/female ratio in euploid miscarriages is that of an immunologic cause, described as an abnormal immune reaction against male-specific minor histocompatibility H-Y antigens (Nielsen et al., 2010a,b; Nielsen, 2011). Nielsen et al. state that sex ratios prior and subsequent to secondary recurrent miscarriage show that birth of a boy predisposes to secondary recurrent miscarriage and male fetuses are more likely to be miscarried. However, agenesis of chorionic villous vascularization, resulting in avascular villi, is more likely to be an embryopathogenic abnormality during organogenesis, rather than the result of an abnormal immunologic reaction.

**Conclusions**

No significant difference in vascularization scores of chorionic villi was found between euploid and noneuploid miscarriages, or between empty sac, yolk sac and embryonic miscarriages. Although the number of samples was limited, we did find the interesting result that among the euploid miscarriages, there were twice as many males as females and that avascular villi were predominantly present in trisomy 16 miscarriages. Avascular villi may indicate abnormal early placentation as a part of embryopathogenesis. Further study is warranted to determine whether a genetic cause can be found to explain these results.

**Authors’ roles**

M.D.S, N.E, and A.D.R. contributed to the study concept and design. A.D.R., M.D.S, F.M.D, R.R.K and N.E participated in the study execution and acquisition of data. A.D.R., M.D.S, M.J. and N.E participated in analysis of the data. All authors participated in the interpretation of the data, manuscript drafting and critical discussion and gave final approval of the submitted manuscript.

The study was supervised by M.D.S, E.A.P.S and N.E.

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**Conflict of interest**

None declared.

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Shimokawa O, Harada N, Miyake N, Sato K, Mizuguchi T, Niikawa N, Matsumoto N. Array comparative genomic hybridization analysis in...