Early pregnancy

First trimester trophoblast and placental bed vascular volume measurements in IVF or IVF/ICSI pregnancies

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STUDY QUESTION: Are first trimester trophoblast volume (TV) and placental bed vascular volume (PBVV) different in IVF or IVF/ICSI pregnancies in comparison with spontaneously conceived pregnancies?

SUMMARY ANSWER: Any possible abnormal placentation in IVF or IVF/ICSI pregnancies in comparison with spontaneously conceived pregnancies is not detected by a difference in PBVV or TV at an early gestational age (GA).

WHAT IS KNOWN ALREADY: Assisted reproductive technology pregnancies have been associated with an increased risk of placenta-related adverse pregnancy outcomes. It is unclear whether these effects originate from infertility or from the technique itself.

STUDY DESIGN, SIZE, DURATION: We performed a retrospective cohort study in which 154 pregnant patients qualified for participation.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Out of 154 pregnant patients, 84 conceived spontaneously and 70 conceived after IVF or IVF/ICSI. We determined the TV at 10 weeks GA by Virtual Organ Computer-aided Analysis measuring application and the PBVV at 12 weeks GA by the virtual reality operating system of BARCO I-Space in both subgroups. The investigators were blinded to the mode of conception during the measurements. Analysis was limited to singleton pregnancies with only one sac ever detectable.

MAIN RESULTS AND THE ROLE OF CHANCE: There were no differences in TV (mean 42.7, SD 15.9 versus mean 41.2, SD 13.9, \( P = 0.70 \)) and PBVV (mean 27.6, SD 16.9 versus mean 24.8, SD 19.9, \( P = 0.20 \)) between IVF or IVF/ICSI pregnancies and spontaneously conceived pregnancies. There was a significant correlation between TV and PBVV (\( r_1 = 0.283, P = 0.004 \)).

LIMITATIONS, REASONS FOR CAUTION: The limitations of the present study concern the small size of the study groups.

WIDER IMPLICATIONS OF THE FINDINGS: IVF or IVF/ICSI does not seem to be associated with abnormal placentation.

STUDY FUNDING/COMPETING INTEREST(S): This study was financially supported by the Erasmus Trustfonds, the Meindert de Hoop foundation and the Fonds NutsOhra. No competing interests are declared.

Key words: assisted reproductive technology / IVF/ICSI / trophoblast volume / placental bed vasculature volume / virtual reality

Introduction

Up to 4% of children are born after assisted reproductive technology (ART) (Eisenberg, 2012); however, there are issues raised concerning the impact of ART on the outcome of pregnancy. ART pregnancies are at higher risk of preterm birth, fetal growth restriction, low birthweight and pre-eclampsia (PE) (Schieve et al., 2002; Helmerhorst et al., 2004; Jackson et al., 2004; Källén et al., 2005; Chaveeva et al., 2011; Cooper et al., 2011). A favourable outcome of pregnancy largely depends on an adequate function of the placenta. Several studies have described differences between placentas of ART and spontaneously conceived pregnancies.
Materials and Methods

Patient selection
From November 2009 until December 2012, a total of 244 healthy pregnant women were enrolled in the study. Patients were recruited from the OB/GYN outpatient clinic and fertility unit at the Erasmus Medical Center. Patients who conceived spontaneously had no known history of infertility. The analysis was limited to singleton pregnancies with only one sac ever detectable. After exclusion of pregnancies with suboptimal quality of ultrasound images due to incomplete framing or too many artefacts \(n = 81\) and pregnancies conceived with intrauterine insemination \(n = 9\), 154 patients remained in our study cohort, consisting of 84 spontaneously conceived pregnancies and 70 IVF \((n = 41)\) or IVF/ICSI \((n = 29)\) pregnancies. Forty of these patients \(37 \text{ IVF or IVF/ICSI and 3 spontaneously conceived pregnancies} \) also participated in our previous study in which PBVV measurements were validated \(\text{(Reus et al., 2014). Fresh embryo transfers} n = 59\) as well as cryopreserved embryo transfers \(n = 11\) were included. Two patients become pregnant by oocyte donation. In 60 patients, 1 embryo was transferred and in 10 patients 2 embryos were transferred; all embryos were transferred on Day 3. GA was determined by the first day of the last menstrual period or by the day of oocyte retrieval, in the case of IVF or IVF/ICSI. There were no patients with large and/or multiple fibroids or known uterine anomalies.

Ethical approval
Written informed consent was obtained from all participants. The study was approved by the Medical Ethics review board of Erasmus Medical Center and is part of an ongoing, prospective, periconception cohort study, called the Rotterdam Predict Study \(\text{(van Uitert et al., 2014).}\)

Increased placental weight as well as an increased placental weight/fetal weight ratio have been described in ART pregnancies \(\text{(Daniel et al., 1999; Haavaldsen et al., 2012). Microscopic examination of placentas has shown a significant increase in villous oedema and an increased incidence of microcalcification in placentas of ART pregnancies} (Lalosevíc et al., 2003). Moreover, an increase in thickness of the placental blood barrier is found in ART pregnancies, suggesting a reduced materno-fetal exchange following ART treatment \(\text{(Zhang et al., 2011). A study on the association of abnormal placentation and ART has displayed significantly increased placental thickness, which has previously been linked to increased perinatal risk} (Joy et al., 2012). Also more placental haematomas have been found in ART pregnancies \(\text{(Joy et al., 2012). In other studies, no difference in first trimester placentation between ART and spontaneously conceived pregnancies has been observed} \text{(Conway et al., 2011).}\)

Given the fact that low birthweight and abnormal placental development are associated, we hypothesized that placental growth and/or placental bed vascular growth is influenced by IVF or IVF/ICSI. We compared the trophoblast volume \(\text{(TV)}\) and the placental bed vascular volume \(\text{(PBVV)}\) at an early gestational age \(\text{(GA)}\) between IVF or IVF/ICSI and spontaneously conceived pregnancies using the latest techniques of three-dimensional virtual reality \(\text{(3D VR)}\) and 3D ultrasound \(\text{(3D US)}\) \(\text{(Verwoerd-Dikkeboom et al., 2008; Rousian et al., 2010, 2011; Reus et al., 2013, 2014; van Uitert et al., 2013). Thus, the aim of this study was to compare TV and PBVV in early pregnancy between IVF or IVF/ICSI pregnancies and spontaneously conceived pregnancies.\)

Placental bed vascular volume
To measure the PBVV, we performed a 3D Power Doppler ultrasound scan at 12 weeks of gestation using a 4–8 MHz transabdominal probe of the GE Voluson E8 \(\text{(General Electrics Medical Systems, Zipf, Austria). We chose 12 weeks of gestation because of safety reasons} \text{(Salvesen et al., 2011). Every scan was performed with standard settings of the ultrasound machine: pulse repetition frequencies of 0.9 kHz, Gain = −2.0, quality ‘high’, wall motion filter ‘low’. Patients were asked to hold their breath while the scan was done to limit disturbance by movement of the abdomen.}\)

3D VR is an imaging technique that actually uses all three dimensions, in contrast to traditional 3D reconstructions displayed on a 2D screen. A fully immersive virtual reality system, the Barco I-Space, is operated at the department of Bioinformatics of the Erasmus MC Rotterdam. The V-Scope volume rendering application creates a hologram of the 3D US volume for optimal depth perception and an intuitive interaction for enlargement and rotation for length and volume measurements \(\text{(Rousian et al., 2010).}\)

For the offline measurement of the vascular volumes in the I-Space, we first set the lower-Doppler threshold at 100 for better perception of the vasculature. This means that all voxels with a grey value between 100 and 250 are coloured and counted in the volume calculation. We removed the normal US signal by making this channel fully transparent, retaining the coloured power doppler signal of the vasculature. After removing artefacts, fetal vasculature and the uterine vessels, we measured the PBVV \(\text{(Fig. 1), as described elsewhere} \text{(Reus et al., 2014). The measurements were performed three times by one experienced investigator (M.S.R.) and the mean values were used for further calculations. In case more than one US dataset was available, the first dataset was used unless this was technically inappropriate. The investigators were blinded to the mode of conception during the measurements.}\)

Trophoblast volume
To measure the TV, a 3D-US scan was performed at 10 weeks of gestation using a transvaginal probe \(\text{(6–12 MHz)}\) of the GE Voluson E8 \(\text{(General Electrics Medical Systems). All scans were performed with standard settings of the ultrasound machine and evaluated offline using specialized 3D software (4D view, GE medical systems), using the Virtual Organ Computer-aided Analysis \(\text{(VOCAL)}\) measuring application \(\text{(Reus et al., 2013).}\) We decided to use the datasets of 10 weeks GA being the optimal period for placental volume measurements. The 15° rotational angle was used to perform TV measurements. A sequence of 12 sections of the trophoblast was obtained, each after a 15° rotation from the previous one. In each plane, the contour was traced manually and at the end the computer provided the reconstruction of the trophoblast and the volume. We first measured the total pregnancy volume \(\text{(TPV)}\) by tracing the total pregnancy along the outside of the placental contour \(\text{(Fig. 2). Subsequently, the gestational sac volume} \text{(GSV) was measured in the same way. The TV was calculated by subtracting the GSV from the TPV. The VOCAL measurements were performed once.}\)

Statistical analysis
Data analysis was performed using the SPSS software \(\text{(version 20, Chicago, IL, USA). The Mann–Whitney U-test was used for evaluating differences between patient characteristics and periconceptional data in our two groups. To check for differences in TV and PBVV between the two groups, the Mann–Whitney U-test was used. The Kruskal–Wallis test was used to check for differences in PBVV and TV between the different groups of gravidity and parity. Spearman’s rank correlation coefficients were calculated to check for associations between the measured data. The impact of BMI or age to TV and the PBVV was tested by using the Spearman’s rank test. Differences were supposed to be significant in case of a \(P\)-value of <0.05.}\)
Results

There were 282 ultrasound datasets obtained from 154 pregnant patients with a mean of 1.83 ultrasound scans per patient (ranging from 0 to 4 datasets) at their 10th week of GA. Additionally, 231 ultrasound datasets were obtained at the 12th week of GA with a mean of 1.50 scan per patient (ranging from 0 to 4 datasets). Patient characteristics, periconceptional data and treatment characteristics of the IVF and IVF/ICSI are summarized in Table I. There were no significant differences in patient characteristics between the included and excluded patients (data not shown). Women who conceived spontaneously had been pregnant more often and had more children compared with women who conceived by IVF or IVF/ICSI. No other significant differences were found. Five women developed either pregnancy-induced hypertension (PIH, $n = 2$), or PE ($n = 2$), or haemolysis elevated liver enzymes and low platelets syndrome (HELLP, $n = 1$). One of the patients who developed PIH conceived spontaneously, and the other four became pregnant after IVF or IVF/ICSI. Four children were born premature and two of these pregnancies were conceived spontaneously, while three children, all conceived spontaneously, had a birthweight below the fifth percentile. The numbers of these abnormal pregnancies are small, but the results of the PBVV and TV measurements for these pregnancies did not differ from that of the other pregnancies.

There was no difference in PBVV between the different groups of gravidity ($P = 0.18$) and parity ($P = 0.37$), neither was there a difference in TV between these groups (gravidity $P = 0.40$ and parity $P = 0.61$). There was no difference in PBVV between nulliparous women and multiparous women. The PBVV and TV measurements are presented in Table II for the IVF or IVF/ICSI group and the spontaneously conceived group. There were no significant differences in TV (mean 42.7, SD 15.9 versus mean 41.2, SD 13.9, $P = 0.70$) or PBVV (mean 27.6, SD 16.9 versus mean 24.8, SD 19.9, $P = 0.20$) between IVF or IVF/ICSI pregnancies and spontaneously conceived pregnancies respectively, nor was there a significant difference in PBVV between the IVF and IVF/ICSI groups.

Figure 1 (A) An image of a placenta of 12 + 3 weeks GA is projected on the wall of the I-Space. (B) Different structures were defined and measured: branches of the uterine arteries are shown in mint, the fetal vascular volume is in purple and the PBVV is in blue; these structures together represent the ‘Total Volume’.
Figure 2 Image illustrating total TV measured by the VOCAL method. The TVs were calculated by subtracting the volume of the gestational sac (A) from the volume of the total pregnancy (B) by using VOCAL.

Table I Patient characteristics, periconceptional data and treatment characteristics of IVF or IVF/ICSI and spontaneous pregnancies.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IVF or IVF/ICSI, N = 70</th>
<th>Spontaneous, N = 84</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years) at US</td>
<td>33.0 (24–44)</td>
<td>31.0 (22–42)</td>
<td>0.149</td>
</tr>
<tr>
<td>BMI (kg/height²)</td>
<td>23.8 (18.6–35.4)</td>
<td>23.2 (17.8–38.5)</td>
<td>0.665</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1 (1–7)</td>
<td>2 (1–12)</td>
<td>0.001</td>
</tr>
<tr>
<td>Parity</td>
<td>0 (0–2)</td>
<td>1 (0–2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3380 (1475–4270)</td>
<td>3395 (1200–4650)</td>
<td>0.378</td>
</tr>
<tr>
<td>GA at birth (weeks)</td>
<td>39 3/7 (31 3/7–41 4/7)</td>
<td>39 2/7 (27 4/7–41 3/7)</td>
<td>0.844</td>
</tr>
<tr>
<td>Cigarette smoking (n)</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption (n)</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>8 (1–23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of embryos created</td>
<td>3 (1–13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The characteristics are given by median and range.
g, gram; N, number.

Table II PBVV (cm³) at the 12th week and TV (cm³) at 10th week of gestation in IVF or IVF/ICSI and spontaneously conceived pregnancies, given in median, interquartile range and range.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>P25</th>
<th>P75</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF or IVF/ICSI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBVV</td>
<td>22.8</td>
<td>14.8</td>
<td>37.6</td>
<td>6.4–82.4</td>
</tr>
<tr>
<td>TV</td>
<td>40.2</td>
<td>30.0</td>
<td>51.9</td>
<td>16.8–79.8</td>
</tr>
<tr>
<td>Spontaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBVV</td>
<td>19.5</td>
<td>13.8</td>
<td>34.1</td>
<td>2.7–133.2</td>
</tr>
<tr>
<td>TV</td>
<td>39.8</td>
<td>29.7</td>
<td>50.4</td>
<td>14.3–78.8</td>
</tr>
</tbody>
</table>

PBVV, placental bed vascular volume; TV, trophoblast volume; P, percentile.
groups (mean 25.1, SD 16.4 versus mean 30.6, SD 17.2, \( P = 0.163 \)). A borderline difference was found in TV between the IVF and IVF/ICSI group (mean 39.6, SD 15.6 versus mean 47.1, SD 15.5, \( P = 0.051 \)). There was no difference in PBVV (\( P = 0.49 \)) or in TV (\( P = 0.16 \)) between the fresh or cryopreserved embryo transfers.

There was a significant correlation between TV and PBVV (\( r_s = 0.283, P = 0.004 \)) when taking all the patients together. This significant correlation was also present in the IVF or IVF/ICSI group separately (\( r_s = 0.351, P = 0.012 \)), whereas it was absent in the group of patients who conceived spontaneously (\( r_s = 0.244, P = 0.081 \)). Similarly, a significant correlation between TV and PBVV among the nulliparous women in the group of patients who conceived spontaneously (\( r_s = 0.0513, P = 0.012 \)) was found, whereas such a relationship was absent in the group of multiparous women (\( r_s = 0.030, P = 0.789 \)). In the IVF or IVF/ICSI group, we found the same significant correlation among the nulliparous women (\( r_s = 0.397, P = 0.011 \)) and the absence of a correlation among the multiparous women (\( r_s = 0.227, P = 0.502 \)).

The mean PBVV of pregnancies complicated by hypertensive disease (PIH, PE and HELLP, \( n = 5 \)) did not differ from the mean PBVV of normal pregnancies (\( n = 90 \)) (27.53 versus 26.16; \( P = 0.58 \)). Neither were significant correlations found between BMI and PBVV (\( r_s = -0.132, P = 0.163 \)) or TV (\( r_s = 0.115, P = 0.176 \)) nor between age and PBVV (\( r_s = 0.076, P = 0.419 \)) or TV (\( r_s = -0.038, P = 0.649 \)).

**Discussion**

ART pregnancies have been associated with an increased risk of placenta-related adverse pregnancy outcomes such as fetal growth restriction and PE (Jackson et al., 2004; Carbone et al., 2011; Pinborg et al., 2013). In this study, we tried to provide further insight, with novel techniques, into early parameters of placentation, including TV as well as placental bed vasculature volume at the 10th and 12th weeks of gestation. Our previous study on PBVV in the first trimester of pregnancy did show larger volumes in multiparous women (Reus et al., 2014). In the present study, no difference was found in PBVV between IVF or IVF/ICSI and spontaneously conceived pregnancies, but among the spontaneously conceived pregnancies were significantly more multiparous women. It is possible that the difference in PBVV is nullified by the difference in parity between the two groups. Another explanation for not finding a difference in PBVV may be that the study size is too small to detect the difference. We did find a significant correlation between TV and PBVV, suggesting that larger placentas are supplied by a more pronounced adaptation of maternal vessels. Nevertheless, no significant differences were found between the IVF or IVF/ICSI-women and those naturally conceived. This is in accordance with previous studies in which uterine artery pulsatility index (PI), as a reflection of placentation in first trimester, was compared between ART and spontaneously conceived pregnancies (Prefumo et al., 2007; Carbone et al., 2011). In these studies, no difference was found in PI indicating that the increased risk of placenta-related complication as early PE in ART pregnancies is not clinically measurable in this way. Moreover, there seems to be no discernible difference in trophoblastic invasion of the spiral arteries between ART and spontaneously conceived pregnancies (Prefumo et al., 2007; Carbone et al., 2011). However, the number of children born premature or with a low birthweight in this study was too small to reflect on any associations with PBVV or TV.

In an effort to elucidate the pathogenic basis of the relationship between ART and increased pregnancy complications, several histopathologic (Jauniaux et al., 1990; Daniel et al., 1999; Lalosević et al., 2003; Zhang et al., 2011; Haavaldsen et al., 2012; Joy et al., 2012) and molecular (Zhang et al., 2008, 2010) placental studies have been performed. Although altered proteomic and gene expression profiles in ART-derived placentas were identified (Zhang et al., 2008, 2010), results were rather inconsistent with regard to histopathologic and macroscopic features. Haavaldsen et al. (2012) found larger placentas and a higher placental weight/birthweight ratios among pregnancies conceived by ART while others did not find this difference (Jauniaux et al., 1990; Daniel et al., 1999). Different studies have shown an increased placental thickness in ART pregnancies (Daniel et al., 1999; Joy et al., 2012). Lalosević et al. (2003) found an increase of villous oedema and an increased incidence of microcalcifications in placentas of ART pregnancies. Zhang et al. (2011) found a difference with respect to the placental blood barrier, while others have not found a difference in histopathological features of the plenta of ART and spontaneously conceived pregnancies (Jauniaux et al., 1990; Daniel et al., 1999).

Although the limitations of the present study concern the small size of the study groups, our findings suggest that IVF or IVF/ICSI conception itself seems not to be associated with abnormal early placentation. Given the reported difference in placental weight independent of GA at delivery between ART and spontaneously conceived pregnancies (Haavaldsen et al., 2012), future research should delineate the mechanisms responsible for such differences in placental growth.

**Conclusions**

The possible abnormal placentation in IVF or IVF/ICSI pregnancies, in comparison with spontaneously conceived pregnancies, is not detected by a difference in PBVV or TV at an early GA. TV and PBVV are significantly correlated, indicating an association of trophoblast growth and uterine maternal vascularization.

**Authors’ roles**

M.S.R. contributed to the data-collection, analysed and interpreted the data, and drafted and revised the paper. A.D.R. was involved in the data-collection, data analysis and revision of the manuscript. A.H.J.K. and P.J.S., respectively, developed the V-Scope software and supported 3D virtual reality and both contributed to the revision of the manuscript. J.S.E.L. was responsible for the IVF/ICSI patients and N.E. supervised the TV and PBVV measurements. Both contributed to the design of the study and revision of the manuscript. E.A.P.S. initiated the 3D US and I-Space facilities for embryonic measurements and revised the manuscript. All authors approved the final version of the manuscript.

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**Conflict of interest**

No conflicts of interest are declared.
References


