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## **Short-Term Exposure to Urban Air Pollution and Influences on Placental Vascularization Indexes**

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**Running title:** Air pollution and placental vascular indexes

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## **ABSTRACT**

**Background:** It has been widely demonstrated that air pollution can affect human health and that certain pollutant gases lead to adverse obstetric outcomes, such as preeclampsia and fetal growth restriction.

**Objectives:** To evaluate the influence of individual maternal exposure to air pollution on placental volume and vascularization evaluated in the first trimester of pregnancy.

**Methods:** This was a cross-sectional study on low-risk pregnant women living in São Paulo, Brazil. The women carried passive personal NO<sub>2</sub> and O<sub>3</sub> monitors in the week preceding evaluation. We employed VOCAL technique using three-dimensional power Doppler ultrasound to evaluate placental volume and placental vascular indexes [vascularization index (VI), flow index (FI), and vascularization flow index (VFI)]. We analyzed the influence of pollutant levels on log-transformed placental vascularization and volume using multiple regression models.

**Results:** We evaluated 229 patients. Increased NO<sub>2</sub> levels had a significant negative association with log of VI ( $p = 0.020$  and  $\beta = -0.153$ ) and VFI ( $p = 0.024$  and  $\beta = -0.151$ ). NO<sub>2</sub> and O<sub>3</sub> had no influence on the log of placental volume or FI.

**Conclusions:** NO<sub>2</sub>, an estimator of primary air pollutants, was significantly associated with diminished VI and VFI in the first trimester of pregnancy.

## **INTRODUCTION**

Air pollution can affect human health, and some of the pollutants that are capable of having that effect are carbon monoxide, nitrogen dioxide (NO<sub>2</sub>), sulfur dioxide, ozone (O<sub>3</sub>), lead, hydrocarbons, and particulate matter (Brook et al. 2010; Costa et al. 2014; de Oliveira et al. 2014; Mayhew 2009; Mirowsky and Gordon 2015; Olmo et al. 2011). Certain pollutant gases lead to adverse obstetric outcomes, such as preeclampsia and fetal growth restriction (Olsson et al. 2013; Pedersen et al. 2014; Romão et al. 2013; Sapkota et al. 2012; Shah et al. 2011; van den Hooven et al. 2011; Yorifuji et al. 2015).

These maternal and fetal complications are consequences of the effects of air pollution on placental function and growth. Indeed, the placenta is an extremely important organ that is responsible for the exchange of gas and fetal nutrients during pregnancy. Maternal air pollution exposure may affect pregnancy by inducing oxidative stress and systemic inflammation, decreasing the degree of placental global DNA methylation, and eliciting suboptimal placentation or placental inflammation (Brook et al. 2010; de Melo et al. 2015; Janssen et al. 2013; Kannan et al. 2006; van den Hooven et al. 2012; Veras et al. 2008). Thereby, the above-described pregnancy complications are suggested to have their origin in abnormal early placentation, which is characterized by impaired trophoblast invasion and a lack of modification of the spiral arteries (Campbell 2007; Kaufmann et al. 2003).

In recent years, a combined method involving three-dimensional (3D) imaging associated with power Doppler ultrasonography was employed to evaluate placental volume and vascularization (Araujo Júnior et al. 2011; Campbell 2007; de Paula et al. 2009; Hafner et al. 2010; Odibo et al. 2011; Pairleitner et al. 1999; Pomorski et al. 2014; Zalud and Shaha 2008). Using 3D power Doppler (3DPD), it is possible to estimate placental volume, flow, and vascularization, and this estimative is related to the real volume, flow, and

vascularization observed in the organ evaluated (Morel et al. 2010; Pairleitner et al. 1999; Raine-Fenning et al. 2008b).

Because air pollution affects placentation (Kannan et al. 2006; van den Hooven et al. 2012; Veras et al. 2008), we hypothesized that higher individual exposure to air pollution would lead to diminished placental volume, flow, and vascularization estimated by 3DPD evaluation.

Thus, the aim of our study was to evaluate the influence of individual short-term exposure to urban air pollution on placental volume and vascularization during the first trimester of pregnancy in low-risk patients to investigate one of the potential pathways by which maternal air pollution exposure may cause adverse pregnancy outcomes.

## **METHODS**

### ***Design:***

This was a cross-sectional study that was part of a larger cohort [“Impact of exposure to air pollution during intrauterine life and postnatal life on respiratory health of children” (PROCRIAR)] designed to verify the effects of air pollution on the health of the maternal–fetal dyad and children.

We recruited low-risk pregnant women from the Health District of Butantan School in São Paulo from October 2011 to January 2014.

The inclusion criteria consisted of the following: single pregnancy, no maternal diseases, and gestational age between 11 and 13 weeks and 6 days as confirmed by the measurement of the crown-rump length (CRL) in the first trimester ultrasound. Exclusion criteria were twin pregnancy, fetal malformation detected on ultrasound, and inappropriate use of the filter.

After inclusion, we performed individual air pollution exposure and sonographic evaluation as described below.

The sonographer was blinded to the values of air pollution exposure, and the personnel conducting air pollution evaluation were blinded to the ultrasound measurements.

This project was approved by the Research Ethics Committee of the University of São Paulo under the number 132/10. Women who agreed to participate signed the informed consent document.

***Measurement of exposure to air pollution:***

Energética brand cellulose filters, with a diameter of 37 mm, were used as previously described by our group (André et al. 2014; Carneiro et al. 2011; Novaes et al. 2007). We evaluated individual short-term exposure to NO<sub>2</sub> and O<sub>3</sub>. A community health agent delivered the passive personal monitor to the patient 7–18 days before the ultrasound scan. We advised pregnant women to use them out of the bag throughout the day. During the night, the filter should be left in the room where the woman sleeps, beside the bed. After the recording period, the personal monitors were returned to the facility where we performed the ultrasound scan: at the Clinical Fetal Medicine of Department of Obstetrics, the hospital school, University of São Paulo, Brazil. The personal monitors were sent to the Laboratory of Experimental Air Pollution, University of São Paulo, Brazil (LPAE), where they were disassembled and stored until the analysis. We analyzed the pollutants separately according to the respective protocols (André et al. 2014; Carneiro et al. 2011; Novaes et al. 2007). The NO<sub>2</sub> filters were extracted within 24 h and analyzed by spectrophotometry. If they could not be analyzed at the same time, they were frozen for a period of 2 weeks. Previous studies used the same method to measure NO<sub>2</sub> (Carneiro et al. 2011; Novaes et al. 2007). We could analyze the O<sub>3</sub> filters at any time after exposure. We calculated the difference between the final and initial reflectance of the filters and

determined the values after and before exposure, respectively, and corrected for the number of days the patient carried the filters (André et al. 2014). Each patient carried two filters of NO<sub>2</sub> and O<sub>3</sub> at the same time, and we considered the average for our analysis. Details of analyses were published by the same group of LPAE researchers (André et al. 2014; Carneiro et al. 2011; Novaes et al. 2007). We selected NO<sub>2</sub> and O<sub>3</sub> as markers of exposure to ambient pollution for several reasons. First, the passive devices could be used without disrupting the daily activities of the individuals enrolled in the study. Second, NO<sub>2</sub> is a pollutant produced by almost every combustion process and correlates well with other primary pollutants, and hence it is a good marker of urban pollution (Conceição et al. 2001; da Silva et al. 2006). O<sub>3</sub> represents a good estimator of the exposure to pollutants generated by photochemical processes. Thus, NO<sub>2</sub> and O<sub>3</sub> were considered proxy estimators of the complex mixture that composes urban pollution in the present study.

***Ultrasound evaluation:***

All patients underwent an ultrasound examination performed by the same operator (KH). The maternal characteristics analyzed included body mass index (BMI = weight/height<sup>2</sup>) at enrollment, smoking status (considered positive if any number of cigarettes was smoked), parity, alcohol consumption, age, ethnicity, and education level. We started the examination with patients in the semi-Fowler's position to avoid postural hypotension (Khatib et al. 2014) and performed two-dimensional (2D) ultrasound using a 2D convex 3.5-MHz transducer. Localization of the placenta and fetal anomaly scan were performed according to standard published techniques, and CRL measurements were used in early pregnancy to evaluate fetal biometry (Hadlock 1990).

We performed the placental assessments using a 3DPD ultrasound using the same machine brand and model (Voluson 730 Expert<sup>TM</sup>; General-Electric, Austria) in all evaluations and according to the method described by de Paula et al. (de Paula et al. 2009). Because

ultrasound parameters influence placental vascular indexes (Martins et al. 2010; Raine-Fenning et al. 2008a), we used the same pre-established 3DPD instrument settings in all cases (angio mode: cent; smooth: 4/5; FRQ: low; quality: 16; density: 6; enhancement: 16; balance: GO150; filter: 2; actual power: 2 dB; pulse repetition frequency: 0.9 kHz) (de Paula et al. 2009). In the case of artifacts during the acquisition of volume due to fetal movement, we repeated the capture process until a good pattern quality could be achieved. The parameters evaluated included the placental volume and placental vascular indexes [vascularization index (VI), flow index (FI), and vascularization flow index (VFI)].

When a 3D acquisition is performed, the primary image acquired is called voxels (which are the smallest volume acquired by the machine), and they construct the image. The 3DPD colors this primary image according to the movement detected, which gives us a sense of the blood flow. The VI measures the number of color voxels within the volume and represents the number of vessels in the 3D image. The FI measures the intensity of the color in the voxel and represents the flow within the evaluated volume. The VFI is a combination of the two previous indexes and represents the association of vascularization and flow within the organ evaluated (Pairleitner et al. 1999).

We started the ultrasound examination by the placental location and identified its long axis, using 2D technique, and then we adjusted the volume box to scan the entire placenta. We performed real-time scanning using a convex volumetric transducer from 4.0 to 8.0 MHz (Voluson 730 Expert<sup>TM</sup> apparatus) using low speed and an angle of 85° to ensure that data from the entire placenta were collected.

After placental acquisition, we calculated the placental volume using VOCAL technique (3D SonoView; GE Medical Systems, Milwaukee, WI, USA) with 30° rotation (Fig. 1-A) (de Paula et al. 2008). Subsequently, we used 3DPD histogram to obtain the VI, FI, and VFI (Fig. 1-B) (Pairleitner et al. 1999).

***Statistical analysis:***

We used descriptive measures such as mean, median, minimum, maximum, and standard deviation for quantitative variables wherever appropriate. We described the qualitative data using absolute and relative frequencies and percentages. We also evaluated the normal distribution using the Shapiro–Wilk test, histogram graphs, and the observed versus expected values. Because data showed a nonparametric distribution, we used the Spearman’s correlation coefficient to quantify the association between NO<sub>2</sub> and O<sub>3</sub> individual exposure.

Logarithmic (log) transformation of the response variables was necessary for normal distribution assumption. To assess the significance of the association between air pollution short-term exposure and the estimate of placental measures (volume, VI, FI, and VFI), we used the linear regression multiple models controlled for BMI, smoking status, parity, localization of the placenta, gestational age, maternal alcohol consumption, maternal age, maternal ethnicity, and maternal education level. We performed a modeling process taking into account the above mentioned control variables. Final models with and without the control variables are presented in order to show the constancy of air pollution influence on the dependent variables (described as Model 1, Model 2, Model 3, and Model 4). We demonstrated the nonstandardized beta-coefficients (95% confidence intervals) and the standardized beta-coefficients (obtained by the standardization of all variables in the model) in all tables to compare the influence of each variable in the regression models (Seber and Lee 2003). Significance was set at p value of <0.05 or, in the multiple regression models, by the absence of zero in the 95% confidence intervals of beta-coefficients. Since smoking may influence placental vascularization (Jauniaux and Burton 2007; Rizzo et al. 2009), we presented a fourth model evaluating placental vascularization

and control variables excluding pregnant smokers. This model was performed to evaluate the influence of air pollution on placental vascularization, regardless of smoking.

For each adjusted model, we performed the residual analysis to verify the linear regression multiple model assumptions. We did not find any evidence of violation of the model assumptions. We also performed the outlier analysis to identify the influential points in the adjusted model. When the influential points were detected, we adjusted the model by removing these points; however, we found no change in the results. We used SPSS for Windows, version 22, to perform the statistical analyses.

## **RESULTS**

We included a total of 288 patients in this study; 59 (20%) were excluded for the following reasons: 21 women did not attend the first-trimester scan, 8 patients had spontaneous abortions, and 30 patients had inappropriate use of the filter. A total of 229 patients were included in the final sample (Fig. 2).

The women carried the filter for a median number of 12 days (range: 7–18 days); 57.6% of the patients had 12 days of exposure and 91.1% of patients stayed with the filter for 10–14 days.

Results of the descriptive analyses of maternal data, placental variables, and pollutants are shown in Table 1. We observed that there was no significant difference in the population included and not included in the study. Table 2 shows that there was no correlation between the pollutants NO<sub>2</sub> and O<sub>3</sub>.<sup>1</sup>

The association between the pollutants NO<sub>2</sub> and O<sub>3</sub> and the placenta is shown in Tables 3–6, considering as the outcome variables the placental volume, VI, FI, and VFI, respectively.

Exposure to NO<sub>2</sub> had a negative association with the log of VI and VFI (Tables 4 and 6). It is important to notice that the fourth model, excluding pregnant smokers, showed similar results to the other models, confirming the consistency of the influence of NO<sub>2</sub> on these parameters. Similarly, in the models including smokers, there was no association of NO<sub>2</sub> and O<sub>3</sub> with the log of placental volume and FI.

Evaluation of the control variables showed that gestational age had a positive association with placental volume. BMI was also associated with all the vascular indexes. Multiparity had a positive association with IF. Placenta location and tobacco use showed no influence, in the first trimester, on the placental volume and vascular indexes. Furthermore, alcohol consumption, maternal age, ethnicity, and education level had no significant association with the outcome variables.

## **DISCUSSION**

To the best of our knowledge, this is the first study to investigate the effect of air pollution on vascular indexes and placental volume, considering individual short-term exposure to pollutants in the first trimester of pregnancy. In the present study, exposure to NO<sub>2</sub> had a negative influence on VI and VFI. This finding suggests that the placental vasculature can be impaired by exposure to air pollution at ambient levels, specifically to NO<sub>2</sub>, which correlates well with other primary pollutants, and is a good marker of urban pollution (Conceição et al. 2001; da Silva et al. 2006). We observed that the influence of NO<sub>2</sub> was present for VI and VFI, but not FI. This influence probably occurs because the number of vessels is affected, whereas the flow is not affected in this specific point of gestation. Studies that have evaluated placental vascularization in the first trimester and have predicted pregnancy complications observed that women with diminished VI and VFI showed an increased tendency to have pregnancies that are later complicated with preeclampsia and fetal growth restriction (de Almeida Pimenta et al. 2014; Odeh et al.

2011; Odibo et al. 2011). Short-term exposure to air pollution seems to affect the same indexes, which indicates the possible pathway by which air pollution may provoke these complications (Olsson et al. 2013; Pedersen et al. 2014; Romão et al. 2013; Sapkota et al. 2012; Shah et al. 2011; van den Hooven et al. 2011; Yorifuji et al. 2015).

As already shown in the literature, gestational age had a positive influence on the placental volume (Paula et al. 2008). On the other hand, we observed that NO<sub>2</sub> and O<sub>3</sub> did not have any influence on placental volume at this stage. At the first trimester, vascularization is being formed by branching of immature intermediate villi (Demir et al. 2007; Y. and S. 2010), and the major process occurring at this point is vasculogenesis. Placental volume increases more substantially in volume in the second and third trimester (de Paula et al. 2008; Y. and S. 2010). This might be reason why the volume was not influenced in the first trimester by the concentration of NO<sub>2</sub>, but only the placental vascularization.

BMI has an influence on the vascular indexes, as previously demonstrated by other authors (Hafner et al. 2010; Odibo et al. 2011). Similarly, as demonstrated previously (Zalud and Shaha 2008), multiparity had a positive association with FI in this study. On the other hand, placental location and tobacco use showed no significant effect, as described previously (de Paula et al. 2009; Guiot et al. 2008; Hafner et al. 2010; Odibo et al. 2011).

Numerous clinical studies conducted around the world have examined the hypothesis that air pollution damages health and, in particular, can negatively affect pregnancy and placental functioning. Possible mechanisms for this association include the following: oxidative stress; inflammation; systemic alterations in the hematocrit and blood viscosity; coagulation; endothelial dysfunction and hemodynamic responses. These mechanisms are regarded as leading to a loss in placentation and placental dysfunction (Brook et al. 2010; de Melo et al. 2015; Kannan et al. 2006; Slama et al. 2008; Sørensen et al. 2003; Veras et

al. 2008). Because adequate placentation and placental functioning are essential for a normal pregnancy, impairment of these processes, reflected by alterations in markers of placental growth and function, represent a risk factor for fetal adverse outcomes (Schlembach et al. 2007; Veras et al. 2008). In 2012, Van den Hooven et al. showed that maternal exposure to particulate matter  $\leq 10$  microns in aerodynamic diameter and NO<sub>2</sub> exposure were associated with changes in fetal soluble fms-like tyrosine kinase 1 (sFlt-1) and placental growth factor (PlGF) levels at delivery, which is consistent with an anti-angiogenic state (van den Hooven et al. 2012). These changes may influence placental development by decreasing vascularization, which may be demonstrated by 3DPD evaluation.

Indeed, these results are consistent with published experimental findings (de Melo et al. 2015; Veras et al. 2008). Veras et al. (2008) showed that pregnant mice exposed to air pollution compared with unexposed mice had decreased placental vasculature in maternal side and decreased flow resistance in the fetal side in order to try to maintain the proper flow. de Melo et al. (2015) demonstrated that pregnant rats exposed to air pollution, before and during pregnancy, had an increase of interleukin-4 in the fetal portion of the placenta, suggesting an anti-inflammatory placental response to previous inflammatory process induced by the pollutants.

The quantification of placental flow and vascularization by 3DPD may vary with ultrasound presets. Therefore, standardization of power Doppler parameters is critical. In our study, all presets were fixed in all evaluations (Huster et al. 2010; Jones et al. 2011; Martins et al. 2010; Raine-Fenning et al. 2008a). Another factor already known to influence the vascular indexes is the distance between the probe and the volume of interest. In our study, we controlled this influence using BMI and placental location, both related to placental depth (Martins et al. 2010). When presets are fixed and the distance

between the probe and the structure evaluated is taken into account, this technique has a good reproducibility and has satisfactory correlation with real vascularization of the organ (Bernardes et al. 2011; Huster et al. 2010; Jones et al. 2009; Jones et al. 2011; Raine-Fenning et al. 2003; Raine-Fenning et al. 2008b).

One limitation of our study is that placental histology was not evaluated to assess the influence of air pollution on actual vascularization. However, because placental indexes correlate with real vascularization and flow (Morel et al. 2010; Pairleitner et al. 1999; Raine-Fenning et al. 2008b), our results suggest that these factors are diminished when the mother is exposed to higher values of NO<sub>2</sub>. Diminished placental vascularization may be the underlying cause of the impaired fetal growth and adverse pregnancy outcomes are related to higher levels of air pollution exposure during pregnancy (Olsson et al. 2013; Pedersen et al. 2013; Pedersen et al. 2014; Romão et al. 2013; Sapkota et al. 2012; Shah et al. 2011; van den Hooven et al. 2011; van den Hooven et al. 2012; Yorifuji et al. 2015).

Another limitation of this study is the lack of a control group in a non-urban center. However, because exposure to air pollution was measured using individual filters, different lifestyle habits led to different air pollution exposures for each woman evaluated (Steinle et al. 2013).

## **CONCLUSIONS**

This study showed that the placental VI and placental VFI are significantly decreased in the first trimester in pregnant women exposed to higher concentrations of NO<sub>2</sub>, which suggests that this pollutant and other primary pollutants that are associated with NO<sub>2</sub> influence placentation and decrease placental vascularization. Because placentation permits normal pregnancy and fetal development, these findings suggest that this negative influence may be the underlying cause of pregnancy complications related to short-term air pollution exposure.

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**TABLES**

**Table 1 – Comparison of characteristics of population in included (n=229) and excluded patient’s groups (n=59). Placental Volumes and Vascularization Indexes, Pollutants (n=229)**

<b>Descriptive measures</b>	<b>Included Patients</b>	<b>Excluded Patients</b>	<b>P value</b>
<b>Mean ± SD/N (%)</b>			
<b>Body Mass Index (kg/m<sup>2</sup>)</b>	26.1 ± 5.6	26.4 ± 5.0	NS (0.478)
<b>Maternal age (years)</b>	25.5 ± 6.2	24.6 ± 6.2	NS (0.350)
<b>Smoking</b>			
NO	193 (84.3%)	51 (86.4%)	NS (0.696)
YES	36 (15.7%)	8 (13.6%)	
<b>Alcohol consumption</b>			
NO	206 (90.7%)	52 (88.1%)	NS (0.622)
YES	21 (9.3%)	7 (11.9%)	
Missing	2		
<b>Maternal ethnicity</b>			
White	83 (36.7%)	24 (41.4%)	NS (0.545)
No – white	143 (63.3%)	34 (58.6%)	
Missing	3	1	
<b>Education level</b>			
< 9 years	101 (44.3%)	25 (42.4%)	NS (0.883)
> 9 years	127 (55.7%)	34 (57.3%)	
Missing	1		
<b>Parity</b>			
Nulliparous	122 (53.3%)	32 (54.2%)	NS (1.000)
Multiparous	107 (46.7%)	27 (45.8%)	
<b>Placental location</b>			
No – Posterior	125 (54.6%)	-	-
Posterior	104 (45.4%)	-	-
<b>Gestational age (w)</b>	12.5 ± 0.64	-	-
<b>Placental volume (cm<sup>3</sup>)</b>	52.2± 20.3	-	-
<b>VI (%)</b>	7.7 ± 6.2	-	-
<b>FI</b>	35.7 ± 5.4	-	-
<b>VFI</b>	2.7 ± 2.0	-	-
<b>NO<sub>2</sub> (ug/m3)</b>	40.50± 7.72	-	-
<b>O<sub>3</sub> (ug/m3)</b>	8.22 ± 1.15	-	-

SD = standard deviation; VI = vascularization index; FI = flow index; VFI = vascularization flow index; NO<sub>2</sub> = nitrogen dioxide; O<sub>3</sub> = ozone; NS = No significant.

**Table 2 - Spearman's correlation coefficient between the measured pollutants in the first trimester.**

	<b>O<sub>3</sub></b>
<b>NO<sub>2</sub></b>	0,088

NO<sub>2</sub> = nitrogen dioxide; O<sub>3</sub> = ozone.

**Table 3 – Estimates of the effects of the NO<sub>2</sub> and O<sub>3</sub> and significant control variables on placental volume (n=229)**

Placental Variable	Pollutant and SV	Model 1			
Log Volume	n=228	Beta*	p-value	Beta	95% CI
	NO <sub>2</sub>	0.061	0.315	0.001	-0.001 – 0.004
	O <sub>3</sub>	0.016	0.790	0.005	-0.034 – 0.044
	GA	0.454	<0.001	0.271	0.201 – 0.341
Placental Variable	Pollutant and SV	Model 2			
Log Volume	n=228	Beta*	p-value	Beta	95% CI
	NO <sub>2</sub>	0.070	0.246	0.002	-0.001 – 0.004
	O <sub>3</sub>	0.018	0.759	0.006	-0.033 – 0.045
	GA	0.459	<0.001	0.274	0.204 – 0.345
Placental Variable	Pollutant and SV	Model 3			
Log Volume	n=222	Beta*	p-value	Beta	95% CI
	NO <sub>2</sub>	0.080	0.200	0.002	-0.001 – 0.004
	O <sub>3</sub>	0.007	0.909	0.002	-0.037 – 0.042
	GA	0.446	<0.001	0.264	0.192 – 0.336
Placental Variable	Pollutant and SV	Model 4			
Log Volume	n=187	Beta*	p-value	Beta	95% CI
	NO <sub>2</sub>	0.031	0.658	0.001	-0.002 – 0.003
	O <sub>3</sub>	0.018	0.793	0.005	-0.035 – 0.046
	GA	0.433	<0.001	0.248	0.171 – 0.325

Log = logarithm; NO<sub>2</sub> = Nitrogen dioxide; O<sub>3</sub> = Ozone; \* = standardized beta; SV = significant variable; CI = confidence intervals; GA = gestational age;

Model 1: Exposure to both pollutants, controlling for fetus's gestational age;

Model 2: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, smoking and placental location;

Model 3: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, smoking, placental location, maternal alcohol consumption, maternal age, maternal ethnicity and maternal education level.

Model 4: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, placental location, maternal alcohol consumption, maternal age, maternal ethnicity and maternal education level.

**Table 4 – Estimates of the effects of the NO<sub>2</sub> and O<sub>3</sub> and significant control variables on placental vascularization index (n=229)**

<b>Placental Variable</b>	<b>Pollutant and SV</b>	<b>Model 1</b>			
<b>Log VI</b>	n=228	<b>Beta*</b>	<b>p-value</b>	<b>Beta</b>	<b>95% CI</b>
	NO <sub>2</sub>	-0.168	<b>0.013</b>	-0.008	-0.014 – -0.002
	O <sub>3</sub>	0.025	0.708	0.017	-0.073 – 0.108
<b>Placental Variable</b>	<b>Pollutant and SV</b>	<b>Model 2</b>			
<b>Log VI</b>	n=228	<b>Beta*</b>	<b>p-value</b>	<b>Beta</b>	<b>95% CI</b>
	NO <sub>2</sub>	-0.153	<b>0.020</b>	-0.007	-0.013 – -0.001
	O <sub>3</sub>	0.013	0.842	0.009	-0.079 – 0.096
	BMI	0.268	<b>&lt;0.001</b>	0.038	0.020 – 0.056
<b>Placental Variable</b>	<b>Pollutant and SV</b>	<b>Model 3</b>			
<b>Log VI</b>	n=222	<b>Beta*</b>	<b>p-value</b>	<b>Beta</b>	<b>95% CI</b>
	NO <sub>2</sub>	-0.137	<b>0.042</b>	-0.006	-0.012 – -0.0002
	O <sub>3</sub>	0.012	0.851	0.009	-0.082 – 0.099
	BMI	0.280	<b>&lt;0.001</b>	0.040	0.021 – 0.059
<b>Placental Variable</b>	<b>Pollutant and SV</b>	<b>Model 4</b>			
<b>Log VI</b>	n=187	<b>Beta*</b>	<b>p-value</b>	<b>Beta</b>	<b>95% CI</b>
	NO <sub>2</sub>	-0.213	<b>0.004</b>	-0.009	-0.015 – -0.003
	O <sub>3</sub>	0.056	0.430	0.036	-0.054 – 0.126
	BMI	0.239	<b>0.001</b>	0.034	0.014 – 0.055

Log = logarithm; VI = vascularization index; NO<sub>2</sub> = Nitrogen dioxide; O<sub>3</sub> = Ozone; \* = standardized beta; SV = significant variable; CI = confidence interval; BMI = body mass index;

Model 1: Exposure to both pollutants, controlling for fetus's gestational age;

Model 2: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, smoking and placental location;

Model 3: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, smoking, placental location, maternal alcohol consumption, maternal age, maternal ethnicity and maternal education level.

Model 4: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, placental location, maternal alcohol consumption, maternal age, maternal ethnicity and maternal education level.

**Table 5 – Estimates of the effects of the NO<sub>2</sub> and O<sub>3</sub> and significant control variables on placental flow index (n=229)**

Placental Variable	Pollutant and SV	Model 1			
Log FI	n=228	Beta*	p-value	Beta	95% CI
	NO <sub>2</sub>	-0.001	0.991	- 6 x 10 <sup>-6</sup>	-0.001 – 0.001
	O <sub>3</sub>	-0.096	0.154	-0.012	-0.030 – 0.005
Placental Variable	Pollutant and SV	Model 2			
Log FI	n=228	Beta*	p-value	Beta	95% CI
	NO <sub>2</sub>	0.006	0.927	4.9 x 10 <sup>-5</sup>	-0.001 – 0.001
	O <sub>3</sub>	-0.079	0.197	-0.010	-0.026 – 0.005
	BMI	-0.430	<0.001	-0.011	-0.015 – -0.008
	Parity	0.135	0.037	0.040	0.002 – 0.078
Placental Variable	Pollutant and SV	Model 3			
Log FI	n=222	Beta*	p-value	Beta	95% CI
	NO <sub>2</sub>	0.011	0.858	9.7 x 10 <sup>-5</sup>	-0.001 – 0.001
	O <sub>3</sub>	-0.073	0.246	-0.009	-0.026 – 0.007
	BMI	-0.427	<0.001	-0.011	-0.015 – -0.008
	Parity	0.190	0.011	0.057	0.013 – 0.101
Placental Variable	Pollutant and SV	Model 4			
Log FI	n=187	Beta*	p-value	Beta	95% CI
	NO <sub>2</sub>	0.008	0.907	7 x 10 <sup>-5</sup>	-0.001 – 0.001
	O <sub>3</sub>	-0.090	0.185	-0.012	-0.029 – 0.006
	BMI	-0.432	<0.001	-0.012	-0.016 – -0.009
	Parity	0.209	0.007	0.065	0.018 – 0.112

Log = logarithm; FI = flow index; NO<sub>2</sub> = Nitrogen dioxide; O<sub>3</sub> = Ozone; \* = standardized beta; SV = significant variable; CI = confidence interval; BMI = body mass index;

Model 1: Exposure to both pollutants, controlling for fetus's gestational age;

Model 2: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, smoking and placental location;

Model 3: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, smoking, placental location, maternal alcohol consumption, maternal age, maternal ethnicity and maternal education level.

Model 4: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, placental location, maternal alcohol consumption, maternal age, maternal ethnicity and maternal education level.

**Table 6 – Estimates of the effects of the NO<sub>2</sub> and O<sub>3</sub> and significant control variables on placental vascularization and flow index (n=229)**

Placental Variable	Pollutant and SV	Model 1			
		Beta*	p-value	Beta	95% CI
Log VFI	n=228				
	NO <sub>2</sub>	-0.167	<b>0.014</b>	-0.008	-0.014 – -0.002
	O <sub>3</sub>	0.007	0.913	0.005	-0.086 – 0.096
Placental Variable	Pollutant and SV	Model 2			
		Beta*	p-value	Beta	95% CI
Log VFI	n=228				
	NO <sub>2</sub>	-0.151	<b>0.024</b>	-0.007	-0.013 – -0.001
	O <sub>3</sub>	-0.002	0.981	-0.001	-0.091 – 0.089
	BMI	0.185	<b>0.006</b>	0.026	0.008 – 0.045
Placental Variable	Pollutant and SV	Model 3			
		Beta*	p-value	Beta	95% CI
Log VFI	n=222				
	NO <sub>2</sub>	-0.136	<b>0.048</b>	-0.006	-0.012 – -5.5 x 10 <sup>-5</sup>
	O <sub>3</sub>	-0.001	0.982	-0.001	-0.094 – 0.092
	BMI	0.196	<b>0.005</b>	0.028	0.009 – 0.048
Placental Variable	Pollutant and SV	Model 4			
		Beta*	p-value	Beta	95% CI
Log VFI	n=187				
	NO <sub>2</sub>	-0.211	<b>0.004</b>	-0.009	-0.016 – -0.003
	O <sub>3</sub>	0.037	0.601	0.025	-0.069 – 0.118

Log = logarithm; VFI = vascularization flow index; NO<sub>2</sub> = Nitrogen dioxide; O<sub>3</sub> = Ozone; \* = standardized beta; SV = significant variable; CI = confidence interval; BMI = body mass index;

Model 1: Exposure to both pollutants, controlling for fetus's gestational age;

Model 2: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, smoking and placental location;

Model 3: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, smoking, placental location, maternal alcohol consumption, maternal age, maternal ethnicity and maternal education level.

Model 4: Exposure to both pollutants, controlling for fetus's gestational age, BMI, parity, placental location, maternal alcohol consumption, maternal age, maternal ethnicity and maternal education level.

## **FIGURE LEGENDS**

Fig. 1 – Three-dimensional power Doppler (3DPD) ultrasound: A-Placental capture by 3DPD ultrasonography with the VOCAL method. B- Assessment of placental volume using the rotational technique (VOCAL) and a 3DPD histogram showing the vascular indexes.

Fig. 2 – Flow chart of study subjects.

Figure 1 – A and B

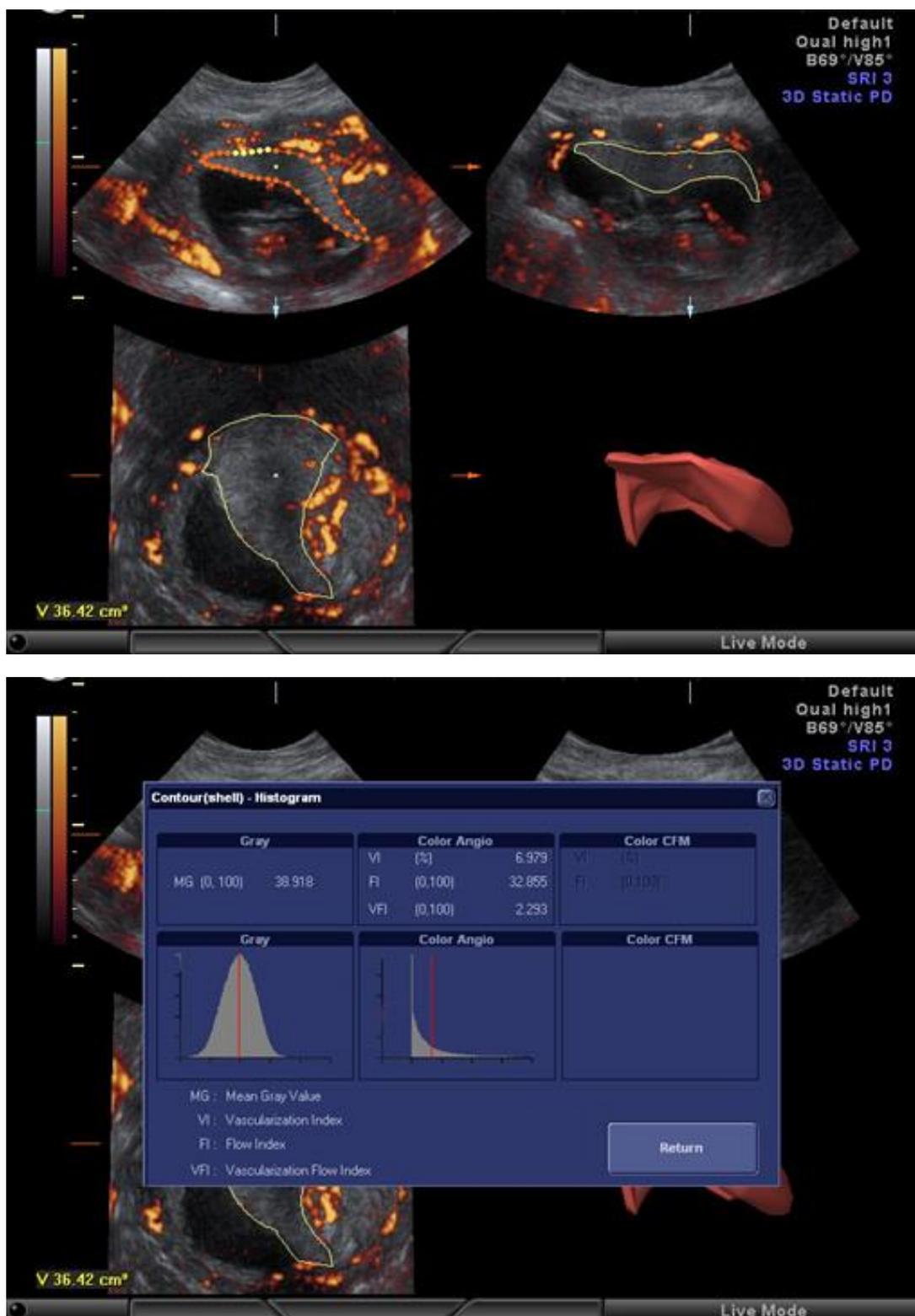


Figure 2

