

## Original Article

# Association between fetoplacental Doppler results, placental pathology, and angiogenic factors among pregnant women with anxiety

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## ABSTRACT

**Objective:** This study aimed to evaluate whether state and trait anxiety among pregnant women were associated with fetoplacental Doppler findings, abnormal placental pathology, and placental angiogenic factors.

**Materials and methods:** A total of 102 pregnant women at 32–35 gestational weeks were recruited and examined prospectively. State and trait anxiety were measured using the State-Trait Anxiety Inventory. Using Doppler ultrasound, pulsatility index (PI) of the umbilical artery (UA), middle cerebral artery (MCA), and uterine artery (UtA) and cerebroplacental ratio (CPR) were determined. Doppler parameters were converted into multiples of the median (MoM). Abnormal placental pathology was classified into 2 groups: vascular underperfusion (VU) and histological chorioamnionitis (HCA). Immunohistochemical analysis was performed to examine placental cells staining positive for placental growth factor (PLGF) and hypoxia-inducible factor-1- $\alpha$  (HIF-1 $\alpha$ ), which are markers for angiogenesis and hypoxic status, respectively.

**Results:** Women with high state anxiety scores had low MCA-PI MoM and CPR MoM, while those with high trait anxiety scores had low MCA-PI MoM. VU was associated with a higher incidence of high trait anxiety scores, and HCA was associated with a higher incidence of high state and trait anxiety scores. Regression analysis showed a relationship between maternal state anxiety on MCA-PI MoM and HCA after controlling for covariates. Maternal trait anxiety exhibited relationships with VU and HCA after adjustment.

**Conclusion:** Our results demonstrated that maternal anxiety is associated with altered fetal cerebral blood flow and abnormal placental pathology but is not associated with uteroplacental insufficiency and placental angiogenic factors.

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## Introduction

Several studies suggest that psychosocial factors, such as high anxiety and stress among pregnant women, can negatively affect the success of pregnancy and fetal development. Maternal anxiety during pregnancy is associated with preterm delivery and adverse implications for fetal growth and neurodevelopment [1–4]. Other authors reported that a mother's emotional state during pregnancy can predispose the child to a range of psychiatric, behavioral, and intellectual problems in later life, such as increased risk of anxiety

and cognitive deficits [5–7]. Ongoing research addresses the mechanisms through which maternal anxiety and stress affect offspring outcomes; however, the mechanisms underlying the link remain unclear.

Several mechanisms explain the association between maternal anxiety and adverse offspring outcomes. One of them proposed is impaired maternal blood flow to the fetus. A previous study showed the association between maternal anxiety in pregnancy and increased uterine artery resistance index (UtA-RI) [8], which resulted in impaired blood flow to the fetus and placenta. Another study suggested that maternal anxiety influenced fetal cerebral circulation [9]. However, the relationship between maternal anxiety and fetoplacental blood flow is unclear and controversial. It is clinically important to prove the associations between antecedent maternal psychological anxiety and downstream changes in

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fetoplacental blood flow to clarify the underlying mechanisms related to maternal anxiety and adverse offspring outcomes.

Impaired fetoplacental blood flow is known to be associated with abnormal placentation. Fetoplacental Doppler indices, which are tools for measuring fetoplacental blood flow, have long been considered surrogates of uteroplacental underperfusion [10]. The placenta mirrors the pathophysiologic condition of both the mother and fetus; thus, pathologic assessment of the placenta may be indicative of adaptive changes in the intrauterine environment during pregnancy [11]. In addition, angiogenic imbalance is common in the setting of uteroplacental underperfusion [10]. Placental growth factor (PLGF) is one of the placental angiogenic biomarkers for predicting and characterizing early-onset preeclampsia and fetal growth restriction, the pathophysiology of which is known as uteroplacental underperfusion [12,13]. Hypoxia-inducible factor-1- $\alpha$  (HIF-1 $\alpha$ ) is one of the key mediators that responds to compromised oxygen conditions during physiological or pathologic processes [14] and can mediate the differentiation of human placental trophoblasts based on oxygen availability [15]. In this study, we hypothesized that maternal anxiety causes impaired fetoplacental blood flow, which resulted in abnormal placentation, such as placental underperfusion and hypoxia.

Therefore, this study aimed to evaluate the effect of maternal state and trait anxiety on impaired fetoplacental blood flow and placental pathology. In addition, we sought to explore whether maternal anxiety influenced placental underperfusion and hypoxia by measuring placental angiogenic factors.

## Materials and methods

### Study population

A prospective study was conducted among women who received prenatal care and delivery at Bucheon St. Mary's Hospital between 2012 and 2014. Women aged >18 years and who had singleton pregnancies were included. Exclusion criteria were pre-existing hypertension, renal disease, diabetes mellitus, congenital major malformations, aneuploidies, and depression or bipolar disorder before pregnancy. The study was approved by the ethics committee of the Clinical Research Coordinating Center of the Catholic Medical Center (HC15TISI0017). Written informed consent was obtained from each participant after explaining the study protocol. The participants underwent Doppler assessment at the 3rd trimester between 32 and 35 gestational weeks, and were blinded to the results of the Doppler assessment. In our hospital, obstetricians used the Doppler result in clinical practice in cases of absent or reversed end-diastolic umbilical artery (UA) and high peak systolic velocity of the middle cerebral artery (MCA), and the participants in this study did not have those findings. Data on trait and state anxiety symptoms were collected through self-reported questionnaires, which the mothers in the current sample filled out between 32 and 35 gestational weeks. Data on patient characteristics, including sociodemographic factors and obstetric complications, were collected from medical records in the hospital database. Information about delivery and placental pathology was also collected. Preterm delivery was defined as delivery before 37 gestational weeks, and the criterion for intrauterine growth restriction was fetal weight lower than the 10th percentile.

### Measures

#### Doppler ultrasonography

Doppler ultrasonography was performed by a single examiner using the Accuvix XQ (Samsung Medison Co, Seoul, South Korea) ultrasound machine equipped with a 6–2 MHz linear curved-array

transducer. The UA, fetal MCA, and maternal UtA were measured using color and spectral Doppler, according to the standard protocol [16–18]. Pulsatility index (PI) was calculated automatically using spectral Doppler from three or more consecutive waveforms. The cerebroplacental ratio (CPR) was defined as the ratio between the MCA-PI and UA-PI [19]. The values of Doppler examination were calculated to multiples of the median (MoM) to adjust for gestational age.

#### State and trait anxiety assessments

Symptoms of anxiety were measured using the State-Trait Anxiety Inventory, which comprises two parts, each containing 20 items: state anxiety and trait anxiety. It is the most widely used tool for measuring anxiety during pregnancy in the obstetric and psychological settings [20]. State anxiety is defined as the transitory emotional condition characterized by subjective feeling of tension and apprehension, generally fluctuating over time [21]. Trait anxiety refers to the relatively stable anxiety proneness, i.e., an individual's tendency to respond to situations perceived as threatening [21]. The cut-off point for high anxiety among participants was determined as 40, low anxiety (score <40) and high anxiety (score  $\geq$ 40), based on a previous study, where a score of 40 as indicated as significant anxiety levels [9]. In the present study, Cronbach's alpha coefficients of the State-Trait Anxiety Inventory for state and trait were 0.786 and 0.734, respectively.

#### Placental evaluation

All areas of the placenta were examined in the Division of Pathology, following the standard protocol. The placenta was grossly examined, for abnormalities in the umbilical cord, maternal and fetal surfaces of the placenta, and membranes. After removal of the umbilical cord and membranes, placental weight was measured. Thereafter, the placenta was sliced and examined for abnormal lesions. The representative tissues were embedded in paraffin. The histological sections were 4- $\mu$ m thick and stained by hematoxylin and eosin. All pathological examinations were made by examiners blinded to the maternal characteristics and perinatal outcomes.

Abnormal histopathological features were classified into 2 groups based on the presence of abnormal vascular lesions (vascular underperfusion, VU) and evidence of infection and inflammation (histologic chorioamnionitis, HCA), according to previously published study [22]. Detailed histologic findings of placental lesions associated with VU or HCA were reported, according to the previous study [22].

#### Immunohistochemistry

Placental samples were fixed in formalin and embedded in paraffin; thereafter, slides were prepared with 4- $\mu$ m paraffin sections. Immunohistochemistry was performed using an automated immunohistochemical stainer (Ventana Medical Systems, Inc., Tucson, AZ, USA) following the manufacturer's protocol. The primary antibodies were diluted in Dako antibody diluent (DakoCytomation, Glostrup, Denmark) with background-reducing components and were used at the following dilutions: rabbit polyclonal anti-PLGF antibody (diluted 1:100; 30  $\mu$ g/ml; ab9542; Abcam, Cambridge, UK) and mouse monoclonal anti-HIF-1 $\alpha$  antibody (diluted 1:300; 5  $\mu$ g/ml; ab6489; Abcam). For negative controls, the primary antibody was replaced by the anti-mouse immunoglobulin G (IgG) isotype and anti-rabbit IgG isotype. The sections were observed under a light microscope (BX50; Olympus, Tokyo, Japan).

Staining was evaluated in a blinded fashion by a pathologist. The scoring scale was in accordance with that in a previous study [23]. The intensity of immunoreactivity was semi-quantitatively evaluated by grouping positively stained cells according to the following

categories: 0 (no staining), +1 (mild), +2 (moderate), and +3 (intense). Each slide was evaluated under a microscope using 400× original magnification. For each tissue, an H-score was calculated by adding the percentage of cells grouped in one intensity category and multiplying this number with the weighted intensity of the staining, using the formula ( $H\text{-score} = P_c(s+1)$ ), where “s” represents the intensity scored and  $P_c$  is the corresponding percentage of the cells).

### Statistical analysis

All statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Chi-square analysis or Fisher's exact test was used for categorical data and Student's *t*-test or the Mann–Whitney *U* test for continuous data. Multiple regression model analysis was used to examine the relationship between maternal state and trait anxiety and fetoplacental Doppler findings and abnormal placental pathology. Maternal and offspring covariates that may have been associated with the outcomes were included in all models. Covariates were maternal age, self-reported smoking, alcohol consumption, preeclampsia, preterm delivery, IUGR, and neonatal sex. For all tests, the significance level was defined as a *p* value < 0.05.

### Results

The final population comprised 102 women, in whom the incidence of preterm delivery was 34% (35 women). Some women had complications including preeclampsia (10%) and intrauterine growth restriction (7%) (Table 1). Women with high state anxiety scores had a higher percentage of alcohol consumption (12.5% versus 1.3%,  $p = 0.013$ ), higher body mass index ( $24.02 \pm 4.14$  versus  $21.40 \pm 5.26$ ,  $p = 0.030$ ), and higher percentage of preterm delivery (54.2% versus 28.2%,  $p = 0.019$ ) than those with low state anxiety score. Women with high trait anxiety scores had a lower percentage of nulliparity (36.4% versus 71.2%,  $p = 0.003$ ) than those with low trait anxiety scores.

Table 2 shows the fetoplacental Doppler index and placental pathology in groups of women with different levels of anxiety. Women with high state anxiety had lower MCA-PI MoM ( $0.74 \pm 0.13$  versus  $0.89 \pm 0.25$ ,  $p = 0.028$ ), CPR ( $1.54 \pm 0.24$  versus

$1.92 \pm 0.55$ ,  $p = 0.006$ ), and CPR MoM ( $0.70 \pm 0.09$  versus  $0.85 \pm 0.28$ ,  $p = 0.013$ ). Women with high trait anxiety scores showed lower MCA-PI ( $1.21 \pm 0.11$  versus  $1.61 \pm 0.45$ ,  $p < 0.001$ ) and MCA-PI MoM ( $0.64 \pm 0.02$  versus  $0.88 \pm 0.24$ ,  $p < 0.001$ ). In placental pathology, VU was associated with a higher incidence of high trait anxiety scores, while HCA was associated with a higher incidence of high state and trait anxiety scores.

Fig. 1 shows the placental expression of PLGF and HIF-1 $\alpha$ . The immunohistochemistry analysis of HIF-1 $\alpha$  revealed that this molecule was expressed both in the nucleus and cytoplasm of the placental cells. PLGF staining was localized in the cytoplasm. Immunohistochemistry analysis revealed that neither PLGF nor HIF-1 $\alpha$  showed differential placental expression based on the anxiety level.

H-score analysis was performed for the comparison of the placenta between the low anxiety group and the high anxiety group, by assessing the nuclear and cytoplasmic levels of PLGF and HIF-1 $\alpha$  based on anxiety levels. The H-score of PLGF revealed that the expression in the cytoplasm did not significantly differ based on the levels of anxiety (Table 3). Both nuclear and cytoplasmic expressions of HIF-1 $\alpha$  were found to be similar between groups with different levels of anxiety.

Table 4 shows the results of the regression analysis for examining the association of maternal anxiety with fetal brain blood supply and abnormal placental pathology. The associations of maternal state anxiety with MCA-PI MoM and HCA were evident, even after controlling for maternal age, smoking, alcohol consumption, preeclampsia, preterm delivery, IUGR, and neonatal sex. Maternal trait anxiety exhibited relationships with VU and HCA, after adjustment for covariates.

### Discussion

Our study showed that prenatal maternal anxiety was associated with altered fetal cerebral blood flow and abnormal placental pathologies. However, maternal anxiety was not associated with uteroplacental insufficiency and angiogenic factors in the placenta.

In this study, MCA-PI was lower in mothers with high state anxiety than in those with low state anxiety. The finding is consistent with that of previous studies. One study showed a significant association between trait anxiety and lower MCA-PI [21],

**Table 1**  
Baseline characteristics of the study population.

	Low state anxiety scores (N = 78)	High state anxiety scores (N = 24)	p-value	Low trait anxiety scores (N = 80)	High trait anxiety scores (N = 22)	p-value
<b>Maternal characteristics</b>						
Maternal age, years	33.06 $\pm$ 4.83	32.42 $\pm$ 6.45	0.653	33.05 $\pm$ 4.72	32.41 $\pm$ 6.89	0.685
Education, years	15.36 $\pm$ 2.32	14.54 $\pm$ 2.81	0.204	15.29 $\pm$ 2.29	14.73 $\pm$ 3.01	0.424
Employed	20 (25.6)	8 (33.3)	0.460	23 (28.8)	5 (22.7)	0.575
Smoker	1 (1.3)	2 (8.3)	0.074	2 (2.5)	1 (4.5)	0.615
Alcohol drinker	1 (1.3)	3 (12.5)	0.013	2 (2.5)	2 (9.1)	0.158
Married/cohabiting	76 (97.4)	21 (87.5)	0.050	77 (96.3)	20 (90.9)	0.304
BMI, kg/m <sup>2</sup>	21.40 $\pm$ 5.26	24.02 $\pm$ 4.14	0.030	21.72 $\pm$ 5.48	22.67 $\pm$ 3.60	0.367
Nulliparous	53 (67.9)	12 (50)	0.110	57 (71.3)	8 (36.4)	0.003
Chronic hypertension						
<b>Perinatal outcome</b>						
Gestation at birth, weeks	37.85 $\pm$ 2.38	35.83 $\pm$ 3.14	0.006	37.54 $\pm$ 2.43	36.78 $\pm$ 3.23	0.316
Preterm delivery	22 (28.2)	13 (54.2)	0.019	26 (32.5)	9 (40.9)	0.462
Cesarean delivery	40 (51.3)	14 (58.3)	0.545	43 (53.8)	11 (50)	0.755
Birth weight, kg	2.99 $\pm$ 0.59	2.67 $\pm$ 0.78	0.069	2.93 $\pm$ 0.63	2.84 $\pm$ 0.73	0.609
IUGR	5 (6.4)	2 (8.3)	0.745	6 (7.5)	1 (4.5)	0.627
Preeclampsia	7 (9.0)	3 (12.5)	0.612	9 (11.2)	1 (4.5)	0.349
UA pH < 7.2	7 (9.0)	1 (4.2)	0.485	8 (10)	0 (0)	0.124

Values are expressed as mean  $\pm$  standard deviation or number (%).

BMI, body mass index; IUGR, intrauterine growth restriction; UA, umbilical artery.



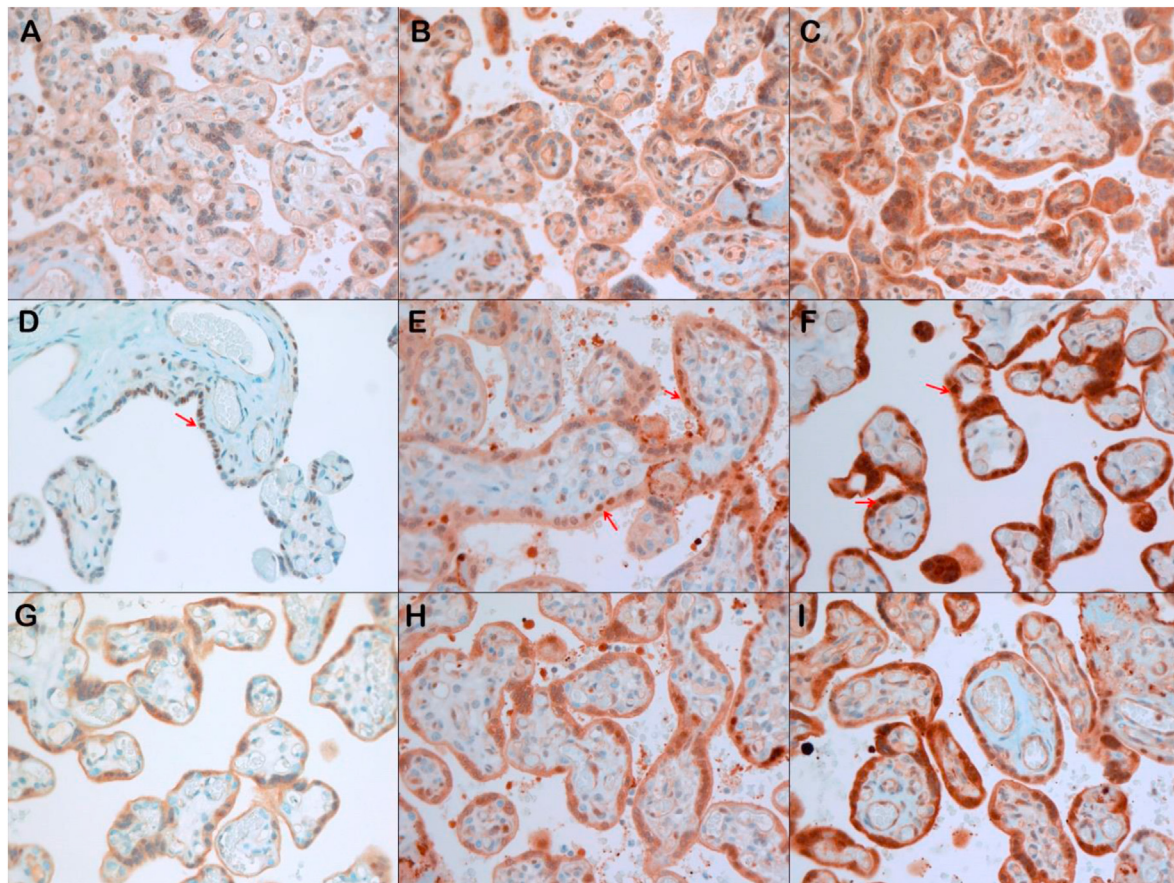
**Table 2**

Fetoplacental Doppler results and placental pathology in the study population.

	Low state anxiety scores (N = 78)	High state anxiety scores (N = 24)	p-value	Low trait anxiety scores (N = 80)	High trait anxiety scores (N = 22)	p-value
<b>Fetoplacental Doppler</b>						
UtA-PI	0.76 ± 0.26	1.00 ± 0.37	0.291	0.79 ± 0.28	0.73 ± 0.38	0.813
UtA-PI MoM	1.10 ± 0.33	1.14 ± 0.51	0.281	1.15 ± 0.38	1.12 ± 0.48	0.947
UA-PI	0.83 ± 0.18	0.87 ± 0.16	0.366	0.85 ± 0.18	0.82 ± 0.15	0.418
UA-PI MoM	0.92 ± 0.19	0.95 ± 0.16	0.575	0.94 ± 0.19	0.91 ± 0.16	0.493
MCA-PI	1.60 ± 0.47	1.43 ± 0.1	0.240	1.61 ± 0.45	1.21 ± 0.11	<0.001
MCA-PI MoM	0.89 ± 0.25	0.74 ± 0.13	0.028	0.88 ± 0.24	0.64 ± 0.02	<0.001
CPR	1.92 ± 0.55	1.54 ± 0.24	0.006	1.88 ± 0.53	1.58 ± 0.36	0.205
CPR MoM	0.85 ± 0.28	0.70 ± 0.09	0.013	0.83 ± 0.27	0.76 ± 0.18	0.506
<b>Placental pathology</b>						
Placental weight, g	647.71 ± 193.14	580 ± 194.08	0.146	632.90 ± 203.98	626.73 ± 160.73	0.882
VU	4 (5.1)	4 (16.7)	0.066	3 (3.8)	5 (22.7)	0.003
HCA	11 (14.1)	13 (54.2)	<0.001	15 (18.8)	9 (40.9)	0.030

Values are expressed as mean ± standard deviation or number (%).

UtA, uterine artery; PI, pulsatility index; MoM, multiples of median; UA, umbilical artery; MCA, middle cerebral artery; CPR, cerebroplacental ratio; VU, vascular under-perfusion; HCA, histologic chorioamnionitis.



**Fig. 1.** Expression of placental growth factor (PLGF) and hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) in the placenta observed using immunohistochemistry. A–C: representative photomicrographs of PLGF in cytoplasm with mild intensity (A), moderate (B), and intense (C). D–F: representative photomicrographs of HIF-1 $\alpha$  in nucleus with mild intensity (D), moderate (E), and intense (F). G–I: representative photomicrographs of HIF-1 $\alpha$  in cytoplasm with mild intensity (G), moderate (H), and intense (I). Arrow shows the expression of protein in nucleus. Magnification 400 $\times$ .

and another study found that a higher state anxiety score was a significant predictor of lower MCA-PI at the 3rd trimester [9]. Sjöström et al. considered that this change in blood flow in response to anxiety may be compensatory for the uteroplacental insufficiency to favor circulation to the fetal brain [21]. However, we failed to prove the association between maternal anxiety and

maternal UtA-PI and CPR, which are known markers of uteroplacental insufficiency [24]. Previous findings regarding the association between anxiety and UtA or CPR are inconsistent. One study found that women in a high state anxiety group at 32 weeks of gestation showed a high mean UtA-RI and maximum RI and notch [8]; however, anxiety scores were not related to UtA-RI at 22 weeks

**Table 3**H-score assessment of PLGF and HIF-1 $\alpha$  in study population.

	Low state anxiety scores (N = 78)	High state anxiety scores (N = 24)	p-value	Low trait anxiety scores (N = 80)	High trait anxiety scores (N = 22)	p-value
PLGF (cytoplasm)	205.71 $\pm$ 67.86	217.39 $\pm$ 49.10	0.449	206.85 $\pm$ 65.25	215.00 $\pm$ 58.71	0.615
HIF-1 $\alpha$ (nucleus)	127.14 $\pm$ 22.47	131.30 $\pm$ 39.58	0.532	126.58 $\pm$ 22.31	134.00 $\pm$ 41.60	0.287
HIF-1 $\alpha$ (cytoplasm)	325.71 $\pm$ 75.54	308.70 $\pm$ 94.93	0.382	326.03 $\pm$ 78.22	305.00 $\pm$ 88.70	0.304

Values are expressed as mean  $\pm$  standard deviation.PLGF, placental growth factor; HIF-1 $\alpha$ , hypoxia-inducible factor-1- $\alpha$ .**Table 4**Regression analysis examining the association of maternal anxiety with fetal brain blood supply and abnormal placental pathology, controlling for covariates<sup>a</sup>.

	MCA-PI MoM $\beta$ (95% CIs)	p	VU $\beta$ (95% CIs)	p	HCA $\beta$ (95% CIs)	p
State anxiety	0.02 (0.01–0.66)	0.028	6.25 (0.65–59.93)	0.112	7.44 (1.13–48.87)	0.037
Trait anxiety	1.83 (0.29–11.50)	0.517	6.55 (1.05–44.06)	0.043	13.34 (1.24–143.99)	0.032

MCA, middle cerebral artery; PI, pulsatility index; MoM, multiples of median; VU, vascular underperfusion; HCA, histologic chorioamnionitis; CI, confidence interval.

<sup>a</sup> Covariates: maternal age, smoking, alcohol consumption, preeclampsia, preterm delivery, IUGR, and neonatal sex.

of gestation in another study [25]. In terms of CPR, one study showed that enhanced levels of anxiety were shown to be related to lower values of CPR at 36 weeks of gestation [21], but paucity of studies has made it difficult to conclude the relevance. Therefore, contrary to our thesis, we can assume that the mechanism of increased blood flow to the fetal brain of a mother with anxiety may not be compensatory to uteroplacental insufficiency.

We showed the relationship between maternal anxiety and fetal brain blood flow. Some animal and human studies supported the idea that maternal anxiety may have adverse effects on neurologic and behavioral outcomes in offspring. These studies demonstrated the relationship between prenatal stress and hindrance to the growth and development of the fetal brain. Considerable evidence exists in rodents and nonhuman primates that maternal stress exposure during gestation has adverse consequences on the developing hippocampus and amygdala [26–28], regions that play a role in affective neurodevelopmental and psychopathological disorders [29,30]. In human studies, high maternal cortisol level, which is one of the primary biomarkers of the physiologically stressed state, was associated with a large volume of amygdala and more affective problems in offspring [31]. Therefore, the present findings suggest that altered fetal brain blood flow may be associated with maternal anxiety in pregnancy with disorders of brain development and affective disorders in offspring.

Although many studies on the relation between maternal anxiety and fetal growth focused on early gestation, some recent studies focused on the association between anxiety in late pregnancy and offspring diseases [16,32]. Some studies showed that maternal anxiety and depression may impact fetal programming by placenta functioning, and those associations were particularly prominent during late gestation and in cases of maternal anxiety [5,33]. Further studies are needed to help clarify the association between the timing of maternal anxiety and offspring outcome.

In the present study, maternal anxiety was not associated with uteroplacental blood flow and angiogenic factors in the placenta but was associated with abnormal placental pathology. Thus, we can suspect that maternal anxiety may affect the placenta but that altered blood supply to the placenta and angiogenesis may not be involved in this pathophysiology. Abnormal placental pathology, known as VU and HCA, is associated with various kinds of pregnancy complications. The mechanism underlying the association between maternal anxiety and abnormal placental pathology is unclear. However, we assumed that abnormal placental pathology

was not owing to impaired fetoplacental blood flow from the mother but from the endogenous hormone of the placenta itself. It is known that pregnancy reflects a complex interaction of different hormonal systems with the placenta as a peripheral hormonal system, which influences the regulation of maternal and fetal hypothalamic–pituitary–adrenal axis [34]. Stress and depression in pregnancy can affect the placental expression of enzymes regulating cortisol levels, thereby stimulating the release of excessive stress hormones, such as cortisol and catecholamines, resulting in excessive hormonal exposure to offspring [31]. These biological changes may result in placental pathologic changes, such as placental hypoperfusion and inflammation, leading to poor pregnancy outcomes [35–38].

This study had some limitations. First, the placenta is a large organ, and pathologic examination of several parts of the placenta may not represent the entire placental pathology. Second, pregnant women present profound alterations in endocrine and immune conditions, and these pregnancy-related changes may disguise or confound a linkage between psychiatric symptoms with the fetus and placenta. Third, our study recruited a small number of women. Fourth, this study was a cross-sectional study, for which the causal pathway underlying the observed relationships cannot be inferred. Finally, the rate of preterm delivery was high. Because our hospital is one of the few tertiary centers in the community, it seems that high-risk patients visited our hospital. Being diagnosed as having a high-risk pregnancy and undergoing follow-up in a tertiary center might have affected the maternal anxiety state. To overcome this limitation, we adjusted the covariates of high-risk pregnancy (preeclampsia, IUGR, and preterm delivery). Further studies that include a greater number of low-risk pregnant women are needed to evaluate the association of maternal anxiety with fetoplacental Doppler findings and placental pathology.

Despite these limitations, our study had several strengths. To the best of our knowledge, the present findings represent the first report linking maternal anxiety level in pregnancy with subsequent abnormal placental pathology. In addition, we compared not only fetoplacental Doppler measurements in terms of different anxiety levels but also compared angiogenic factors in placenta concurrently to assess the association between maternal anxiety and the outcome of offspring.

In conclusion, this study showed that maternal anxiety is associated with altered fetal cerebral blood flow and abnormal placental pathology. However, our findings suggested that

maternal prenatal anxiety did not decrease uteroplacental blood flow, suggesting that other mechanisms may be involved in this pathophysiology. Further prospective studies with large samples are necessary to better clarify the relationship between maternal anxiety and fetal conditions.

### Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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