# 学位論文

**Clinicopathologic Study of Placentas From Women With a Fontan Circulation**

**（フォンタン術後妊娠女性から得られた胎盤の病理学的検討）**

小西　妙

Tae　Konishi

熊本大学大学院医学教育部博士課程医学専攻循環器先進医療学

指導教員

野口　暉夫　客員教授

熊本大学大学院医学教育部博士課程医学専攻循環器先進医療学

2023 年 3 月

# 学　位　論　文

論文題名　：　**Clinicopathologic Study of Placentas From Women With a Fontan Circulation**
（フォンタン術後妊娠女性から得られた胎盤の病理学的検討）

著 者 名　：　小　西　妙
Tae Konishi

指導教員名：熊本大学大学院医学教育部博士課程医学専攻循環器先進医療学　野　口　暉　夫　客員教授

審査委員名　：　産科婦人科学担当教授　近　藤　英　治
病理診断学担当教授　三　上　芳　喜
循環器内科学担当教授　辻　田　賢　一
生体微細構築学担当教授　若　山　友　彦

2023年3月

Clinical Investigation

# A clinicopathologic study of placentas from women with a Fontan circulation

Tae Yokouchi-Konishi, MD[1,2], Keiko Ohta-Ogo, MD, PhD[3], Chizuko A Kamiya, MD, PhD [1], Tadasu Shionoiri, MD[1], Atsushi Nakanishi, MD[1], Naoko Iwanaga, MD, PhD[1], Hideo Ohuchi, MD, PhD[4], Kenichi Kurosaki, MD[4], Hajime Ichikawa, MD, PhD[5], Teruo Noguchi, MD, PhD, FJCS[2,6], Hatsue Ishibashi-Ueda, MD, PhD[3,7] and Jun Yoshimatsu, MD, PhD[1]

1. Department of Obstetrics and Gynecology, National Cerebral and Cardiovascular Center in Japan.

2. Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University, Japan.

3. Department of Pathology, National Cerebral and Cardiovascular Center in Japan.

4. Department of Pediatrics Cardiology, National Cerebral and Cardiovascular Center in Japan.

5. Department of Pediatric Cardiovascular Surgery, National Cerebral and Cardiovascular Center in Japan.

6. Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center in Japan.

7. NCVC Biobank, National Cerebral and Cardiovascular Center in Japan.

**Shorted title**: Placenta in a Fontan circulation

**Disclosure of interests**: All authors have no interests, including financial, personal, political, intellectual or religious interests. Teruo Noguchi is a member of Circulation Journal's editorial team.

**IRB information:** This study was approved by the Institutional Research Ethic Board of the National Cerebral and Cardiovascular Center, Suita, Japan (M30-161) on January 16th 2019.

**Corresponding author**: Tae Yokouchi-Konishi

Obstetrics and Gynecology, National Cerebral and Cardiovascular Center, 6-1 Kishibe-Shinmachi, Suita, Osaka, 564-8565, Japan.

Tel: +81-6-6170-1070, E-mail: yokouchitae@ncvc.go.jp

**Abstract**

**Background:** Pregnant women with Fontan circulation have high risks of obstetric complications such as preterm delivery and small for gestational age (SGA), which might be affected by low blood flow to placenta and hypoxia. The purpose of this study is to investigate placental pathology in a Fontan circulation.

**Method and Results:** Eighteen pregnancies in 11 women with a Fontan circulation were reviewed. Pregnancy outcomes showed 9 miscarriages and 9 live births with 4 preterm deliveries. Five neonates were SGA (<5th centile). Eight placentas from live births in 7 women were available for the study. Five placentas had low weight placenta for gestational age, and 7 grossly showed a chronic subchorionic hematoma. Histological examination revealed all placentas had some form of histological hypoxic lesions: maternal vascular malperfusion in 7, fetal vascular malperfusion in 1, and other hypoxia-related lesions in 8. Quantitative analyses, including immunohistochemistry (CD 31, CD 68, and hypoxia inducible factor-1α antibodies) and Masson's trichrome staining, were also performed and compared with 5 control placentas. Capillary density and the area of fibrosis were significantly greater in placentas from women with a Fontan circulation than control placentas.

**Conclusions:** Placentas in a Fontan circulation were characterized by a high frequency of low

placental weight, chronic subchorionic hematoma, and constant histological hypoxic changes, which could reflect altered maternal cardiac conditions and lead to poor pregnancy outcomes.

**Keywords:** Fontan circulation; Placenta; Placental hypoxia; Pregnancy; Small for gestational age

**Text:**

**Introduction:**

The success of treatment for congenital heart disease (CHD) has increased the number of pregnancies complicated by CHD. CHD can interfere with the normal increases of 40–50 % in plasma volume and 30–50 % in cardiac output during pregnancy[1]. Studies have shown that the risk of obstetric complications in these pregnancies is high, with reported rates of miscarriage, preterm delivery, and small for gestational age (SGA) of 19.4 %, 12.0 %, and 25.0 %, respectively.[2] The Fontan procedure is a palliative operation for patients with CHDs who lack biventricular circulation, and complications of the procedure include high central venous pressure, low cardiac output, and hypoxia. High rates of miscarriage (45.0–54.8 %), preterm delivery (59.0–70.4 %), and SGA babies (20.0–55.6 %) have been reported among pregnant women who have a Fontan circulation.[3, 4]

The placenta is an organ that connects mother and fetus and is essential for successful pregnancy and fetal health. Throughout pregnancy, the placenta has many roles, including in gas exchange, immunity, nourishment, and hormone productions. Abnormal placental function is a cause of pregnancy complications such as miscarriage, preterm delivery, pre-eclampsia (PE), and SGA.

However, little is known regarding the effect of the maternal Fontan circulation on placental histology. Maternal perfusion pressure is crucial to placental diffusion and transfer. We hypothesized that in women with Fontan circulation, the maternal cardiac condition would cause placental hypoxia, which in turn, could be an important cause of the poor obstetric outcomes.

The aim of this study was to investigate placental pathology from pregnancies in women with a

Fontan circulation, with particularly focusing on hypoxic changes.

**Methods:**

**Study population**

The study subjects were 11 women with a Fontan circulation who had 18 pregnancies registered at the National Cerebral and Cardiovascular Center (NCVC) in Suita, Japan between January 2006 and September 2019. Eight placentas from 7 women and chorionic villi from 6 miscarriages in 4 women were also examined. The study was approved by the Institutional Research Ethics Board of the NCVC, Suita, Japan (M30-161).

**Data collection**

Data were obtained from the medical records and samples stored in the NCVC Biobank. The demographic and clinical data of mothers and babies are presented in Table 1. Adverse cardiovascular events were defined as heart failure, arrhythmia requiring medication and/or any intervention, worsening of atrioventricular valve regurgitation (AVVR) requiring termination of pregnancy, thromboembolism, and cardiac arrest or death.

**Placental pathologic examinations**

The placentas were weighed fresh after removal of umbilical cords and membranes. In the gross examination, we evaluated the degree of chronic subchorionic hematoma (SCH) and the presence of disorders of membrane development. Placental histologic examination was performed using a standard protocol. At least 4 samples were taken from each placenta: 1 at the cord insertion; 2 from the central tissue that appeared normal on the gross examination (if the appearance was grossly abnormal, additional samples were obtained), and 1 from the umbilical cord and membrane roll. Specimens were inspected for evidence of placental hypoxia. Placental lesions were classified according to the criteria of the 2014 Amsterdam Placental Workshop Group[5] and previous studies of

placental hypoxia,[6, 7] as follows: 1) Lesions associated with maternal vascular malperfusion (MVM): villous infarcts, increased syncytial knots, villous agglutination, increased intervillous fibrin deposition, distal villous hypoplasia, accelerated villous maturation, and decidual vasculopathy(acute atherosis and mural hypertrophy). 2) Lesions associated with fetal vascular malperfusion (FVM): thrombosis of the chorionic plate and stem villous vessels and avascular villi. 3) Lesions associated with placental hypoxia other than MVM and FVM: villous stromal fibrosis, hypervascularity, chorangiosis, perivillous fibrin deposition, laminar necrosis and delayed villous maturation. Hypervascularity was defined as the presence of 6 to 9 capillaries in the terminal villi of a lesion, whereas chorangiosis was defined as 10 or more capillaries in more than 10 terminal villi in several areas of the placenta visualized using a 10 × objective.[8] In case of miscarriage, we also performed a histological analysis of the chorionic villus from products of conception.

**Quantifying immunohistological findings and villous fibrosis**

Of the 8 placentas, 7 placentas from 7 women with a Fontan circulation had additional tissue sections available, provide by the NCVC Biobank, to quantify the immunohistological findings and villous fibrosis. These palcentas were compared to 5 control placentas obtained from planned cesarean deliveries at term without maternal or fetal complications (all women were non-smokers). Control placentas, delivered by healthy women without any complications at NCVC, were also provided by NCVC Biobank. Formalin-fixed and paraffin-embedded sections were stained by immunohistochemistry with anti-CD 31 antibody (M0823, DAKO) for endothelial cells and anti-CD 68 antibody (M0876, DAKO) for macrophages termed Hofbauer cells in the villi, and were examined using a Leica Bond-III autostainer (Leica Biosystems, Wetzlar, Germany) as previously described.[9] Immunohistochemistry using anti-hypoxia inducible factor (HIF)-1α antibody (ab51608; Abcam) to show evidence of hypoxia was outsourced (Applied Medical Research). Briefly, paraffin-embedded sections were deparaffinized and rehydrated. Antigen retrieval was conducted with EDTA

buffer (pH 9.0) in a water bath. After antigen retrieval at 98 ℃, the sections were treated with 3 % $H_2O_2$ for 10 min and then incubated with anti HIF-1α (diluted 1:100) at 4 ℃ overnight. The sections were then washed and incubated with diaminobenzidine solution. Masson's trichrome stain was used to evaluate villous stromal fibrosis.

Five random 10 × objective field images per slide were taken, and the number of capillaries was counted and the total villous area was determined using Image J software (National Institutes of Health, Bethesda, MD, USA). At first, the images were converted to 8-bit size and binarized using the Image J thresholding algorithm. Then, the number of capillaries was counted using the Image J command of analyzing particles. The total villous area was measured using the Image J command of measuring the area after applying the erosion processes 3 times to binarized images. Capillary density was then calculated as the number of capillaries divided by the total villous area. Five random 40 × objective field images per slide were taken and the number of macrophages was counted by 2 observers (T.K. and K.O.). The observers scored HIF-1α staining as follows: 0, no staining; 1, mild staining; 2, moderate staining; 3, intense staining. The area of fibrosis was analyzed for 20 villi cut along the short axis of 20 × objective field images, and the percentage area of fibrosis was determined automatically using Aperio ImageScope (Leica Biosystems).

**Statistical Analysis**

Data with a normal (Gaussian) distribution are presented as the mean ± standard deviation and data that were not normally distributed are presented as the median with minimum-maximum. Student's t test was used to compare normally distributed continuous variables and Mann-Whitney U test was used for comparison of non-parametric variables. Pearson's correlation coefficient was used to evaluate correlations between capillary density and area of fibrosis. Statistical analyses were performed with GraphPad Prism 7. Two side $P < 0.05$ was considered statistically significant.

## Results

Of 18 pregnancies in 11 women in the present study, 9 pregnancies in 6 women ended before 22 weeks of gestation. From the 9 continuing pregnancies, 8 placentas were available for detailed clinical and histopathological description, and 7 placentas were available for quantifying analysis of immunohistological findings and villous fibrosis. Of the 9 miscarriages, the chorionic villi of 6 miscarriages in 4 women for which tissue was available were evaluated histologically.

### Maternal cardiac characteristics of the eight deliveries

Table 1 provides baseline characteristics of the 8 deliveries whose placentas were available for histological examination. Five of 8 women showed decreased systemic saturation (95 or < 95 %) levels at rest. Regarding systemic ventricular function, all but 1 had preserved ejection function (55 or > 55 %), but, 3 out of the 7 women in whom cardiac index (CI) was measured had a decreased CI, and 3 out of the remaining 4 had a CI just above the lower limit of the normal range (2.6−4.2 l/min/$m^2$). Furthermore, 4 of 6 women had decreased peak $VO_2$. Because of Fontan circulation, central venous pressure was elevated, with a median value of 10 mmHg. Women taking antiplatelet agents before pregnancy continued with this medication, except for 1 woman (Delivery case 1 in Table 1), who stopped taking the antiplatelet agent because of SCH identified on an ultrasound scan. Women taking warfarin before pregnancy changed from warfarin to prophylactic doses of unfractionated heparin during pregnancy. Women who were not taking warfarin before pregnancy were started on heparin only if there was an increased risk of thromboembolism (e.g., due to arrhythmia or hospitalization due to preterm delivery). Three women took a diuretic agent and 2 women took a beta-blocker during pregnancy.

### Cardiovascular complications of the eight pregnancies with placental histology

Two women had cardiovascular events during pregnancy. One (Delivery case 2-1) experienced atrial tachycardia, which required defibrillation and medication at 14 weeks of gestation. Because

this woman also had sick sinus syndrome, she experienced extreme bradycardia with anti-tachyarrhythmic medications and proceeded to pacemaker insertion at 20 weeks of gestation. Another woman (Delivery case 3) suffered worsening AVVR at 36 weeks of gestation, and underwent an emergency cesarean section. Cardiovascular events occurred after delivery in 4 women: atrial tachycardia at 2 days after delivery (n = 1, Delivery case 1); intraventricular thrombosis at 8 days after delivery (n = 1, Delivery case 2-2); and heart failure at 4 weeks (n = 1, case 5) and at 6 weeks (n = 1, Deliery case 7) after delivery. There was no significant factor for predicting maternal cardiovascular complications.

**Obstetrics and neonatal outcomes of the eight pregnancies with placental histology**

In the 8 pregnancies with placental histology, the median gestational age at delivery was 36 weeks. There were 4 preterm deliveries and 4 women delivered at the 37 weeks. Two (25.0 %) women had SCH (Delivery case 1 and case 2-1) and gestational diabetic mellitus (Delivery case 2-1, 2), and 1 (12.5 %) had hypertensive disorders of pregnancy (Delivery case 2-2). All neonates, including the 5 who were SGA (62.5 %), survived to hospital discharge without complications. None of them had CHD. Of the 8 women, 5 were examined by cardiopulmonary exercise testing before pregnancy; the median interval between the examination and pregnancy was 15 months [minimum-maximum 5–39] and 1 case was examined 14 months after delivery. The peak $VO_2$ in the mothers of SGA infants was lower than those that in the mothers of appropriate for gestational age infants (19.35 ± 2.69 vs 26.6 ± 1.27 ml/kg/min, P = 0.026).

**Gross and histological findings of the eight placentas**

Five placentas had low placental weight for gestational age (< 25$^{th}$ percentile) and had a low fetal/placental ratio (< 25$^{th}$ percentile) (Table 2). The presence of a low-weight placenta and SGA status was concordant in 6 of 8 neonates. Of the remaining 2 neonates, 1 baby with SGA had a normal-weight placenta and the other had a low-weight placenta but a normal birth weight. Of the 8

placentas, all but 1 showed chronic SCH (massive, n = 1; multifocal, n = 6); 4 were circummarginate and 1 was circumvallate. Histologically, all specimens showed evidence of placental hypoxia (MVM, n = 7; FVM, n = 1). None showed decidual vasculopathy. All 8 placentas showed hypervascularity, and 7 had chorangiosis and villous stromal fibrosis (Table 2, Figure 1).

**Quantifying analysis of immunohistological findings and stromal fibrosis**

The quantitative findings of 7 of the 8 placentas in women with a Fontan circulation and 5 control placentas are presented in Table 3. Placentas of the study group had significantly greater capillary density and area of fibrosis than control placentas (capillary density (number/$mm^2$): 537 [minimum-maximum 471–639] vs 424 [minimum-maximum 292–543], P = 0.03, area of fibrosis (%): 44.3 [minimum-maximum 39.2–50.8] vs 32.4 [minimum-maximum 29.1–43.8], P = 0.01), and was a significant correlation between capillary density and area of fibrosis (P = 0.0007, Figure 2). Although there were no significant differences in HIF-1α the scores in the villi between placentas of women with a Fontan circulation and control placentas (1.0 [minimum-maximum 0–2] vs 0.0 [minimum-maximum 0–1], P = 0.07), seven of eight placentas in women with a Fontan circulation expressed HIF-1α, with three showing a score > 2. The number of macrophages in the villous area was not significantly different between the placentas in women with a Fontan circulation and control placentas (number): 8.2 [minimum-maximum 3.4–12.6] vs 5.8 [minimum-maximum 5.4–10.2], P = 0.66).

**Interaction between hemodynamic features and placental pathology**

Most placentas in women with a Fontan circulation showed both MVM and other hypoxic lesions, but 1 did not show MVM (Delivery case 5). This woman had the highest peak $VO_2$ and systemic saturation among the cases studied.

**Interaction between placental pathology and obstetric outcome**

Both MVM lesions and other hypoxic lesions were seen in all 5 cases of SGA. Specifically,

among other hypoxic lesions, all cases showed hypervascularity, and 4 of 5 cases showed villous stromal fibrosis and chorangiosis.

**Miscarriage**

Nine pregnancies in 6 women resulted in miscarriage: 7 at very early gestation (including 1 twin pregnancy), and 1 each at 12 and 17 weeks of gestation. There were no therapeutic terminations. Of the 6 women, 3 miscarried twice, and 2 of these 3 did not have a live birth during the study period. The chorionic villi in 6 miscarriages were examined and the baseline characteristics are presented in Table 4. In 1 miscarriage at 17 weeks of gestation, SCH was present from 8 weeks of gestation, fetal weight was normal, and the placental weight was large for gestational age. Histologically, villous stromal fibrosis, perivillous fibrin deposition and chronic SCH were observed. There was no evidence of infection. The histological findings in the chorionic villi in the other 5 miscarriages were similar to those seen in miscarriages in mother with normal hearts.

**Discussion**

In this study, we observed 2 important findings in the placentas from mothers with a Fontan circulation that could be associated with poor pregnancy outcomes: First, they frequently showed low weight for gestational age and chronic SCH. Second, all placentas demonstrated some histological evidence of placental hypoxia, particularly MVM, hypervascularity/chorangiosis and villous stromal fibrosis.

Placental weight is an indicator of placental growth and a surrogate for placental function.[5] The high frequency of low placental weight in this study (n = 5/8, 62.5 %) suggests that women with a Fontan circulation could be more susceptible to impaired placental growth, which is presumably

associated with underlying maternal hypoxia and cardiac dysfunction. Four of 5 newborns with a low-weight placenta were SGA. The reported risk factors for SGA in mothers with CHD are maternal cyanosis,[10] low cardiac output, and low peak $VO_2$.[11] The present study also identified low peak $VO_2$ as a good marker for SGA. Macroscopically, most placentas (n = 7/8, 87.5 %) had noticeable chronic SCH, consistent with a previous report,[12] indicating that even if it is not detected clinically, subclinical SCH could be common in placentas of women with a Fontan circulation. SCH might have had negative effects on placental function, and is possibly associated with aspirin and heparin use during pregnancy.

As we hypothesized, all placentas demonstrated some histological evidence of placental hypoxia, most commonly MVM (n = 7/8, 87.5 %). A previous study identified MVM in 8.4 % of 856 healthy women and found a link between MVM lesions and higher rates of PE, SGA, and preterm delivery compared with the non-MVM group.[13] Our results are in agreement with this observation, as shown by the high rate of SGA (n = 5/8, 62.5 %) and preterm delivery (n = 4/8, 50.0 %). The most common MVM lesion in PE is decidual vasculopathy.[14] However, no PE or decidual vasculopathy was observed in the placentas of women with a Fontan circulation in the present study. Although both PE and a Fontan circulation are associated with placental hypoxia, the mechanisms are different: PE occurs due to the failure of remodeling of the spiral artery, whereas in a Fontan circulation the pre-placental problems are more likely to manifest as MVM lesions other than decidual vasculopathy. In a previous study of 954 placentas of SGA with a healthy mother, the rates of any MVM, multiple MVM or any FVM were 84.8 %, 46.0 %, and 25.1 %, respectively.[15] Compared with these cases of SGA, placentas of women with a Fontan circulation were much more likely to have MVM (any, 87.5 %; multiple, 87.5 %) and less likely to have FVM (12.5 %). This comparison also indicates that the high rates of MVM in placentas of women with a Fontan circulation are caused by the maternal cardiac conditions.

Among hypoxic lesions other than MVM and FVM, the high occurrence of chorangiosis (n = 7/8, 87.5 %) in placentas of women with a Fontan circulation is intriguing. Chorangiosis is found in 6.6 % of the general population,[16] in 13.3 % of the population at high altitude,[17] and in 21.2 % of smokers.[18] When it occurs in such populations, it is associated with low-grade hypoxia and is considered an adaptive mechanism to increase gas exchange.[19] Although it is reported that placentas with chorangiosis induced placenta to become large for gestational age,[21] in the present study, 4 of 7 (57.1 %) placentas were low weight despite having chorangiosis. Furthermore, chorangiosis was present in all but 1 placenta, regardless of SGA status. These data suggested that, in many cases, the chorangiosis induced in the placentas of women with a Fontan circulation is not enough to compensate for the hypoxic state and impaired placental growth resulting from maternal cardiac conditions. Regarding the Delivery case 5, which showed a small placenta and normal weight baby, the chorangiosis could have compensated for the placental insufficiency because this case did not show MVM. As shown in this case, in addition to placental weight, placenteal quality is important for normal fetal growth. MVM and other hypoxic lesions, including chrangiosis, show placental insufficiency. Conversely, chorangiosis results from compensation for placental insufficiency, so whether the fetus appears small or not depends on both the degree of chorangiosis and the degree of other lesions.

The quantifying histological findings confirmed that placentas of women with a Fontan circulation had a greater capillary density and area of stromal fibrosis than control placentas, and these findings are considered to be the result, in part, of the hypoxic state. There was a significant correlation between capillary density and the area of fibrosis. This may be one piece of evidence that indicates that chorangiosis is the result of placenta’s response against the other lesions. Placental hypoxia in women with a Fontan circulation in this study is also supported by the immunohistochemical expression of HIF-1α expression in most of the placentas.

Therefore, the placentas of women with a Fontan circulation are considered highly exposed to hypoxia and low perfusion, which could influence placental growth and function. Because uteroplacental blood flow is perfusion-dependent, improving cardiac output to the level required by pregnancy is important to improve pregnancy outcomes. However, there is no way to directly increase cardiac output. Bed rest might improve pregnancy prognosis by increasing the blood supply to the uterus. With regard to hypoxia, oxygen supply and correcting any iron deficiency anemia might improve obstetric outcomes.

This study has some limitations. First, the dataset was small. Second, we quantified only hypervascularity, villous stromal fibrosis, the number of macrophages, and expression of HIF-1α expression. Third, because the pathophysiology of a Fontan circulation involves multiple organs, we cannot completely rule out the effect of factors other than hypoxia. However, we found no blood test abnormalities suggestive of undernutrition or abnormal liver function.

**Conclusion:**

The placentas in women with a Fontan circulation were characterized by hypoxic changes, growth impairment, and chronic SCH, which could play important roles in poor pregnancy outcomes.

**Acknowledgments**: This study was supported by the Intramural Research Fund for Cardiovascular Diseases of the National Cerebral and Cardiovascular Center (30-6-17) and was performed using the National Cerebral and Cardiovascular Center Biobank resource (NCVC Biobank URL http://www.ncvc.go.jp/biobank/). T.K., K.O., and J.Y. designed the study. T.K., K.O., and H.I-U. analyzed pathological examination. T.K., K.O., and C.K. analyzed the clinical data. T.K. and K.O. wrote the manuscript through a fruitful discussion with and supervision by C.K., T.S., A.N., N.I.,

H.O., K.K., H.I., T.N., H.I-U., and J.Y. Assistance with editing the final manuscript was provided by Prof P J Steer.

**Data-Availability:** The deidentified participant data will not be shared.

**References**

1. Pritchard JA. Changes in the blood volume during pregnancy and delivery. *Anesthesiology* 1965; **26:** 393-399.

2. Drenthen W, Boersma E, Balci A, Moons P, Roos-Hesselink JW, Mulder BJ, et al. Predictors of pregnancy complications in women with congenital heart disease. *Eur Heart J* 2010; **31:** 2124-2132.

3. Cauldwell M, Steer PJ, Bonner S, Asghar O, Swan L, Hodson K, et al. Retrospective UK multicentre study of the pregnancy outcomes of women with a Fontan repair. *Heart* 2018; **104:** 401-406.

4. Garcia Ropero A, Baskar S, Roos Hesselink JW, Girnius A, Zentner D, Swan L, et al. Pregnancy in women with a Fontan circulation: a systematic review of the literature. *Circ Cardiovasc Qual Outcomes* 2018; **11:** e004575.

5. Khong TY, Mooney EE, Ariel I, Balmus NC, Boyd TK, Brundler MA, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. *Arch Pathol Lab Med* 2016; **140:** 698-713.

6. Parks WT. Placental hypoxia: the lesions of maternal malperfusion. *Semin Perinatol* 2015; **39:** 9-19.

7. Redline RW. Classification of placental lesions. *Am J Obstet Gynecol* 2015; **213:** 21-28.

8. Altshuler G. Chorangiosis. An important placental sign of neonatal morbidity and mortality. *Arch Pathol Lab Med* 1984; **108:** 71-74.

9. Nakayama T, Sugano Y, Yokokawa T, Nagai T, Matsuyama TA, Ohta-Ogo K, et al. Clinical impact of the presence of macrophages in endomyocardial biopsies of patients with dilated cardiomyopathy.

*Eur J Heart Fail* 2017; **19:** 490-498.

10. Gelson E, Curry R, Gatzoulis MA, Swan L, Lupton M, Steer P, et al. Effect of maternal heart disease on fetal growth. *Obstet Gynecol* 2011; **117:** 886-891.

11. Ohuchi H, Tanabe Y, Kamiya C, Noritake K, Yasuda K, Miyazaki A, et al. Cardiopulmonary variables during exercise predict pregnancy outcome in women with congenital heart disease. *Circ J* 2013; **77:** 470-476.

12. Phillips AL, Cetta F, Kerr SE, Cheek EH, Rose CH, Bonnichsen CR, et al. The placenta: a site of end-organ damage after Fontan operation. A case series. *Int J Cardiol* 2019; **15:** 52-55.

13. Wright E, Audette MC, Ye XY, Keating S, Hoffman B, Lye SJ, et al. Maternal vascular malperfusion and adverse perinatal outcomes in low-risk nulliparous women. *Obstet Gynecol* 2017; **130:** 1112-1120.

14. Weiner E, Feldstein O, Tamayev L, Grinstein E, Barber E, Bar J, et al. Placental histopathological lesions in correlation with neonatal outcome in preeclampsia with and without severe features. *Pregnancy Hypertens* 2018; **12:** 6-10.

15. Kibel M, Kahn M, Sherman C, Kingdom J, Zaltz A, Barrett J, et al. Placental abnormalities differ between small for gestational age fetuses in dichorionic twin and singleton pregnancies. *Placenta* 2017; **60:** 28-35.

16. Ogino S, Redline RW. Villous capillary lesions of the placenta: distinctions between chorangioma, chorangiomatosis, and chorangiosis. *Hum Pathol* 2000; **31:** 945-954.

17. Soma H, Murai N, Tanaka K, Oguro T, Kokuba H, Yoshihama I, et al. Review: Exploration of placentation from human beings to ocean-living species. *Placenta* 2013; **34:** 17-23.

18. Akbulut M, Sorkun HC, Bir F, Eralp A, Duzcan E. Chorangiosis: the potential role of smoking and air pollution. *Pathol Res Pract* 2009; **205:** 75-81.

19. Barut A, Barut F, Kandemir NO, Aktunc E, Arikan I, Harma M, et al. Placental chorangiosis:

the association with oxidative stress and angiogenesis. *Gynecol Obstet Invest* 2012; **73:** 141-151.

20. URL: jspe.umin.jp/medical/keisan.html. 08/15/2020.

21. Kraus FT. Placental pathology. Washington (DC): *The American Registry of Pathology*; 2004.

**Legends for Figures**

Figure 1. Representative findings of placental specimens (H&E staining: hematoxylin and eosin staining). A. Control placenta (H&E staining, bar = 200 μm). B. Increased number of syncytial knots. Note the aggregation of syncytiotrophoblastic nuclei in tight clusters (H&E staining, bar = 100 μm). C. Villous stromal fibrosis. Chorionic villi show increased extracellular matrix (H&E staining, bar = 50 μm). D. Perivillous fibrin deposition. Large deposits of fibrin are seen surrounding the villi (H&E staining, ba r= 200 μm). E. Chorangiosis. More than 10 terminal villi with 10 or more capillaries are seen in several areas of the placenta (H&E staining, bar = 100 μm). F. Representative histological findings of placental specimens (Masson's trichrome staining, bar = 100 μm). Representative microscopic immunohistochemical findings of placental specimens. G. Endothelial cells are stained by CD 31 (bar = 200 μm). H. Macrophages are stained by CD 68 (bar = 50 μm). I. Moderate HIF-1α expression in the nuclei of trophoblast cells (bar = 20 μm).

Figure 2. Correlation between capillary density and area of fibrosis.

**Table 1.** Baseline characteristics of eight deliveries in seven women with a Fontan circulation

| Case | Age years | BMI kg/m$^2$ | CHD | Operation type (age) | Systemic Ventricle | SVEF % | AVVR | NYHA | Peak $VO_2$ ml/kg/min | $SpO_2$ % | CVP† mmHg | CI † l/min/m$^2$ | Medication Before pregnancy | Medication During pregnancy | Delivery week | Delivery mode | Indication for CS | Birth weight g (percentile) [20] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 27 | 18.8 | Right iso SV | TCPC (16) | RV | 68* | Moderate | II | NA | 96 | 10 | 2.76 | ASA<br>D | H (–22w) | 30w3d | CS | pPROM | 1450 (44.1) |
| 2-1 | 28 | 22.3 | TA | APC (11)<br>TCPC (23) | LV | 72* | Mild | II | 22.3 | 94 | 11 | 2.75 | ASA<br>D<br>AAD | ASA<br>H (–20w)<br>B<br>D | 35w6d | CS | NRFS | 1760 (2.9) |
| 2-2 | 34 | 21.6 | TA | APC (11)<br>TCPC (23) | LV | 55† | Trivial | II | 18.6 | 96 | 14 | 2.70 | ASA<br>A<br>D<br>AAD | ASA<br>B<br>D | 33w0d | CS | NRFS | 1451 (3.1) |
| 3 | 29 | 19.9 | MS hypoLV | APC (5) | RV | 72* | Moderate | I | 25.6‡ | 94 | NA | NA | – | H (–28w) | 36w4d | CS | Worsening TR | 2456 (36.8) |
| 4 | 19 | 20.7 | Right iso SV | TCPC (3) | RV | 60* | Mild | I | NA | 91 | 10 | 2.25 | ASA | ASA | 37w0d | VD | – | 1620 (0.2) |
| 5 | 33 | 23.5 | Left iso DILV | TCPC (15) | LV | 57† | Trivial | I | 27.4 | 96 | 16 | 2.19 | W<br>ASA<br>A<br>D | ASA<br>H<br>D | 37w0d | CS | Breech | 2312 (23.9) |
| 6 | 28 | 20.8 | TA | APC (8)<br>TCPC (23) | LV | 58† | Mild | II | 16.0 | 95 | 8 | 2.51 | ASA | ASA (–27w)<br>H (27w–) | 37w5d | CS | NRFS | 1726 (0.1) |
| 7 | 28 | 23.9 | DIRV | TCPC (7) | RV | 44† | Mild | II | 20.5 | 95 | 9 | 3.67 | W<br>ASA | ASA<br>H | 37w6d | CS | Delivery arrest | 1902 (0.4) |

BMI; body mass index, CHD; congenital heart disease, SVEF; systematic ventricular ejection function, AVVR; atrioventricular valve regurgitation, NYHA; New York Heart Association, CVP; central venous pressure, CI; cardiac index, CS; cesarean section, Right iso; right isomerism, SV; single ventricle, TCPC; total cavopulmonary connection, RV; right ventricle, NA; not available, ASA; acetylsalicylic acid, D; Diuretic agent, H; heparin, pPROM; preterm premature rupture of membranes, TA; tricuspid atresia, APC; atriopulmonary connection, LV; left ventricle, AAD; antiarrhythmic drugs, B; β-blocker, NRFS; non reassuring fetal status, A; angiotensin converting enzyme inhibitor/angiotensin II receptor blocker, MS; mitral stenosis, TR; tricuspid regurgitation, VD; vaginal delivery, Hypo LV; hypoplastic left ventricle, Left iso; left isomerism, DILV; double inlet left ventricle, W; Warfarin, DIRV; double inlet right ventricle. *estimated by echocardiography or cardiac magnetic resonance imaging, †measured by catheterization study. ‡examined 14 months after the delivery.

**Table 2.** Placental findings of eight deliveries in seven women with a Fontan circulation

| Case | 1 | 2-1 | 2-2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| Circummarginate or circumvallate | Circumvallate | – | – | Circummarginate | – | Circummarginate | Circummarginate | Circummarginate |
| Chronic subchorionic hematoma | Focal | Focal | Focal | Massive | – | Focal | Focal | Focal |
| Placental weight (g)/ Percentile [21] | 365/ 50–75$^{th}$ | 465/ 50–75$^{th}$ | 220/ 3–5$^{th}$ | 500/ 50–75$^{th}$ | 288/ < 3$^{th}$ | 285/ < 3$^{th}$ | 242/ < 3$^{th}$ | 375/ 10–25$^{th}$ |
| Fetal-placental weight ratio/ Percentile [21] | 3.98/ < 25$^{th}$ | 3.84/ < 3$^{th}$ | 6.59/ > 50$^{th}$ | 4.91/ < 10$^{th}$ | 5.62/ < 25$^{th}$ | 8.11/ > 75$^{th}$ | 7.13/ > 50$^{th}$ | 5.07/ < 5$^{th}$ |
| Maternal vascular malperfusion | | | | | | | | |
| Villous infarcts | + | – | – | + | – | – | + | – |
| Increased syncytial knots | + | + | + | + | + | – | + | + |
| Villous agglutination | – | – | + | – | – | – | + | + |
| Increased intervillous fibrin deposition | – | + | – | + | + | – | – | + |
| Distal villous hypoplasia | – | – | – | – | – | – | + | – |
| Accelerated villous maturation | – | – | – | – | – | – | – | – |
| Decidual vasculopathy | – | – | – | – | – | – | – | – |
| Fetal vascular malperfusion | | | | | | | | |
| Fetal vessels thrombosis | – | – | – | – | – | – | + | – |
| Avascular villi | – | – | – | – | – | – | – | – |
| Others | | | | | | | | |
| Villous stromal fibrosis | + | + | + | + | + | + | – | + |
| Hypervascularity | + | + | + | + | + | + | + | + |
| Chorangiosis | + | + | + | + | + | + | + | – |
| Perivillous fibrin deposition | + | – | + | + | + | – | – | + |
| Laminar necrosis | – | – | + | – | + | – | – | – |
| Delayed villous maturation | – | + | – | – | – | – | – | – |

The cases of small for gestational age are Case 2-1, 2-2, 4, 6 and 7.

**Table 3.** Quantifying immunohistological and villous fibrosis data in seven out of eight placentas of study group (Case 2-2 was not available) and five in control group.

| Case | 1 | 2-1 | 3 | 4 | 5 | 6 | 7 | Control | Control | Control | Control | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Density of capillaries (number/mm$^2$) | 537 ± 40 | 581 ± 121 | 527 ± 108 | 639 ± 76 | 476 ± 67 | 563 ± 44 | 471 ± 61 | 444 ± 27 | 424 ± 49 | 543 ± 64 | 292 ± 73 | 378 ± 45 |
| Number of macrophages (number) | 3.4 ± 2.0 | 6.6 ± 4.1 | 9.2 ± 1.9 | 8.2 ± 3.1 | 12.6 ± 5.6 | 8.2 ± 3.7 | 7.4 ± 2.9 | 5.8 ± 1.4 | 9.2 ± 3.1 | 5.6 ± 3.6 | 5.4 ± 3.9 | 10.2 ± 3.8 |
| HIF-1α score | 2 | 2 | 1 | 1 | 1 | 2 | 0 | 1 | 0 | 0 | 1 | 0 |
| Area of fibrosis, % | 41.9 ± 4.9 | 41.0 ± 5.8 | 39.2 ± 7.2 | 50.8 ± 9.2 | 45.9 ± 6.9 | 44.3 ± 10.8 | 46.1 ± 7.1 | 36.9 ± 8.8 | 32.4 ± 6.3 | 43.8 ± 8.2 | 29.1 ± 5.7 | 30.5 ± 3.9 |

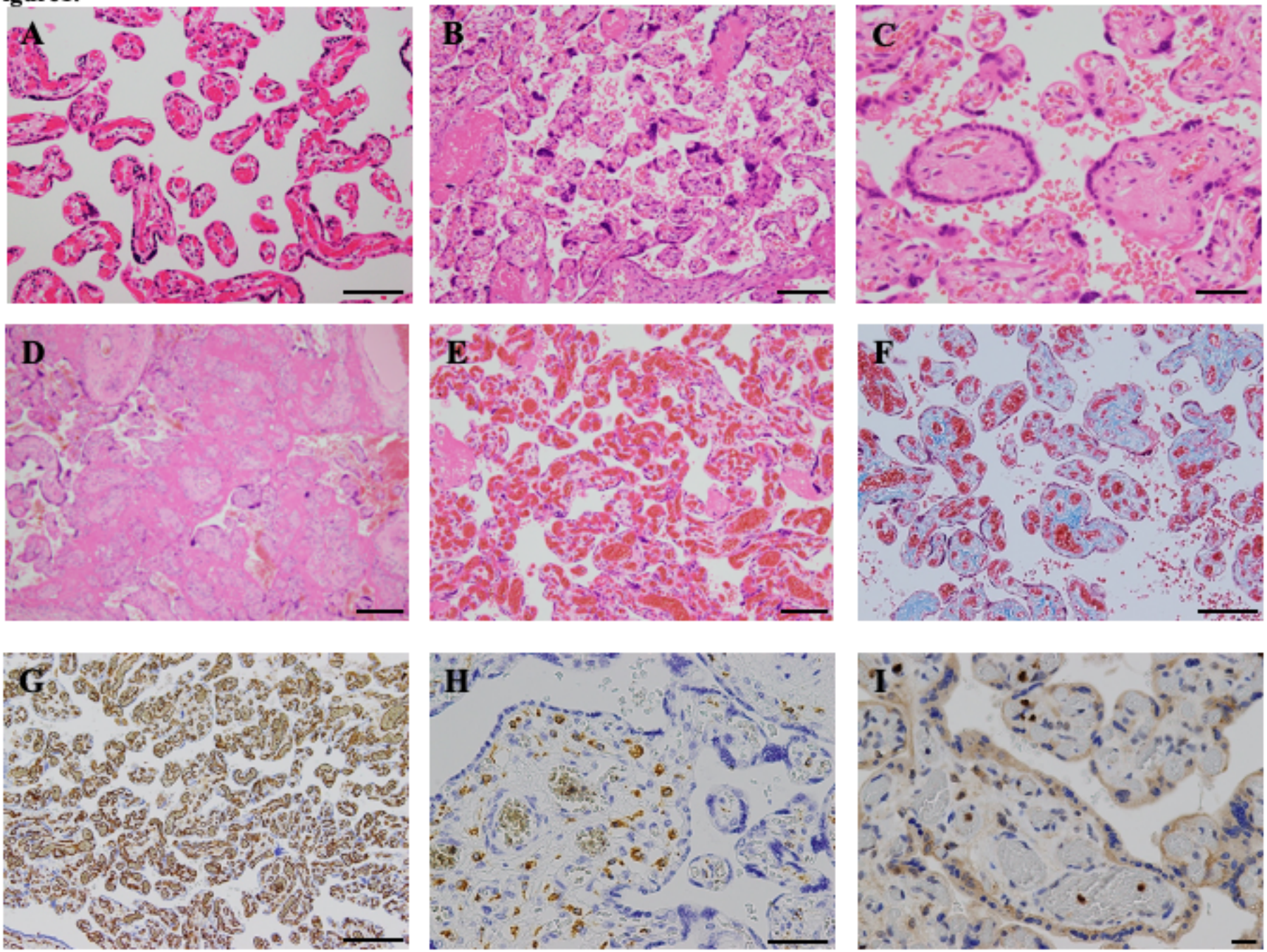
Data are shown as mean ± standard deviation, HIF: hypoxia inducible factor.

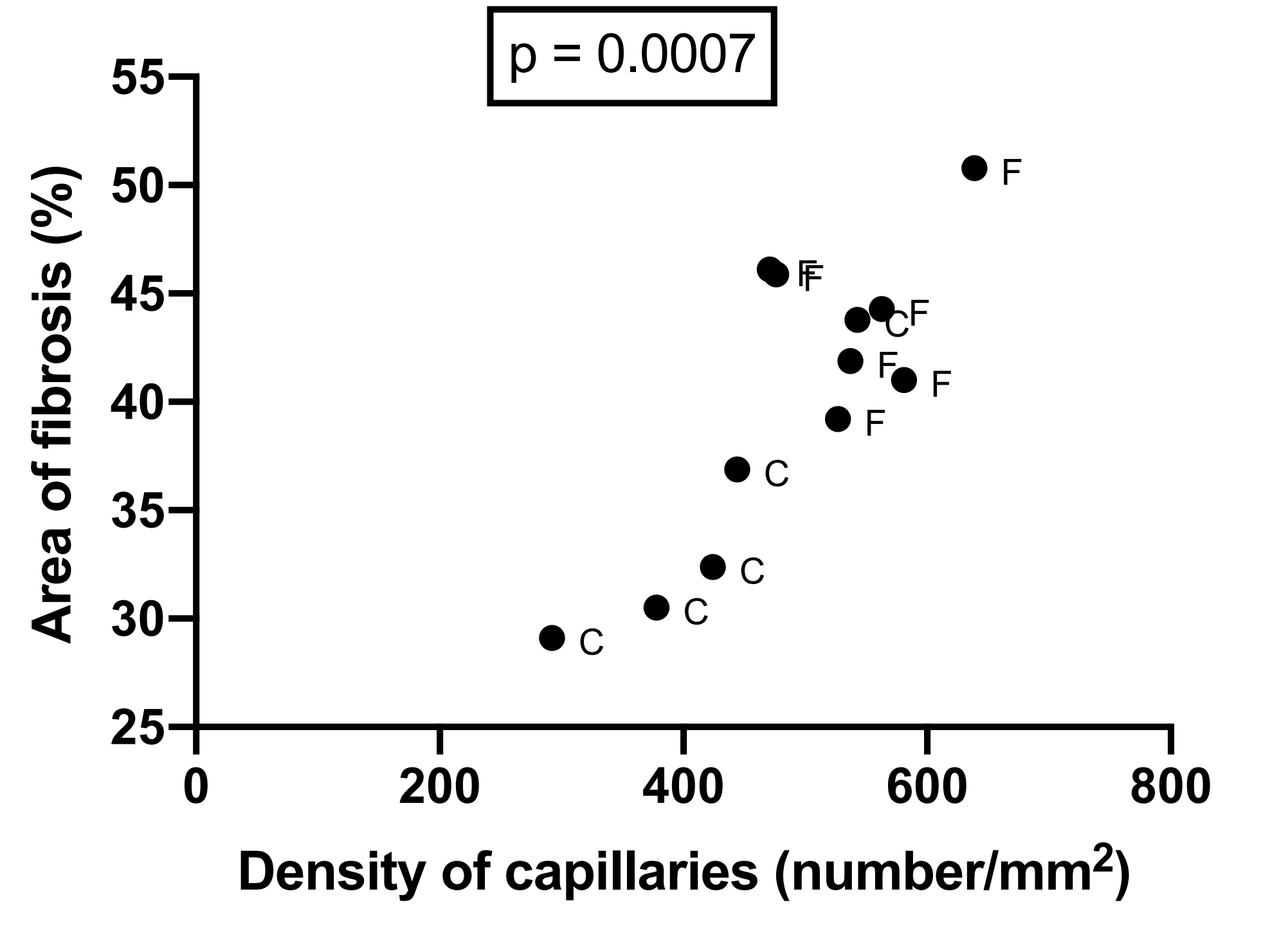
**Table 4.** Baseline characteristics of four women with six miscarriages

| Woman with miscarriage | Corresponding delivery case | Miscarriage week | Age years | BMI kg/m$^2$ | CHD | Operation type (age) | Systemic Ventricle | SVEF % | AVVR | NYHA | Peak $VO_2$ ml/kg/min | $SpO_2$ % | CVP† mmHg | CI † l/min/m$^2$ | Medication Before pregnancy |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2-1, 2-2 | 7 | 30 | 21.4 | TA | APC (11)<br>TCPC (23) | LV | 73* | Mild | II | 18.6 | 94 | 13 | 2.75 | ASA<br>D |
| 2 | 6 | 5<br>8 | 25<br>26 | 20.8 | TA | APC (8)<br>TCPC (23) | LV | 58† | Mild | II | 21.7 | 95 | 8 | 3.25 | _ |
| 3 | _ | 9 | 31 | 18.8 | Right iso CAVC | TCPC (1) | RV | 62† | Mild | II | 18.5 | 97 | 7 | 2.34 | B<br>AAD |
| 4 | _ | 17<br>8 | 28<br>29 | 24.3 | TA | APC (3)<br>TCPC (21) | LV | 60* | Mild | I | 18.1 | 95 | 13 | 2.63 | ASA<br>D |

BMI; body mass index, CHD; congenital heart disease, SVEF; systemic ventricular ejection function, AVVR; atrioventricular valve regurgitation, NYHA; New York Heart Association, CVP; central venous pressure, CI; cardiac index, Right iso; right isomerism, SV; single ventricle, TCPC; total cavopulmonary connection, RV; right ventricle, ASA; acetylsalicylic acid, D; Diuretic agent, TA; tricuspid atresia, APC; atriopulmonary connection, LV; left ventricle, AAD; antiarrhythmic drugs, B; β-blocker, A; angiotensin converting enzyme inhibitor/angiotensin II receptor blocker. *estimated by echocardiography or cardiac magnetic resonance imaging, †measured by catheterization study.

**Figure1.**




p = 0.0007
Area of fibrosis (%)
55
50
45
40
35
30
25
0
200
400
600
800
Density of capillaries (number/mm2)
F
F
F
C
F
F
F
F
C
C
C
C