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Risk factors for non-previa placenta accreta spectrum in pregnancies conceived through frozen embryo transfer during a hormone replacement cycle in Japan

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Abstract

Purpose: Non-previa placenta accreta spectrum (PAS) is associated with assisted reproductive technology (ART), particularly frozen embryo transfer during hormone replacement therapy (HRC-FET). We especially aimed to evaluate the prevalence and risk factors for non-previa PAS in HRC-FET pregnancies.

Methods: Overall, 279 women who conceived through ART at three ART facilities and delivered at a single center were included in this retrospective study. Data regarding endometrial thickness at embryo transfer, previous histories, and type of embryo transfer—HRC-FET, frozen embryo transfer during a natural ovulatory cycle (NC-FET), and fresh embryo transfer (Fresh-ET)—were collected. Univariable logistic regression analyses were conducted.

Results: The prevalence of non-previa PAS was 27/192 (14.1%) in the HRC-FET group and 0 (0.0%) in both the NC-FET and Fresh-ET groups. Significantly high odds ratio [95% confidence interval] of non-previa PAS was associated with a history of artificial abortion (6.45 [1.98–21.02]), endometrial thickness <8.0 mm (6.11 [1.06–35.12]), resolved low-lying placenta (5.73 [2.13–15.41]), multiparity (2.90 [1.26–6.69]), polycystic ovarian syndrome (2.62 [1.02–6.71]), and subchorionic hematoma (2.49 [1.03–6.04]).

Conclusions: A history of artificial abortion, endometrial thickness <8.0 mm, and resolved low-lying placenta may help in antenatal detection of a high-risk population of non-previa PAS in HRC-FET pregnancies.

KEYWORDS
assisted reproductive technology, frozen embryo transfer, hormone replacement cycle, non-previa placenta accreta spectrum, placenta accreta spectrum

1 | INTRODUCTION

Placenta accreta spectrum (PAS) causes potentially life-threatening bleeding and requires blood transfusion without spontaneous detachment of the placenta.[1] PAS is histopathologically diagnosed using hysterectomy specimens in cases of placenta accreta, increta, and percreta. The main risk factors for PAS are placenta previa and prior cesarean section, and screening for PAS is focused on women with placenta previa and ≥1 cesarean delivery.[2] A systematic review has reported that ultrasonography has high accuracy in detecting previa PAS antenatally with a sensitivity of 90.72% and specificity of 96.94%.[3] Magnetic resonance imaging (MRI) is another supplementary diagnostic tool, although its cost-effectiveness remains unknown.[4] Recent advances in antenatal diagnostics have enabled multidisciplinary management and improved the prognosis of previa PAS.[1] Therefore, a treatment strategy for previa PAS is now being formulated. Meanwhile, the incidence of non-previa PAS has been reported to be on the rise,[5,6] and its significance in clinical settings has been increasingly recognized.[7,8] However, non-previa PAS is challenging to detect antenatally,[7,8] which hinders its optimal management by comprehensive multidisciplinary care teams. The maternal morbidity of non-previa PAS has been reported to be similar to that of previa PAS.[7] Therefore, the identification of women at high risk for non-previa PAS is urgently needed to improve their prognosis.[7]

Assisted reproductive technology (ART) is one of the highest risk factors for non-previa PAS.[6–8] Japan is one of the world's leading countries in ART with 69 797 (8.6%) ART-assisted births in 2021.[9] Further increase is anticipated as a result of the expansion of the health insurance coverage for ART in Japan since April 2022. Frozen embryo transfer (FET) constitutes approximately 74%–89% of the ART treatment cycle as an alternative to the conventional fresh embryo transfer (Fresh-ET) since the freeze-all strategy was proposed to reduce the risk of ovarian hyperstimulation syndrome.[9,10] Emerging evidence suggests that ART, especially FET, is associated with critical pregnancy complications, including hypertensive disorders of pregnancy (HDP), postpartum hemorrhage (PPH), and PAS.[11–15] In FET, a hormone replacement cycle (HRC) elevates the PAS risk (adjusted odds ratio: 5.76–6.91) compared with a natural ovulatory cycle (NC).[16–18] The reported frequencies differ greatly due to varying definitions; Sakai et al. reported a risk of 31.7%, and Saito et al. reported a risk of 0.9% with respect to the increased risk of PAS in HRC.[16,17] However, those studies included both previa and non-previa PAS, and the risk factors for non-previa PAS need to be identified. It is also not practical to screen all pregnant women who have conceived with HRC-FET for non-previa PAS.

Therefore, in this study, we aimed to examine the frequency and identify the factors associated with non-previa PAS in HRC-FET pregnancies. We have collected and analyzed the clinical findings, including those during ART and early gestation.

2 | MATERIALS AND METHODS

2.1 | Study population

In this retrospective study, 279 women who conceived through ART at Nagoya University Hospital ($n=40$), Asada Ladies Clinic ($n=90$), or Narita Clinic ($n=149$) and had live births after 22 weeks of gestation at Nagoya University Hospital between January 2010 and December 2020 were included. ART was defined as infertility treatment that included in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). The participants were divided into three groups based on the method—HRC-FET, NC-FET, and Fresh-ET ($n=204$, $n=33$, and $n=42$, respectively) (Figure 1). None of the women underwent preimplantation genetic testing or egg donation.

2.2 | Data collection

The following data were both manually and digitally collected from the clinical records:

A. ART: indications for ART, ART protocol, endometrial thickness, number of previous transfer cycles, IVF or ICSI, cleavage stage or blastocyst transfer, embryo grade, assisted hatching, and number of transferred embryos. While serum estradiol and progesterone

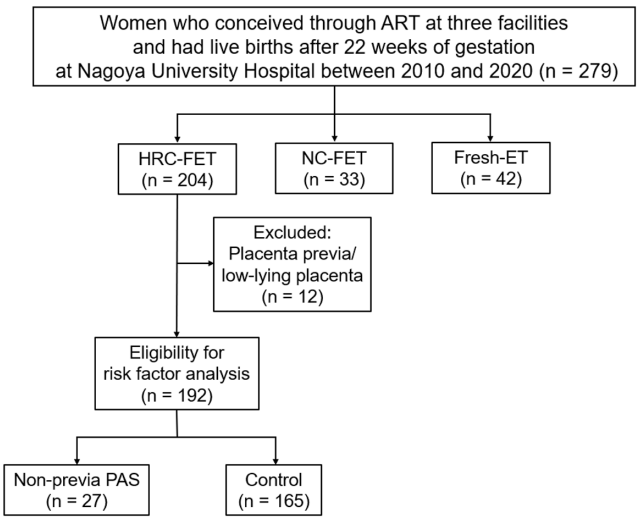

FIGURE 1 Flow diagram depicting the study design. We first analyzed 279 women who conceived via ART at three facilities and delivered after 22 weeks of gestation at Nagoya University Hospital between 2010 and 2020. Risk factor analysis was performed in HRC-FET pregnancies after exclusion of 12 women with placenta previa/low-lying placenta. ART, assisted reproductive technology; HRC-FET, frozen embryo transfer during a hormone replacement cycle; NC-FET, frozen embryo transfer during a natural ovulatory cycle; Fresh-ET, fresh embryo transfer; PAS, placenta accreta spectrum.

levels were collected, they could not be analyzed because of the paucity of data and the disparate timing of blood sampling.

B. Maternal baseline characteristics: age, parity, previous uterine surgeries, history of artificial or spontaneous abortion, previous cesarean sections, history of PAS, and weight and height before pregnancy.
C. Characteristics of the current pregnancy: PAS, placental position at delivery, subchorionic hematoma (SCH), vanishing twin, resolved low-lying placenta, HDP, preeclampsia, twin pregnancy, and gestational diabetes mellitus (GDM).
D. Characteristics of delivery: gestational age at delivery, type of labor onset and mode of delivery, methods of delivery, birth weight, sex of neonates, and placental weight.
E. Maternal outcomes: blood loss, blood transfusion, hysterectomy, and uterine artery embolization (UAE).

2.3 | Outcomes and definition

The primary outcome was the prevalence of non-previa PAS. All participants in this study were not antenatally diagnosed and were diagnosed at delivery. Non-previa PAS was diagnosed when at least one of the following criteria was met according to a previous study: (i) histopathological examination; (ii) ≥1 obstetrician required at the time of delivery for manual removal of the placenta or strong cord traction following the delivery of the fetus; or (iii) manual removal of a retained placenta with bleeding from the site of placental detachment and need for hemostatic maneuvers including uterine balloon tamponade.[16,19]

The secondary outcome was the prevalence of PPH. PPH was defined into two categories; PPH-1 was defined as bleeding >1500 mL within 2 h after delivery (both vaginal delivery and cesarean section),[20] and PPH-2 was defined as bleeding >800 mL in vaginal deliveries for singleton and 1500 mL for twins or intraoperative bleeding in cesarean section >1600 mL for singleton and 2300 mL for twins.[21]

2.4 | Definitions of others

The following definitions were used in this study: good quality embryo, defined as Veeck classification grade 1–3 for cleavage stage[22] or blastocysts with > stage 2, inner cell mass > grade C, and trophectoderm > grade C (≥3BB) according to the Gardner and Schoolcraft's system[23,24]; endometrial thickness, measured using transvaginal ultrasound on the day of transfer; HDP, hypertension (≥140/90 mmHg) during pregnancy according to the guideline[25]; preeclampsia, hypertension (≥140/90 mmHg) during pregnancy complicated with maternal organ damage or uteroplacental insufficiency according to the guidelines[25]; GDM, diagnosed using a 75-g oral glucose tolerance test[26]; small for gestational age (SGA), birth weight < 10th percentile for gestational age according to sex-specific Japanese neonatal anthropometric chart in 2000[27]; and low-lying placenta, internal os distance between the placental edge and the cervical os ≤20 mm.[28] Low-lying placentas were included in the previa group.[29]

Vanishing twins were diagnosed in cases where one of the twins was aborted before 14 weeks of gestation. Resolved low-lying placenta was defined as patients who were diagnosed with placenta previa or low-lying placenta during the screening of cervical length at 17–22 weeks,[30] which resolved by the third trimester.[15] Thin endometrium was defined into two categories: endometrial thickness < 7.0 mm according to a previous report,[31] and endometrial thickness < 8.0 mm, which was the 10th percentile of endometrial thickness in this study population.

2.5 | Statistical analysis

All statistical analyses were performed using SPSS v29 (IBM, Armonk, NY, USA). A chi-squared test was applied to compare categorical variables, and a Fisher's exact test was applied to analyze cases with small numbers, as appropriate. To compare continuous variables, an unpaired *t*-test or Mann–Whitney *U*-test was used to compare two groups. To compare between three groups, one-way analysis of variance or the Kruskal–Wallis test was utilized for normal and non-normal distribution, respectively. If a significant difference was detected in the three groups, Bonferroni correction was used to test whether there was a difference between all possible pairs. Statistical significance was defined as a p-value < 0.05.

Univariable logistic regression analyses were used to investigate the association of susceptible risk factors with the prevalence of non-previa PAS and PPH and to determine the odds ratios with 95% confidence intervals. Multivariable logistic regression analysis was performed by selecting three explanatory variables depending on the number of samples. Univariable and multivariable logistic regression analyses were only performed in the HRC-FET group, which excluded cases of placenta previa or low-lying placenta at delivery (n = 192, Figure 1).

3 | RESULTS

3.1 | Maternal and neonatal outcomes with each ET protocol

Detailed data regarding ART, maternal baseline characteristics, and maternal and neonatal outcomes were compared between the three groups of HRC-FET, NC-FET, and Fresh-ET. Detailed ART data and maternal baseline characteristics are presented in Table S1. The rate of ICSI was the highest in the HRC-FET group, while the rates of blastocyst transfer and assisted hatching were the lowest in the Fresh-ET group. Other factors, including the rate of the good quality embryo, indication for ART, and endometrial thickness, were similar between the three groups. Among the maternal baseline characteristics, maternal age, pre-pregnancy BMI, previous histories of uterine surgery,

artificial or spontaneous abortion, and PAS were not significantly different between the three groups. However, the prevalence of multiparity was the highest in the NC-FET group and a previous history of cesarean section was the least common in the Fresh-ET groups.

Maternal and neonatal outcomes between the three groups are shown in Table 1. There were no significant differences in most of the maternal and neonatal outcomes, including the frequencies of twin pregnancies, HDP, GDM, preterm delivery, placenta previa, induction of labor, cesarean section, gestational age at delivery, and birth weight. The prevalence of SCH was the highest in the HRC-FET group and was significantly higher in the HRC-FET group than in the Fresh-ET group ($p=0.03$). The incidences of both total and non-previa PAS were also the highest in the HRC-FET group (16.7% and 14.1%, respectively). In contrast, non-previa PAS was not detected in either the NC-FET or Fresh-ET groups. HRC-FET group also had the highest volume of blood loss at delivery and the highest frequencies of both PPH-1 (26.0%) and PPH-2 (32.8%). Meanwhile, SGA was the lowest in the HRC-FET group.

TABLE 1 Maternal and neonatal outcomes of the HRC-FET, NC-FET, and Fresh-ET groups.

	HRC-FET	NC-FET	Fresh-ET	
	n = 204	*n* = 33	*n* = 42	*p*-Value
Maternal outcomes				
Twin pregnancy	15/204 (7.4%)	4/33 (12.1%)	1/42 (2.4%)	0.26
Vanishing twin	8/204 (3.9%)	0/33 (0%)	1/42 (2.4%)	0.85
Subchorionic hematoma*	**50/197 (25.4%)**	4/33 (12.1%)	3/41 (7.3%)	**0.02**
HDP	34/204 (16.7%)	4/33 (12.1%)	3/42 (7.1%)	0.26
Preeclampsia	19/204 (9.3%)	2/33 (6.1%)	2/42 (4.8%)	0.72
GDM	18/204 (8.8%)	1/33 (3.0%)	3/42 (7.1%)	0.67
Preterm delivery (<37 weeks)	34/204 (16.7%)	9/33 (27.3%)	9/42 (21.4%)	0.31
Resolved low-lying placenta	38/160 (23.8%)	3/27 (11.1%)	8/30 (26.7%)	0.30
Placenta previa	12/204 (5.9%)	1/33 (3.0%)	6/42 (14.3%)	0.15
Induction labor	63/204 (30.9%)	6/33 (18.2%)	10/42 (23.8%)	0.25
Cesarean section	140/204 (68.6%)	21/33 (63.6%)	24/42 (57.1%)	0.34
Gestational age at delivery (weeks)	38.1 ± 2.7	37.1 ± 3.8	38.0 ± 2.9	0.15
PAS*	**34/204 (16.7%)**	0/33 (0%)	3/42 (7.1%)	**0.01**
Non-previa PAS*	**27/192 (14.1%)**	0/32 (0%)	0/36 (0%)	**<0.01**
Blood loss (mL) *	**1125 ± 674**	761 ± 422	914 ± 631	**<0.01**
PPH-1*	**53/204 (26.0%)**	2/33 (6.1%)	4/42 (9.5%)	**<0.01**
PPH-2*	**67/204 (32.8%)**	4/33 (12.1%)	10/42 (23.8%)	**0.04**
Blood transfusion	13/204 (6.4%)	1/33 (3.0%)	0/42 (0%)	0.20
Hysterectomy	3/204 (1.5%)	0/33 (0%)	0/42 (0%)	1.00
UAE	2/204 (1.0%)	0/33 (0%)	0/42 (0%)	1.00
Neonatal outcomes				
Birth weight (g)	2858 ± 596	2649 ± 825	2680 ± 669	0.10
SGA*	**16/187 (8.6%)**	6/29 (20.7%)	9/39 (23.1%)	**0.01**
Male	89/188 (47.3%)	17/29 (58.6%)	18/40 (45.0%)	0.48
Placental weight (g)	529 ± 143	492 ± 137	518 ± 129	0.40

Note: Data are shown as mean ± standard deviation or *n* (%). Statistical analyses were performed using the chi-squared test or Fisher's exact test for categorical variables and one-way analysis of variance or Kruskal–Wallis test for continuous variables.

Abbreviations: Fresh-ET, fresh embryo transfer; GDM, gestational diabetes mellitus; HDP, hypertensive disorders of pregnancy; HRC-FET, frozen embryo transfer during a hormone replacement cycle; NC-FET, frozen embryo transfer during a natural ovulatory cycle; PAS, placenta accreta spectrum; PPH, postpartum hemorrhage; SGA, small for gestational age; UAE, uterine artery embolization.

*Bold values, Statistically significant. Bonferroni post hoc analysis was also performed when significant differences were noted on the chi-squared test or Fisher's exact test. The prevalence of SGA was lower in HRC-FET than Fresh-ET group ($p=0.04$), and there was no significant difference between NC-FET and HRC-FET groups. The prevalence of subchorionic hematoma was higher in HRC-FET than Fresh-ET group ($p=0.03$), and there was no significant difference between NC-FET and HRC-FET groups. The prevalence of PAS was higher in HRC-FET than NC-FET group ($p=0.01$), and there was no significant difference between Fresh-ET and HRC-FET groups. The prevalence of non-previa PAS was higher in HRC-FET than NC-FET ($p=0.04$) or Fresh-ET ($p=0.02$) groups. The prevalence of PPH-1 and PPH-2 was higher in HRC-FET than NC-FET ($p=0.04$), and there was no significant difference between Fresh-ET and HRC-FET groups.

3.2 | Associated factors of non-previa PAS in HRC-FET pregnancies

Further analyses were only conducted in HRC-FET pregnancies (Figure 1), and the characteristics of the non-previa PAS group were compared with those of the control group (Table 2). Among ART-related factors, compared with the controls, the prevalence of polycystic ovarian syndrome (PCOS) and endometrial thickness <7.0 mm were significantly higher in the non-previa PAS group ($p = 0.04$ and $p = 0.03$, respectively). Endometrial thickness <8.0 mm was commoner in the non-previa PAS group ($p = 0.06$), but the difference was not significant. However, the ART methods, treatment protocols, and the number of previous transfers were similar in both groups. Among the maternal baseline characteristics, the prevalence of multiparity ($p = 0.01$) and a history of artificial abortion ($p < 0.01$) were significantly higher in the non-previa PAS group than those in the control group. Additionally, a history of cesarean section was commoner in the non-previa PAS group ($p = 0.05$); however, no significant differences were noted in age, pre-pregnancy BMI, and histories of spontaneous abortion and PAS. Among pregnancy outcomes, the incidences of SCH ($p = 0.04$) and resolved low-lying placenta ($p < 0.01$) were significantly higher in the non-previa PAS group than in the control group. The blood loss and PPH in the HRC-FET pregnancy were also compared between the non-previa PAS and control groups (Table S2). The non-previa PAS group had a higher blood loss (1568 ± 784 vs. 994 ± 566 mL, respectively; $p < 0.01$) and significantly higher frequencies of PPH-1 and PPH-2 than the control group (51.9 vs. 18.2% and 59.3 vs. 25.5%, respectively; $p < 0.01$) with more than half of the women presenting with PPH. The incidence of hysterectomy was not significantly different between the two groups. Two women underwent hysterectomy for cervical cancer or amniotic fluid embolization in the control group. However, blood transfusion ($p = 0.07$) and UAE ($p = 0.02$) were commoner in the non-previa PAS group. The neonatal outcomes were similar between the groups, except for placental weight (Table S3).

Univariable logistic regression analysis revealed several factors (crude odds ratio [95% confidence interval]) that were significantly associated with non-previa PAS in HRC-FET pregnancies: SCH (2.49 [1.03–6.04]), PCOS (2.62 [1.02–6.71]), multiparity (2.90 [1.26–6.69]), resolved low-lying placenta (5.73 [2.13–15.41]), endometrial thickness <8.0 mm (6.11 [1.06–35.12]), and a history of artificial abortion (6.45 [1.98–21.02]) (Table 3). The crude odds ratio of endometrial thickness <7.0 mm could not be calculated using univariable logistic regression analysis because none of the patients in the control group had endometrial thickness <7.0 mm. The endometrial thickness was also compared between the two groups as a continuous value. In the non-previa PAS group, the endometrial thickness was thinner compared with that in the control group, but the difference was not significant (Figure 2). Furthermore, 25 (92.6%) women in the non-previa PAS group had one or more associated factors identified on univariable analysis, but two patients had no such associated factors (case no. 26 and 27) (Figure 3A). No relationship could be identified between the combination of PAS-associated risk factors and non-previa PAS or the amount of blood loss (Figure 3A). The number of associated factors did not appear to be associated with the volume of blood loss (Figure 3B). When stratified by the number of associated factors, the frequency of non-previa PAS was higher in women with a higher number of associated factors; the prevalence of non-previa PAS was 26.7%, 40.0%, and 100% in those with two, three, and four of the associated six factors, respectively (Figure 3C). The associated factors for PPH in the eligible population ($n = 192$) are listed in Table S4. Except for a history of artificial abortion and SCH, associated factors for PPH were different from those for non-previa PAS. Although a history of artificial abortion and resolved low-lying placenta were significantly associated with non-previa PAS, PCOS and SCH were not significantly associated with non-previa PAS in multivariable analysis (Table S5).

4 | DISCUSSION

The primary finding of this retrospective study was that the following six factors were found to be associated with non-previa PAS in HRC-FET pregnancies: history of artificial abortion, endometrial thickness <8.0 mm, resolved low-lying placenta, multiparity, PCOS, and SCH. Additionally, the frequency of non-previa PAS was higher in women with more risk factors. Moreover, non-previa PAS was significantly more common in HRC-FET than in NC-FET and Fresh-ET pregnancies. Non-previa PAS significantly increased the prevalence of PPH and UAE for PPH treatment. Blood transfusions were also commoner in non-previa PAS.

Previous histories of dilation and curettage and multiparity are known risk factors for both non-previa and previa PAS.[32,33] Dilation and curettage are often performed in artificial abortions in Japan.[34] The most important risk factors for previa PAS are previous cesarean sections and placenta previa.[33] Previous cesarean sections also tended to be associated with non-previa PAS, but the difference was not statistically significant ($p = 0.05$) in the present study. A previous study suggested that a previous cesarean section was the most important factor for previa PAS but not for non-previa PAS, which is consistent with our findings.[6] A recent meta-analysis on non-previa PAS also reported that previous uterine procedures (dilation and curettage, hysteroscopy, endometrial ablation, and manual removal of placenta) were significant associated factors along with ART.[8] Several threshold values of endometrial thickness have been reported to be risk factors for PAS in FET pregnancies, such as <6.0 mm[15] and <9.0 mm.[14] The present findings revealed that endometrial thickness <8.0 mm was associated with non-previa PAS. This inconsistency might be due to the differences in the ethnicity of the patient and the small study population. Furthermore, in FET, endometrial thickness <9.0 mm is related to placenta previa.[35] Additionally, endometrial thickness is associated with ART success; endometrium thickness ≤8.0 mm is related to lower clinical pregnancy rates and live birth rates in FET, and good live birth rates were associated with an endometrium thickness of 8.7–14.5 mm in HRC-FET.[36,37] These findings suggest

TABLE 2 ART-related factors, maternal baseline characteristics, and pregnancy outcomes in women with non-previa PAS compared with those in the control group.

	Non-previa PAS	Control	
	n = 27	*n* = 165	*p*-Value
ART-related factors			
ICSI	20/23 (87.0%)	135/158 (85.4%)	0.57
Blastocyst	14/26 (53.8%)	80/160 (50.0%)	0.72
Good quality embryo	24/25 (96.0%)	140/160 (87.5%)	0.19
Assisted hatching	19/27 (70.4%)	119/163 (73.0%)	0.78
Two embryos transferred	8/27 (29.6%)	48/165 (29.1%)	0.95
Transdermal estrogen administration	21/23 (91.3%)	111/137 (81.0%)	0.19
Oral progesterone administration	10/16 (62.5%)	83/105 (79.0%)	0.13
Number of the previous transfer cycle			0.62
0	4/25 (16.0%)	46/164 (28.0%)	
1–2	10/25 (40.0%)	52/164 (31.7%)	
3–5	8/25 (32.0%)	46/164 (28.0%)	
≥ 6	3/25 (12.0%)	20/164 (12.2%)	
Indication for ART			
Male factor	7/25 (28.0%)	59/164 (36.0%)	0.44
PCOS*	**8/25 (32.0%)**	**25/164 (15.2%)**	**0.04**
Tubal factor	8/25 (32.0%)	31/164 (18.9%)	0.13
Endometriosis	4/25 (16.0%)	26/164 (15.9%)	0.59
Unexplained infertility	4/25 (16.0%)	25/164 (15.2%)	0.56
Endometrial thickness (mm)	10.1 ± 3.1	10.9 ± 2.3	0.30
Endometrial thickness <7.0 mm*	**2/12 (16.7%)**	**0/58 (0.0%)**	**0.03**
Endometrial thickness <8.0 mm	3/12 (25.0%)	3/58 (5.2%)	0.06
Maternal baseline characteristics			
Maternal age (years)	38.2 ± 2.8	37.3 ± 3.9	0.25
Maternal age ≥ 35 (years)	24/27 (88.9%)	125/165 (75.8%)	0.13
Multiparity*	**13/27 (48.1%)**	**40/165 (24.2%)**	**0.01**
Height (cm)	157.7 ± 5.7	158.6 ± 5.6	0.43
Pre-pregnancy BMI (kg/m^2)	20.6 ± 2.2	21.3 ± 3.4	0.25
Previous history			
Cesarean section	8/27 (29.6%)	24/165 (14.5%)	0.05
Uterine surgery	4/27 (14.8%)	17/165 (10.8%)	0.34
Artificial abortion*	**6/27 (22.2%)**	**7/165 (4.2%)**	**<0.01**
Spontaneous abortion	9/27 (33.3%)	52/165 (31.5%)	0.85
PAS	1/27 (3.7%)	3/165 (1.8%)	0.46
Pregnancy outcomes			
Twin pregnancy	2/27 (7.4%)	13/165 (7.9%)	0.65
Vanishing twin	2/27 (7.4%)	6/165 (3.6%)	0.31
Subchorionic hematoma*	**10/25 (40.0%)**	**34/161 (21.1%)**	**0.04**
HDP	6/27 (22.2%)	27/165 (16.4%)	0.31
Preeclampsia	3/27 (11.1%)	16/165 (9.7%)	0.52
GDM	0/27 (0%)	17/165 (10.3%)	0.07
Preterm delivery (<37 weeks)	8/27 (29.6%)	26/165 (15.8%)	0.08
Resolved low-lying placenta*	**10/22 (45.5%)**	**16/126 (13.0%)**	**<0.01**
Induction labor	6/27 (22.2%)	56/165 (33.9%)	0.23
Cesarean section	17/27 (63.0%)	111/165 (67.3%)	0.66
Gestational age at delivery (weeks)	37.3 ± 3.6	38.3 ± 2.5	0.18

Note: Data are shown as mean ± standard deviation or *n* (%). Statistical analyses were performed using the chi-squared test or Fisher's exact test for categorical variables and the unpaired *t*-test or Mann–Whitney *U*-test for continuous variables according to normal or non-normal distributions. Indications for ART include duplicate responses.

Abbreviations: ART, assisted reproductive technology; BMI, body mass index; GDM, gestational diabetes mellitus; HDP, hypertensive disorders of pregnancy; ICSI, intracytoplasmic sperm injection; PAS, placenta accreta spectrum; PCOS, polycystic ovarian syndrome.

*Bold values, Statistically significant.

TABLE 3 Factors associated with non-previa PAS in HRC-FET pregnancies.

Associated factors	OR	95% CI
Subchorionic hematoma	2.49	1.03–6.04
PCOS	2.62	1.02–6.71
Multiparity	2.90	1.26–6.69
Resolved low-lying placenta	5.73	2.13–15.41
Endometrial thickness <8.0 mm	6.11	1.06–35.12
History of artificial abortion	6.45	1.98–21.02

Note: Univariable logistic regression analysis was performed.

Abbreviations: CI, confidence interval; HRC-FET, frozen embryo transfer during a hormone replacement cycle; OR, odds ratio; PAS, placenta accreta spectrum; PCOS, polycystic ovarian syndrome.

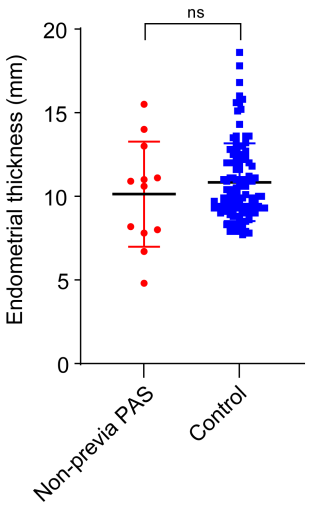

FIGURE 2 Comparison of endometrial thickness on the day of embryo transfer between the non-previa PAS ($n=12$) and control ($n=102$) groups. Only the thickness measured on the day of transfer was used. Student's *t*-test was performed. Error bars represent mean ± standard deviation. PAS, placenta accreta spectrum; ns, not significant.

that achieving adequate endometrial thickness at conception is important in improving live birth rates and reducing obstetric complications. Resolved low-lying placenta has been recently reported as a risk factor for PAS, which was consistent with our results.[15]

PCOS and SCH were identified for the first time as risk factors for non-previa PAS in the present study. Endometrial thickness is reported to be similar or thicker in PCOS than in the controls.[38,39] In our study, women with PCOS also tended to have a thicker endometrium than women without PCOS, but the difference was not significant (12.7 ± 3.1 vs. 10.7 ± 2.4 mm, $p=0.07$, data not shown). It is suggested that PCOS can have detrimental effects on endometrial function irrespective of endometrial thickness, thus potentially leading to PAS.[38,39] SCH has been reported to be common in ART, especially in FET.[40] In this study, we found it particularly prevalent in HRC-FET. SCH is thought to be a vascular disruption during the process of trophoblastic invasion into the endometrium, which subsequently leads to abnormal placental invasion and may be related to the pathological mechanism of PAS in HRC-FET.[40,41] In multivariable analyses, a history of artificial abortion and resolved low-lying placenta were significantly associated with non-previa PAS while PCOS and SCH were not the significant factors associated with non-previa PAS. Due to the small sample size, only three explanatory variables could be used in these analyses, and these results should be interpreted with caution. Although a larger study population is needed to adequately examine the association, a history of artificial abortion and resolved low-lying placenta would be independent risk factors for non-previa PAS in HRC-FET pregnancies. Of the six associated factors for non-previa PAS identified in this study, only the prevalence of SCH was high in HRC-FET. Therefore, we speculate that HRC-FET itself may be adding to the risk. Our results also suggested that endometrium-related factors are more relevant to the pathogenesis of non-previa PAS than embryo-related factors. Therefore, we intend to further investigate endometrial molecules associated with non-previa PAS.

Regarding the pathologies behind ART being a risk factor for PAS, some have suggested that infertility may be the primary cause; however, that speculation cannot explain the particularly high risk in HRC-FET. Placenta accreta is characterized by the absence of decidua and chorionic villi directly adjacent to the myometrial fibers. Although it remains unclear whether it is the cause or the result, it has been reported that placental histology revealed more defects in the decidua in HRC-FET, which is consistent with the results of this study.[42] The absence of corpus luteum in HRC may result in a lack of important substances produced by the corpus luteum other than estradiol and progesterone, which may trigger PAS.[43] Estradiol and progesterone have been reported to be associated with extravillous trophoblast invasion. Therefore, there is a possibility that abnormal invasion may occur as a result of exogenous administration of these hormones.[44,45]

Previous studies have reported that the prevalence of non-previa PAS was higher in HRC-FET compared to NC-FET and Fresh-ET.[16,17] There were no women with non-previa PAS in NC-FET and Fresh-ET groups in this study. In the HRC-FET group, the frequency of non-previa PAS was as high as 14.1%, which was lower than the rate of 31.7% previously reported.[16] In contrast, the non-previa PAS frequency in the general population is 0.4%, thus suggesting that non-previa PAS is markedly more common in HRC-FET pregnancies than in the general population.[6]

These findings suggest that the risk of non-previa PAS should be considered in obstetric management of HRC-FET pregnancies. However, not all HRC-FET pregnancies are at high risk for non-previa PAS. The factors identified in this study might be helpful in detecting high-risk populations in HRT-FET pregnancies. Especially, a history of artificial abortion, endometrial thickness <8.0 mm, and resolved low-lying placenta appear to be important factors since they were associated with >5-fold increased prevalence of non-previa PAS. We also found that having multiple factors was associated with a further increased risk of non-previa PAS. Therefore, it is recommended that women who conceive using HRC-FET with ≥2 associated factors should be

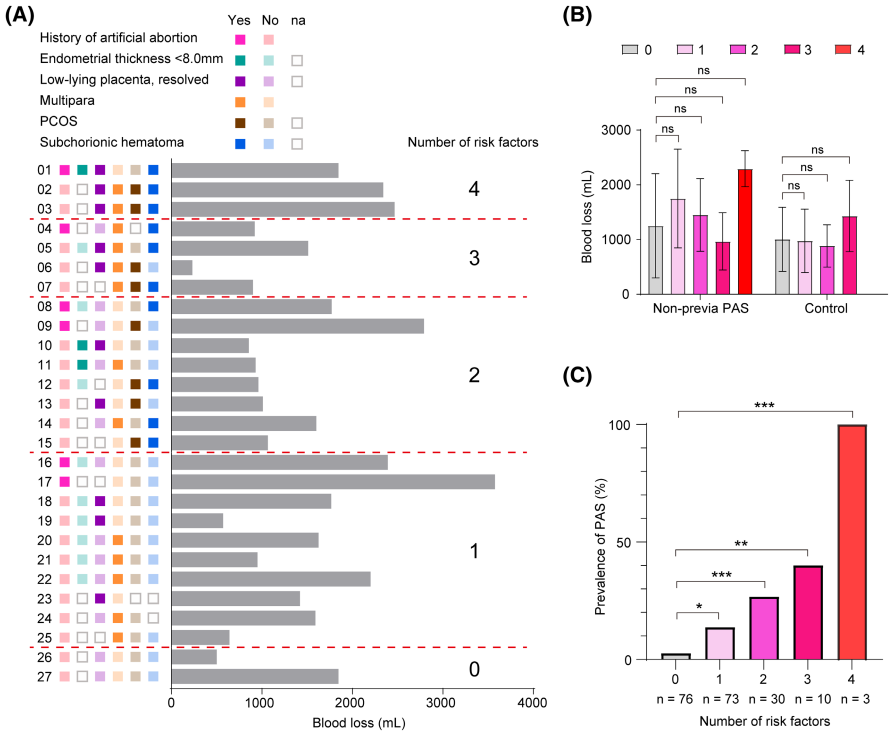

FIGURE 3 Analysis of the number of risk factors for non-previa PAS. (A) Risk characteristics and the amount of blood loss in women with non-previa PAS. (B) The amount of bleeding stratified by the number of risk factors. $^{*}p < 0.05$, Student's *t*-test. (C) Prevalence of PAS stratified by the number of risk factors. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, chi-squared test or Fisher's exact test. PAS, placenta accreta spectrum; PCOS, polycystic ovarian syndrome; na, not applicable.

managed at a tertiary center for delivery, although further studies are needed to validate these results in a large study population. Additionally, routine endometrial assessments and setting a target endometrial thickness in the HRC-FET protocol can be considered to reduce obstetric complications in the future. Routine ultrasound evaluation around the placenta may also be recommended to detect SCH and resolved low-lying placenta in pregnant women who conceived by HRC-FET.

The current study has several strengths. To the best of our knowledge, this is the first report to determine the factors associated with non-previa PAS in HRC-FET pregnancies. It was a single-center study with two ART institutions. Therefore, we could obtain detailed infertility treatment data and information before delivery, including SCH and placental position in the second trimester, which are not included in the perinatal registry database by the Japan Society of Obstetrics and Gynecology.

There are also some limitations to the current study. First, this study may lack generalizability due to the small sample size and inclusion of deliveries at a single tertiary facility. The women who referred to our hospital from other ART facilities are not limited to high-risk pregnancies but are mostly at the request of the patient's request. No significant difference was shown in the background between patients in Nagoya University Hospital and those referred from ART facilities (data not shown). However, the study population may not be representative of the general pregnancy population. Second, endometrial thickness data have several missing values and may be biased. Finally, since the sample size is too small for a multivariable analysis, future studies on a large scale are required to confirm the reproducibility of the present result and, in particular, to investigate the association of PCOS or SCH with non-previa PAS.

In conclusion, we identified six factors associated with non-previa PAS in HRC-FET pregnancies: history of artificial abortion, endometrial thickness <8.0 mm, resolved low-lying placenta, multiparity, PCOS, and SCH. The prevalence of non-previa PAS was 14.1% in our study. Although further large-scale studies are needed to corroborate our results, we believe that these findings will be helpful in creating prediction models for non-previa PAS and optimal transplantation protocols to reduce non-previa PAS in ART.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interests for this article.

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

The study was approved by the Institutional Ethics Board of Nagoya University (approval number: 2021-0037) and the requirement for informed consent was waived because of the retrospective nature of the study. The study was conducted in accordance with the principals of the Helsinki Declaration of 1964 and its later amendments.

ANIMAL STUDIES

This article does not contain any studies with human subjects performed by any of the authors.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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