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Uterine endometrium microbiota and pregnancy outcome in women with recurrent pregnancy loss

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Abstract

To evaluate whether uterine endometrium microbiota (UEM) is associated with pregnancy outcome in women with recurrent pregnancy loss (RPL). This prospective cohort study enrolled 67 women who had a history of two or more RPL. They underwent endometrial biopsy at midluteal phase for UEM analyses with 16S ribosomal RNA sequence. Four women with inappropriate specimens were excluded. Therefore, 63 women were followed up for >14 months; 44 became pregnant, while 19 did not. Thirty of the 44 pregnancies ended in live births, including 24 full-term and six preterm deliveries. Three pregnancies were ongoing, and the remaining 11 ended in miscarriages, including eight miscarriages with normal chromosome karyotype and three miscarriages with abnormal karyotype. Clinical characteristics and UEM associated with risks for non-pregnancy, miscarriage with normal karyotype, and preterm delivery in subsequent pregnancies were evaluated. Multivariable logistic regression analyses revealed that the number of previous miscarriages (odds ratio 42.2, 95% CI 1.19-1490, p=0.040) and relative dominance rate of *Ureaplasma* species (odds ratio 24.2, 95% CI 1.55-377, p=0.023) in UEM were independent risk factors for subsequent miscarriage with normal karyotype; and relative dominance rate of *Ureaplasma s*pecies in UEM was a risk factor for preterm delivery (odds ratio 109, 95% CI 1.07-1110, p=0.047). This study demonstrated for the first time that increases in *Ureaplasma s*pecies in UEM of women with RPL were risks of miscarriage with normal chromosome karyotype and preterm delivery in subsequent

pregnancies. UEM analysis for women with RPL before pregnancy may identify microbiota associated with adverse pregnancy outcomes.

Key words: microbiota, miscarriage, recurrent pregnancy loss, uterine endometrium

1. Introduction

Recurrent pregnancy loss (RPL) is defined as the loss of two or more pregnancies and affects 0.8%–1.4% of couples who attempt to have a baby(Sugiura-Ogasawara et al., 2013)(Bender Atik et al., 2018) (Sugiura-Ogasawara et al., 2013; Atik et al., 2018). A variety of factors are involved in the pathogenesis of RPL, such as abnormal uterine morphology, thyroid dysfunction, antiphospholipid syndrome, thrombophilic disorder, and chromosome abnormality. However, the etiology of >50% of RPL is unknown and is therefore designated as unexplained RPL (Practice Committee of the American Society for Reproductive Medicine, 2012; Morita et al., 2019)(Practice and Medicine, 2012). The mechanisms underlying the pathology of unexplained RPL remain poorly understood.

Recently, microbiome analysis has become possible with 16S ribosomal RNA (rRNA) analysis using a next-generation sequencer. A study assessed the uterine endometrium microbiota (UEM) in infertility women by 16S rRNA sequence method (Vitale et al., 2022), and found that women with *Lactobacillus*-dominant microbiota (>90%) yielded higher rates of implantation, pregnancy, and live birth in in-vitro fertilization and embryo transfer (IVF-ET) compared with those with non-*Lactobacillus*-dominant microbiota (Moreno et al., 2016).

However, whether UEM assessed by 16S rRNA sequence method is associated with subsequent pregnancy outcomes in women with RPL remains undetermined. This prospective

cohort study assessed UEM in women with RPL prior to pregnancy for the first time, and associations with subsequent pregnancy outcomes were evaluated.

2. Material and Method

2.1 Study participants

This prospective cohort study was approved by the institutional review board of Kobe University Hospital. All of the participants provided informed consent. This study consecutively enrolled 67 women who had a history of two or more RPL and underwent endometrial biopsy at the midluteal phase in the university hospital from January 2018 to December 2020.

All women with RPL underwent ultrasound and hysterosalpingography to detect uterine abnormalities. Serum testosterone, estradiol, prolactin (normal; 6.12–30.5 ng/ml), early follicular phase follicle-stimulating hormone, luteinizing hormone, and midluteal phase progesterone levels were measured. Blood analyses were performed for thyroid function (normal; TSH 0.61–4.23 mIU/l, free T4 0.90–1.70 ng/dl), antinuclear antibody, lupus anticoagulant (normal; phospholipid neutralization <1.16, diluted Russel's viper venom time <1.3), anticardiolipin antibody (aCL) IgG (normal; <10 U/ml), aCL IgM (normal; <8 U/ml), β2-glycoprotein I-dependent aCL (aCLβ2GPI) IgG (normal; <3.5 U/ml), antiphosphatidylethanolamine antibody (aPE) IgG (normal; kininogen (+) <0.30), and aPE IgM

(normal; kininogen (+) <0.45). Additionally, hemostatic molecular markers, including activated partial thromboplastin time (normal; 26–38 seconds), protein C activity (normal; 64–146%), protein S activity (normal; 63.5–149%), and coagulation factor XII (normal; 46–156%) were measured. All couples underwent chromosome karyotyping of the peripheral blood.

Pregnant women received standard therapies based on each risk factor/etiology of RPL. Women with thyroid dysfunction received thyroid hormone or antithyroid medications; women with antiphospholipid syndrome received low dose aspirin and unfractionated heparin; women with low aCL IgG/IgM levels received low dose aspirin and/or unfractionated heparin; women with positive tests for aPE IgG/IgM received low dose aspirin and/or unfractionated heparin; women with polycystic ovary received induction of ovulation and luteal support therapy; women with hyperprolactinemia received cabergoline or terguride; women with active autoimmune diseases received glucocorticoid; women with protein S/C deficiency and low levels of factor XII received low dose aspirin and/or unfractionated heparin; and women with antinuclear antibody or unknown etiology received tender loving care and/or low dose aspirin as a placebo. Couples with chromosome abnormalities underwent genetic counseling. Women with septate uterus received surgical treatment, if informed consent was obtained.

Pregnancy outcomes were followed up until February 2022. When an index pregnancy ended in miscarriage, chromosomal karyotyping of the villi was performed by G-banding with informed consent.

2.2 Study protocol and methods of endometrial tissue sampling

Women with RPL underwent endometrial biopsy at the midluteal phase which was confirmed by basal body temperature and ultrasound. Their vaginal wall and fornix were disinfected by Povidone iodine before inserting the sampling pipette (Pipet Curet^{TM}, CooperSurgical, Inc., Trumbull, USA) into the external os. The endometrial tissue was aspirated, and the specimen was immersed in OMNIGENE®-VAGINAL (DNA Genotek Inc., Ottawa, Canada), a container kit containing DNA/RNA stabilizer. The specimen of the midsection in an aspiration tube was used. The specimens were immediately transferred to Varinos Inc, Tokyo, Japan, where uterine endometrial microbiota was analyzed by 16S ribosomal RNA sequence method.

Simultaneously, vaginal secretion was assessed for Nugent score in our hospital laboratory. The Nugent score is one of the microscopic criteria for bacterial vaginosis (Nugent et al., 1991). The Nugent score (0 to 10) was assigned based on the quantitative presence of morphotypes of *Lactobacilli, Gardnerella* species and *Bacteroides*, curved Gram-variable rods. A score of seven or higher was indicative for bacterial vaginosis.

The participants were followed up for at least one year, and subsequent pregnancy outcomes were assessed. Pregnancy was defined as confirmation of the gestational sac in the uterus by ultrasound. Ectopic pregnancy or biochemical pregnancy loss was excluded. Whether

clinical characteristics and UEM were associated with risks of non-pregnancy, miscarriage with normal chromosome karyotype, and preterm delivery in subsequent pregnancies were evaluated. The relative dominance rates of *Lactobacillus, Gardnerella, Ureaplasma* species, biodiversity, Nugent score, and the number of bacterial species were assessed.

2.3 Analysis of uterine endometrial microbiota by 16S ribosomal RNA sequence

The variable region 4 (V4), hypervariable region of 16S ribosomal RNA gene, was amplified by polymerase chain reaction (PCR) using DNA extracted from tissue specimens (Moreno et al., 2016). Amplified PCR sample was identified according to the Illumina 16S Metagenomic Sequencing Library Preparation protocol, as described previously (Kyono et al., 2018).

2.4 Evaluation of biodiversity of bacterial community Shannon diversity index

The biodiversity of a bacterial community in a certain niche can be characterized and quantified by its richness and evenness (Kim et al., 2017). In this study, species richness refers to the number of bacterial species present, and evenness refers to relative abundance of living organisms. The relative abundance of bacterial species indicates their relative dominance rate. Furthermore, mathematical indicators, including both species richness and evenness, should be used for comparison. Because the microbiota samples were taken from only a single niche,

uterine endometrium, we applied Shannon diversity index to compare UEM diversity. The Shannon diversity index (SDI) is commonly and historically used estimator of species richness and evenness (Schloss and Handelsman, 2006). $\sum_{i=1}^{S} piLNpi$

The equation for SDI is expressed as $-$

2.5 Statistical analysis

All statistical analyses in this study were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were compared by Fisher's exact test, chi-square analysis and the Mann-Whitney U test. Univariate and multivariable logistic analyses were performed for risk assessment. All P-values were two-sided, and p-values of 0.05 or less were considered statistically significant (Kanda, 2013).

3. Results

3.1 Pregnancy outcome

Endometrial tissue specimens were inappropriate for UEM analyses in four of 67 women. Therefore, pregnancy outcomes in 63 women were followed up for at least 14 months after UEM analyses. Among the 63 women, seven (11.1%) had thyroid dysfunction, four (6.3%) had chromosome abnormality, four (6.3%) had septate uterus, nine (14.3%) had

antiphospholipid antibody positivity, three (4.8%) had antiphospholipid syndrome, 11 (17.5%) had protein S deficiency, two (3.2%) had protein C deficiency, one (1.6%) had factor XII deficiency, one (1.6%) had cervical incompetence, and the remaining 32 (50.8%) had unexplained etiology.

Forty-four (69.8%) of the 63 women became pregnant, while 19 did not. Thirty of the 44 pregnancies ended in live births including 24 full-term deliveries and six preterm deliveries. The reasons of the 6 preterm deliveries were as follows: hypertensive disorders of pregnancy at 35 weeks of gestation; exacerbation of antiphospholipid syndrome, hypertensive disorders of pregnancy and non-reassuring fetal status at 29 weeks; antiphospholipid syndrome and non-reassuring fetal status at 30 weeks; premature rupture of the membranes (PROM) at 28, 36, and 36 weeks. Three ongoing pregnancies progressed beyond 22 weeks of gestation (Figure 1).

The remaining 11 pregnancies ended in miscarriages at less than ten weeks of gestation, including eight miscarriages with normal chromosome karyotype and three miscarriages with abnormal chromosome karyotype. Three women whose pregnancies ended in miscarriages with abnormal chromosome karyotype had unexplained etiology, and one of the three received low dose aspirin and unfractionated heparin. Among eight women whose pregnancies ended in miscarriages with normal chromosome karyotype, one had septate uterus, two had thyroid dysfunction, one had chromosome abnormality of a couple, one had antiphospholipid antibody positivity, two had protein S deficiency, one had factor XII

deficiency, and three had unexplained etiology. They received standard therapies before and /or during pregnancy.

No woman received antibiotic treatment or probiotics before pregnancy or during the first trimester.

3.2 Clinical characteristics and uterine endometrium microbiota in women with RPL who became pregnant and those who did not become pregnant

Body mass index in women with RPL who became pregnant was higher than in women who did not become pregnant (p=0.0004). The number of bacterial species, Shannon diversity index, relative dominance rate of *Lactobacillus, Gardnerella, or Ureaplasma* species were not different between two groups. The relative dominance rates of *Lactobacillus* species were more than 90% in both groups (Table 1).

3.3 Clinical characteristics and uterine endometrium microbiota in women with RPL whose pregnancies ended in live birth and those whose pregnancies ended in miscarriage with normal chromosome karyotype

Gravidity (p=0.007) and the number of previous miscarriages (p=0.023) in women with RPL whose subsequent pregnancies ended in live birth were higher than in women whose pregnancies ended in miscarriage with normal chromosome karyotype. The relative dominance

rate of *Ureaplasma* species was higher in women with miscarriage with normal chromosome karyotype (p=0.004) (Table 2).

Univariate and multivariable logistic regression analyses revealed that the number of previous miscarriages (odds ratio 42.2, 95% CI 1.19-1490, p=0.040) and relative dominance rate of *Ureaplasma* species (odds 24.2, 95% CI 1.55-377, p=0.023) in UEM were independent risk factors for subsequent miscarriage with normal chromosome karyotype.

UEM in women whose pregnancies ended in miscarriage with normal chromosome karyotype is depicted in Figure 2.

3.4 Clinical characteristics and uterine endometrium microbiota in women with RPL whose pregnancies ended in full-term delivery and those whose pregnancies ended in preterm delivery

A history of preterm delivery in women whose subsequent pregnancies ended in preterm delivery was more frequent than in women whose subsequent pregnancies ended in full-term delivery (p=0.035). The relative dominance rate of *Lactobacillus* species (p=0.049) was lower, while relative dominance rates of *Gardnerella* (p=0.047) and *Ureaplasma* species (p=0.040) were higher in women whose subsequent pregnancies ended in preterm delivery compared with women whose subsequent pregnancies ended in full-term delivery (Table 3).

Univariate logistic regression analysis revealed that relative dominance rates of *Gardnerella* (odds ratio 1.05, 95% CI 1.00-1.10, p=0.032) and *Ureaplasma s*pecies (odds ratio 72.8, 95% CI 1.23-4310, p=0.039) in UEM were associated with preterm delivery. Multivariable logistic regression analysis revealed that the relative dominance rate of *Ureaplasma s*pecies in UEM was a risk factor for preterm delivery (odds 109, 95% CI 1.07-1110, p=0.047).

UEM in women whose pregnancies ended in preterm delivery is depicted in Figure 2. The relative dominance rate of *Lactobacillus* species was higher in three women with PROM than in the other three women without PROM (median 98.0%, range 66.4-98.0% vs. 13.6%, 4.7-51.2%, p=0.046). The relative dominance rate of *Gardnerella* species was lower in women with PROM than in those without PROM (median 0%, range 0-0.7% vs. 78.4%, 30.5-86.5%, p=0.046). The relative dominance rate of *Ureaplasma* species (median 0.5%, range 0-0.8% vs. 0%, 0-0.2%, p=0.25) or Shannon diversity index (median 0.120, range 0.475-1.17 vs. 0.694, 0.0847-1.17, p=0.7) was not different between women with and those without PROM.

3.5 Other bacteria species

Besides *Lactobacillus*, *Gardnerella*, and *Ureaplasma*, the following bacterial species were detected in UEM of the 63 women: *Prevotella* 31.7%, *Dialister* 30.2%, *Streptococcus* 28.6%, *Atopobium* 23.8%, *Bifidobcterium* 11.1%, *Aerococcus* 7.9%, *Mycoplasma*, 6.3%,

Clostridiles 3.2%, *Gemella* 3.2%, *Megasphaera* 3.2%, *Alloscardovia* 1.6%, *Cloacibacterium* 1.6%, *Lachno* 1.6%, *Parvimonas* 1.6%, and *Sneathia* species 1.6%. There were no statistical differences in relative dominance rates of these species between pregnancy and non-pregnancy, between live birth and miscarriage with normal chromosome karyotype, or between full term and preterm deliveries.

4. Discussion

The present cohort study examined the uterine endometrium in women with RPL prior to pregnancy by16S rRNA sequence method, and demonstrated that UEM was not associated with whether they subsequently became pregnant. However, decreases in *Lactobacillus* species and increases in *Gardnerella* species in UEM were associated with preterm delivery in subsequent pregnancy. Multivariable logistic analyses demonstrated that increases in *Ureaplasma* species in UEM were associated with miscarriage with normal chromosome karyotype as well as preterm delivery in subsequent pregnancy. For the first time, this study revealed that UEM in women with RPL prior to pregnancy was associated with risks of miscarriages with normal chromosome karyotype and preterm delivery in their subsequent pregnancy, but not with pregnancy rates. These findings suggest that new preconception care to decrease *Ureaplasma* and *Gardnerella* species and to increase *Lactobacillus* species in the uterine endometrium of women with RPL prior to pregnancy may be beneficial to subsequent

pregnancy outcomes. Randomized controlled trials to investigate whether intervention with antibiotic treatment or probiotics/prebiotics for those women prior to pregnancy is effective can be performed in the future.

It is known that resident bacteria in the lower genital tract can be the causative agent of intrauterine infection, and chorioamnionitis is responsible for 25%–40% of preterm delivery (Goldenberg et al., 2000). *Ureaplasma* species cause intrauterine infection, chorioamnionitis, and adverse pregnancy outcome (Sweeney et al., 2017). *Gardnerella* species, while less isolated in cases of chorioamnionitis, are relevant bacteria of bacterial vaginosis which is a crucial risk for premature labor. Therefore, the present study focused primarily on analyses of *Lactobacillus*, *Ureaplasma*, and *Gardnerella* species. Recently, our cohort study revealed that increases in *Ureaplasma* species and decreases in *Lactobacillus* species in vaginal microbiota analyzed by 16S rRNA sequence method were causally associated with preterm delivery in women with threatened premature labor (Shi et al., 2020).

In the present study, however, decreases in *Lactobacillus* species, increases in *Gardnerella* species in UEM were associated with preterm delivery due to hypertensive disorders of pregnancy, antiphospholipid syndrome and non-reassuring fetal status, but not preterm delivery due to PROM. Dysbiosis of UEM before pregnancy in women with RPL might be causally associated with abnormal placentation early in pregnancy and subsequent preterm delivery.

A study assessed the uterine endometrium in infertility women by 16S rRNA sequence method, and found that women with *Lactobacillus*-dominant microbiota (>90%) yielded higher rates of implantation, pregnancy, and live birth in IVF-ET than those with non-*Lactobacillus*-dominant microbiota (Moreno et al., 2016). However, other studies using 16S rRNA sequence method determined no associations between UEM and pregnancy outcome of IVF-ET in infertility women. Pregnancy rates in infertile women who underwent IVF-ET were not associated with UEM analyzed by 16S rRNA sequence (Franasiak et al., 2016). There were no differences in pregnancy or miscarriage rates in infertile women who underwent IVF-ET between women with *Lactobacillus*-dominant microbiota (>80%) and women with non-*Lactobacillus*-dominant microbiota (Hashimoto and Kyono, 2019). In addition, pregnancy rates in infertile women who underwent IVF-ET were not associated with UEM (Riganelli et al., 2020). Therefore, the significance or usefulness of UEM assessment in infertility women remained unproven. Similarly, the present cohort study revealed that UEM prior to pregnancy in women with RPL was not associated with whether they subsequently became pregnant. UEM in women with infertility or RPL may not be associated with implantation, regardless of spontaneous pregnancy or artificial ET.

The findings in this study will provide useful information for clinical practitioners to investigate etiologies and risk factors of RPL. However, this study has several limitations. The number of participants is not enough. The study was conducted in a single university hospital.

The UEM was analyzed at levels of the genus but not the species. The samples might be contaminated with the vaginal flola during endometrial aspiration. Povidone iodine has a wide range spectrum of disinfection effect, and reduces vaginal bacteria (Vorherr H et al, 1984; Duffy et al., 2020); however, its effect on UEM sampling remains unclear. Further studies are needed to confirm our results.

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Conflict of interest

None of the authors have any conflicts of interest to declare.

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Figure legends

Figure 1 A flowchart of this cohort study and pregnancy outcome

RPL, recurrent pregnancy loss

Figure 2 Uterine endometrial microbiota in miscarriage with normal chromosome karyotype and preterm delivery

Lane 1–8, miscarriage with normal chromosome karyotype;

Lane 9, preterm delivery at 29 weeks due to exacerbation of antiphospholipid syndrome, hypertensive disorders of pregnancy and non-reassuring fetal status;

Lane 10, preterm delivery at 35 weeks due to hypertensive disorders of pregnancy;

Lane 11, preterm delivery at 30 weeks due to antiphospholipid syndrome and non-reassuring fetal status;

Lane 12–14, preterm delivery at 36, 28 and 36 weeks due to premature rupture of the membranes

Table 1 Clinical characteristics and uterine endometrium microbiota in women with recurrent pregnancy loss who became pregnant and those who did not become pregnant

	Women who became pregnant n=44	Women who did not become pregnant n=19	p value*
Clinical characteristics			
Age, years	33 (25-42)	37 (26-45)	0.13
Body mass index, kg/m^2	20.8 (17.1-31.0)	19.5 (16.2-21.4)	0.0004
Gravity	3 (2-6)	3 (2-8)	0.066
Parity	0 (0-3)	0 (0-2)	0.013
Number of previous miscarriage	2 (0-6)	2 (2-7)	0.6
Number of previous stillbirth	0 (0-2)	0 (0-1)	0.59
Nugent score	0 (0-8)	0 (0-10)	0.64
Month until pregnancy	4 (1-24)	N.A.	
Assited reproductive technology	7 (16%)	N.A.	
Uterine endometrium microbiota			
Number of bacterial species	5 (1-16)	6 (1-16)	0.66
Shannon diversity index	0.19 (0-1.99)	0.23 (0-2.10)	0.79
Relative dominance rate of *Lactobacillus* species	96.4% (0-100%)	94.1% (0-100%)	0.73
Presence of *Lactobacillus species*	41 (93.2%)	18 (94.7%)	1
Relative dominance rate of *Gardnerella* species	0% (0-86.5%)	0% (0-63.8%)	0.31
Presence of *Gardnerella species*	14 (31.8%)	7 (36.8%)	0.65
Relative dominance rate of *Ureaplasma* species	0% (0-26.1%)	0% (0-1.9%)	0.61
Presence of *Ureplasma* species	14 (31.8%)	4 (21.1%)	0.57

Median (range)

*Mann-Whitney U test; chi-square test

N.A. not applicable

Table 2 Clinical characteristics and uterine endometrium microbiota in women with recurrent pregnancy loss whose pregnancies ended in live birth and those whose pregnancies ended in miscarriage with normal chromosome karyotype

	Women whose pregnancies ended in live birth	Women whose pregnancies ended in miscarriage with normal chromosome karyotype	p value*	Multivariable logistic regression analysis	
	n=30	n=8		Odds ratio (95% CI)	p value
Clinical characteristics					
Age, years	33 (25-40)	33.5 (28-37)	0.79		
Body mass index, kg/m^2	20.6 (17.1-31.0)	21.4 (19.5-29.6)	0.53		
Gravity	2 (2-5)	3.5 (2-6)	0.007		
Parity	0 (0-1)	0 (0-3)	0.37		
Number of previous miscarriage	2 (1-4)	3.5 (0-6)	0.023	42 .2 (1.19-1490)	0.04
Number of previous stillbirth	0 (0-2)	0 (0-2)	0.79		
Nugent score	0 (0-8)	0 (0-4)	0.78		
Month until pregnancy	4 (1-24)	2.5 (1-12)	0.61		
Assisted reproductive technology	5 (16.7%)	0 (0%)	0.56		
Uterine endometrium microbiota					
Number of bacterial species	4.5 (1-16)	6 (1-13)	0.48		
Shannon diversity index	0.18 (0-1.99)	0.49 (0.001-0.92)	0.41		
Relative dominance rate of *Lactobacillus* species	96.6% (0-100)	58.7% (0-99.9)	0.12		
Presence of *Lactobacillus* species	29 (96.7%)	6 (75%)	0.11		
Relative dominance rate of *Gardnerella* species	0% (0-86.5)	19.3% (0-55.6)	0.31		
Presence of *Gardnerella species*	13 (43.3%)	3 (37.5%)	1		
Relative dominance rate of *Ureaplasma* species	0% (0-0.8)	1.2% (0-26.1)	0.004	24.2 (1.55-377)	0.023
Presence of *Ureaplasma* species	8 (26.7%)	5 (62.5%)	0.09		
Median (range)	*Mann-Whitney U test; Fisher's exact test; chi-square test				

Table 3 Clinical characteristics and uterine endometrium microbiota in women with recurrent pregnancy loss whose pregnancies ended in full-term delivery and those whose pregnancies ended in preterm delivery

	Women whose pregnancies ended in full-term delivery	Women whose pregnancies ended in preterm delivery	p value	Multivariable logistic regression analysis	
	n=24	n=6		Odds ratio (95% CI)	p value
Clinical characteristics					
Age, years	32.0 (25-40)	34.5 (33-39)	0.053		
Body mass index, kg/m^2	20.5 (17.1-31.0)	21.6 (20.4-24.4)	0.11		
Gravity	2 (2-5)	3 (2-4)	0.06		
Parity	0 (0-1)	0 (0-1)	0.11		
Number of previous miscarriage	2 (1-3)	2 (1-4)	0.91		
Number of previous stillbirth	0 (0-1)	0 (0-2)	0.49		
History of preterm delivery	0 (0%)	2 (33.3%)	0.035		
Nugent score	0 (0-8)	1 (0-7)	0.14		
Weeks of gestation at delivery	39.3 (37.0-41.1)	32.9 (28.9-36.6)	0.0002		
Uterine endometrium microbiota					
Number of bacterial species	3.5 (1-13)	6 (4-16)	0.08		
Shannon diversity index	0.12 (0-1.99)	0.584(0.085-1.17)	0.11		
Relative dominance rate of *Lactobacillus* species	97.7% (0-100)	58.8% (4.7-98.0)	0.049	1.02 (0.94-1.10)	0.66
Presence of *Lactobacillus species*	23 (95.8%)	6 (100%)	1		
Relative dominance rate of *Gardnerella* species	0% (0-41.6)	30.5% (0-86.5)	0.047	1.07 (0.97-1.18)	0.16
Presence of *Gardnerella species*	9 (37.5%)	4 (66.7%)	0.36		
Relative dominance rate of *Ureaplasma* species	0% (0-0.7)	0.2% (0-0.8)	0.04	109 (1.07-1110)	0.047
Presence of *Ureaplasma* species	5 (20.8%)	3 (50.0%)	0.3		
Median (range)		Mann-Whitney U test; Fisher's exact test			

Figure 1 A flowchart of this cohort study and pregnancy outcome

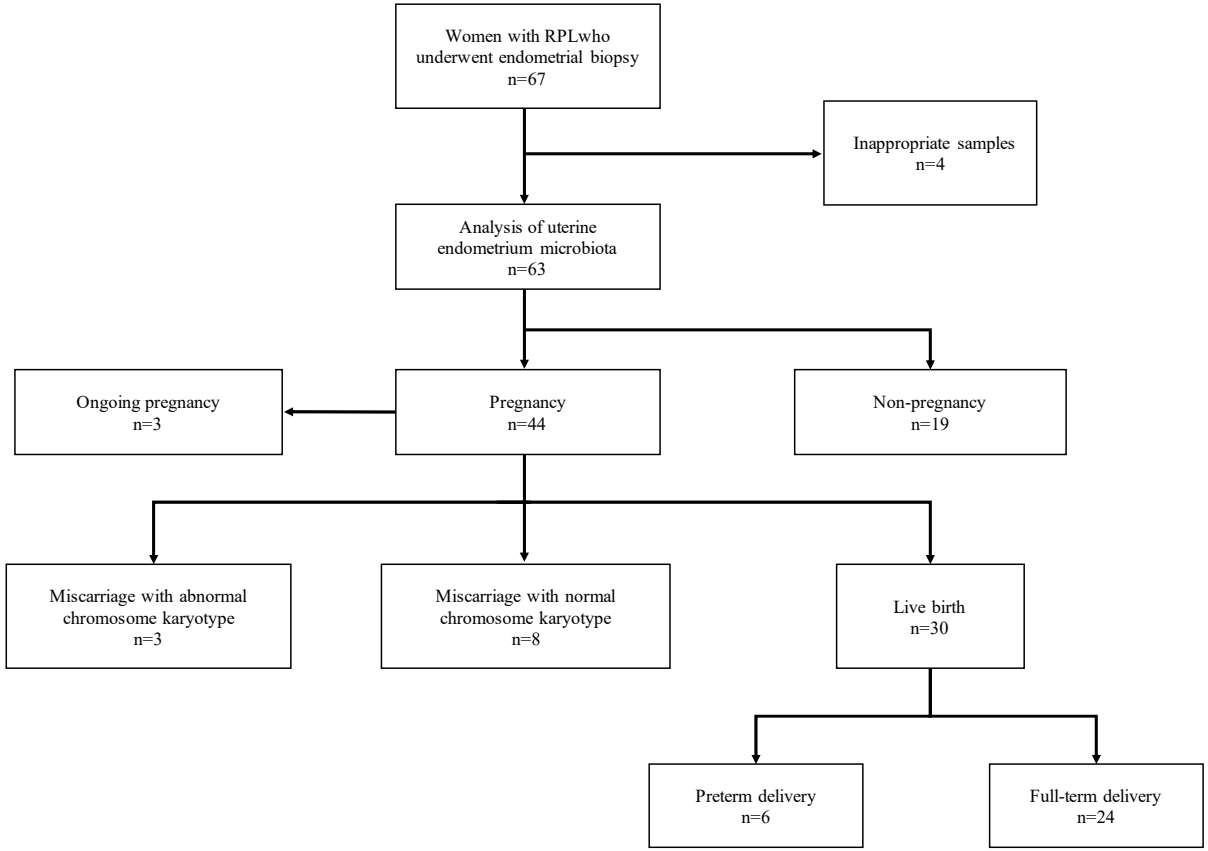

Figure 2　Uterine endometrial microbiota in miscarriage with normal chromosome karyotype and preterm delivery