RESEARCH ARTICLE

COMPARISON OF MICROBIOTA IN THREATENED PRETERM LABOR AND NORMAL PREGNANCY AT RSUP DR.M. DJAMIL PADANG

Muhammad Ryfki SA[1], Roza Sri Yanti[2], Bobby Indra Utama[3]

1. Department of Obstetrics and Gynecology, Faculty of Medicine, Andalas University/ Dr. M. Djamil General Hospital, Padang
2. Subdivision of Fetomaternal Medicine, Department of Obstetrics and Gynecology, Faculty of Medicine, Andalas University/ Dr. M. Djamil Hospital Padang
3. Subdivision of Urogynecology and Reconstructive Surgery, Department of Obstetrics and Gynecology, Faculty of Medicine, Andalas University/ Dr. M. Djamil Hospital Padang

*Korespondensi:*ryfkiadnani92@gmail.com

Abstract

One of the threats in pregnancy is threatened preterm labor. Several studies show that the composition of the microbiota in the vagina of premature births and normal pregnancies is different. This study aims to determine the comparison of microbiota in pregnancies with premature parturition and normal pregnancies at Dr M Djamil General Hospital, Padang. This research is an analytical observational study with a case–control study design conducted at the Department/SMF of Obstetrics and Gynecology, RSUP Dr. M. Djamil Padang from July – September 2023. The study population in this research were all pregnant women with a gestational age of 20-37 weeks who were diagnosed with imminens preterm labor and normal pregnant women with a gestational age of 20-37 weeks who received antenatal care at RSUP Dr. M. Djamil Padang. Respondents had vaginal swabs taken for microbiological culture at the Biomedical Laboratory, Andalas University, Padang. There were 33 respondents each in the imminence premature birth and normal pregnancy groups. Vaginal swab results showed that the most common microorganism found in normal pregnancy was Lactobacilus sp. (73%) while in threatened preterm labor *Staphylococcus aureus* (37%), *Staphylococcus epidermidis* (27%) and *E.coli* (15%) were more commonly found with only a small proportion of *Lactobacilus sp* (12%). Chi square analysis showed a significant difference between the vaginal microbiota of normal pregnant women and pregnant women with PPI. The conclusion of this study is that there is an increase in *Staphylococcus aureus, Staphylococcus epidermidis* and *E.coli* in pregnant women on PPI with a decrease in the number of normal flora, namely *Lactobacilus spp*.

Keywords: Partus Prematurus Iminens; Vaginal microbiota

INTRODUCTION

One of the threats in pregnancy is Partus Prematurus Iminens (PPI). PPI is the occurrence of signs of labor at preterm gestational age (20 weeks-37 weeks), and the baby's birth weight is less than 2500 grams. Premature birth is a problem with a high prevalence in the world and a challenge for doctors, especially obstetricians, to find out the causes and prevention of premature birth. Preventing complications of premature birth requires appropriate treatment. One of them is by handling the threat of premature birth or partus prematurus imminens (PPI) appropriately.[1] Premature births are estimated at 12.9 million or 9.6% of all births worldwide. Data in Indonesia shows that the number of premature births amounting to 10% in 2005. According to WHO, every year, an estimated 15 million babies are born prematurely, and approximately 1 in 10 babies experience premature birth. Premature babies, especially babies with low birth weight, have a high risk of death.[1,2]

Prematurity is the main cause of death in children under 5 years of age. The rate of preterm birth ranges between 5-18%. This rate is increasing worldwide.[3] Preterm birth is a syndrome with various underlying pathological mechanisms. Identification of risk factors and early detection of preterm labor is still being researched and developed.[4] Premature birth occurs for various reasons, ranging from chronic diseases in the mother, pregnancies with indexation, inflammation and rupture of membranes due to bacterial invasion and contamination by microbiota colonization of the vagina.[5]

The relatively acidic vaginal environment is a physiological or normal environment that must be maintained in balance. The hormone estrogen plays an important role in maintaining the acidity level of the vaginal environment. Changes in the pH of the vaginal environment can be caused by various things, such as immunosuppression, poor hygiene, dietary imbalances, sexual partner factors, contraceptive use, and pregnancy. These changes can cause various problems, one of which is infection by pathogenic microorganisms in the vaginal environment.[6,7]

Vaginal microbiota in women of childbearing age is classified into five types of levels or called Community state types (CST). CST I, II, III, and V are dominated by *Lactobacillus Crispatus, L. Gasseri, L. iners*. CST IV has no dominant species and is characterized by low levels of lactobacillus. CST IV is often associated with bacterial vaginosis and inflammation.[4] During pregnancy, a healthy woman's vaginal microbiota will change. Lactobacillus levels will increase as pregnancy progresses. Pregnant women have higher levels of vaginal microbiota. Research conducted by Tamarelle et al. (2020) shows that there are significant differences in the microbiota between pregnant and non-pregnant women, which will cause preterm labor. Lactobacillus plays a role in the homeostasis of the vaginal microbiota during pregnancy.[8] Systemic infections and colonization of the lower reproductive tract in the mother are associated with an increased risk of preterm birth.[9]

Maternal vaginal microbiota plays a role in fetal immune maturation where women with vaginal dysbiosis show increased concentrations of interleukin 12 (IL-12) and decreased concentrations of CD45RO CD4+ T cells in umbilical cord blood.[18] The vaginal microbiota plays a role in the female genital tract for health and disease.[14] Vaginal microbiota in pregnancy is related to the incidence of premature rupture of membranes before delivery due to uterine contractions before 37 weeks of gestation. Premature rupture of membranes before giving birth is the cause of 30% of premature births.[10] Research conducted by Kamgobe et al. in 2015-2016 found that women who experienced premature birth due to premature rupture of membranes had facultative pathogenic bacteria (33.7%). The pattern of bacterial resistance to antibiotics in this study was reported to ampicillin (100%), trimethoprim/sulfamethoxazole (66.7%), and cefotaxime (40%).[11] Research

conducted by Masteryanto et al. in 2015 found that the risk of pregnancy and childbirth premature 80%, growth of *Staphylococcus epidermidis* (30%) and *Escherichia coli* (15%). The number of urinary aerobic bacterial colonies in pregnancies with a risk of preterm labor was higher than in pregnancies without the threat of preterm labor, but was not statistically different. The risk of PPI in pregnant women with a urine bacterial count >105 cfu/mL is 3 times higher than in pregnant women with a colony count <105 cfu/mL.[12]

Research by Giannella et al. (2023) explains that the microbiota of the lower genital tract in women will experience changes during pregnancy. The composition of the microbiota in the vagina of preterm births and normal pregnancies is different.[13] Research by Giannella et al. (2023) shows that around 25% of preterm births are associated with microbial invasion, and the most common source of microbes is the host's microbiota.[13] Other research explains that an increase in vaginal microbiota will increase the risk of premature birth by around two times.[14-16] Based on the explanation above, researchers are interested in seeing the comparison of microbiota in pregnancies with PPI and normal pregnancies in patients at RSUP Dr. M. Djamil Padang.

METHODS

This research is descriptive analysis research using quantitative research methods with a cross sectional research design. This research was conducted at the Department/SMF of Obstetrics and Gynecology, RSUP Dr. M. Djamil Padang. Maternal microbiota examination was carried out at the Biomedical Laboratory, Andalas University, Padang. The research was conducted from March to August 2023. The sample in this study was a collection of pregnant women with a gestational age of 20-37 weeks who were diagnosed with Partus Prematurus Iminens in the Obstetrics and Gynecology Department/SMF at Dr. Central General Hospital. M. Djamil Padang according to the inclusion and exclusion criteria. Sampling was carried out by consecutive sampling.

1. The inclusion criteria for the subjects of this research are as follows:
    *   Pregnant women diagnosed with imminent premature labor with a gestational age at the time of examination between 20 weeks and 37 weeks of gestation (known for certain from a reliable HPHT and by obstetric examination (USG) carried out in the treatment room. In the comparison group are normal pregnant women who perform antenatal care at 20 weeks and 37 weeks of gestation (know for sure from HPHT or from reliable medical records).
    *   Single intrauterine pregnancy.
    *   Willing to become a research subject by signing an agreement after being given an explanation (informed consent).
2. The exclusion criteria for the subjects of this research are as follows:
    *   Pregnant women who experience antepartum bleeding.
    *   Pregnant women with cervical incompetence/uterine deformities.
    *   Pregnant women with multiple fetuses.
    *   Pregnant women with IUFD.

- Pregnant women with polyhydramnios.
- Pregnant women with KPD.
- History of infection of internal and external genital organs before pregnancy.
- Patients with signs of inpartum.

This study examined the comparison of microbiota in pregnancy (independent variable) with PPI (dependent variable) with normal pregnancy. Therefore, the sample formula for the different proportion test from SK. Lwanga and S. Lemeshow was used. The sample size was found to be 33 x 2 groups = 66 patients. To anticipate drop out, the sample size was increased by 10%, namely 6 people. So, the minimum sample size for this study was 72 people (36 pregnant women with premature labor and 36 pregnant women with normal preterm pregnancy).

RESULTS

Characteristics of Premature Imminent Parturition (PPI) patients
Table 1. Research characteristics

No	Characteristic	Group			
		Normal pregnancy		Partus premature Imminens (PPI)	
		f	%	f	%
1	**Age (year**				
	< 20	0	0	0	0
	20 – 35	28	85	29	87,8
	> 35	5	15	4	12,2
2	**Education**				
	Elementary School	0	0	0	0
	Junior High School	2	6	2	6
	Senior High School	26	79	25	75,8
	College	5	15	6	18,2
3	**BMI**				
	Underweight (<18,5)	0	0	0	0
	Normal (18,5-24,9)	22	66,7	23	69,8
	Overweight (25-29,9)	8	24,3	7	21,2
	Obesitas (≥30)	3	9	3	9

Table 1 shows the characteristics of age, education and BMI in normal pregnancy and PPI patients. From this table, in PPI pregnancies, most patients were aged 20-35 years (87.8%), and the remainder were aged >35 years (12.2%), most had a high school education (75.8%), with the remainder had a tertiary education (18.2%) and junior high school (6%). Most patients had a normoweight BMI (69.8%), 21.2% were overweight, and 9% were obese.

In the normal pregnancy group, most patients were also aged 20-35 years (85%), and the remainder were aged >35 years (15%), most had a high school education (79%), with the remainder had a tertiary education (15%) and junior high school (6%). Most patients had a normoweight BMI (66.7%), 24.3% were overweight, and 9% were obese.

Description Of The Microbiota In Imminens Preterm Pregnancies (PPI)

Table 2 shows that the findings in the PPI group where the most microorganisms were dominated by *Staphylococcus aureus* (37%), *Staphylococcus epidermidis* (27%) and *E.coli* (15%), with only a small portion being dominated by *Lactobacilus sp*. (12%). In the PPI group, *Prevotella spp*. was also found. (3%) and *Gardnella vaginalis* (6%).

Table 2. Microbiota in normal PPI

Microorganism	PPI	
	f	%
E. coli	5	15
Staphylococcus epidermidis	9	27
Staphylococcus aureus	12	37
Lactobacilus spp.	4	12
Prevotella spp.	1	3
Gardnella vaginalis	2	6
Total	33	100

Description Of The Microbiota In Normal Pregnancy

Table 3 shows that the most common microorganisms found in normal pregnancy are *Lactobacilus sp.* (73%), with a small percentage of patients found to be *Staphylococcus aureus* (12%), *E. coli (*9%) and *Staphylococcus epidermidis* (6%) microorganisms. There is no finding of *Prevotella spp.* and *Gardnella vaginalis* in normal pregnancy. PPI group also found *Prevotella spp.* (3%) and *Gardnella vaginalis* (6%).

Table 3. Microbiota in normal pregnancy

Microorganism	Normal pregnancy	
	f	%
E. coli	3	9
Staphylococcus epidermidis	2	6
Staphylococcus aureus	4	12
Lactobacilus spp.	24	73
Prevotella spp.	0	0
Gardnella vaginalis	0	0
Total	33	100

Comparison of microbiota and types of germs in pregnancies with imminent premature labor (PPI) and normal pregnancies

In Table 4, there are differences in the microbiota found in the normal pregnant and PPI groups. In normal pregnancy group, there is more *Lactobacilus sp*., whereas in PPI group,

Staphylococcus aureus is mostly found. Chi square analysis showed a significant difference between normal pregnancy microbiota and PPI with a p value of 0.000 (p<0.05).

Table 4. Comparison of microbiota in normal pregnancies and pregnancies with imminent preterm labor (PPI)

Microorganism	Group				*P value
	Normal		PPI		
	f	%	f	%	
E. coli	3	9	5	15	
Staphylococcus Epidermidis	2	6	9	27	
Staphylococcus aureus	4	12	12	37	0,000
Lactobacilus sp.	24	73	4	12	
Prevotella spp.	0	0	1	3	
Gardnella vaginalis	0	0	2	6	

DISCUSSION

Characteristics

Partus Premature Imminent is a threat to pregnancy where signs of labor appear at preterm gestational age (20 weeks-37 weeks).[17] In this study, 33 patients with normal pregnancies and 33 patients with PPI pregnancies were assessed. In both groups, there are similar characteristics where the average age ranges from 20-35 years, most have a high school education and have a normoweight BMI.

Widiana et al., Widandi et al., and Yeni et al. found the same results regarding the highest age in PPI patients, namely the age range of 20-35 years, with the normal pregnant group also found to be mostly 20-35 years old, because this age is the highest reproductive period, so there is no difference in terms of age between normal pregnancy and PPI.[18-20] However, research by Fuchs et al. shows that older maternal age (> 40 years) and older Youth (20-24 years) is associated with an increased incidence of premature birth. Older age is associated with degeneration processes in the uterus, while younger age is mainly associated with women of low socioeconomic status with a higher risk of medical complications of pregnancy.[21] Because the pregnancy rate at an advanced age is lower than the pregnancy rate at a productive age, the population Indirectly, there will also be fewer patients aged 20-35 years in the PPI pregnancy group.

Most of the normal pregnancy and PPI groups had a high school education. It is similar to research by Widandi et al., which found that 62% of PPI patients had a high school education. However, looking at the latest education in normal pregnancies, the same proportion was found between the education of normal pregnant women and PPI. Low education of pregnant women influences the thoughts and decisions that pregnant women will take, and they are also less aware of the importance of information about their pregnancy, so that pregnant women do not know how to care for their pregnancy. Meanwhile, pregnant women with higher education will understand better how to take steps to maintain their pregnancy.[19]

The proportion of normoweight patients was slightly higher in pregnancies with PPI. Previous research by Masteryanto et al. found that the average body mass index of the PPI group was 25.89 ± 5.39 kg/m2, and the control group was 26.78 ± 6.38 kg/m2 where in this study nutritional status did not have a significant influence on occurrence of preterm birth. However, other research suggests that there is an increased risk of premature birth in obese and underweight pregnant women, where obesity is associated with higher levels of inflammation and higher pregnancy complications, and underweight is associated with poor nutrition, which causes the uterus's ability to maintain pregnancy to decrease.[22,23]

Comparison of Microbiota in Pregnancies with Imminent Preterm Labor (PPI) and Normal Pregnancies

The results of this study showed a statistically significant difference between the microbiota found in the normal pregnant and PPI groups, where in normal pregnancies more *Lactobacillus spp.* was found. In PPI pregnancies, *Staphylococcus aureus* is mostly found with *Lactobacillus spp*. which is less (12%).

In this study, there were more *Lactobacillus spp*. in the normal pregnancy group. The reproductive tract microbiota plays an important role in the maintenance of normal pregnancy and influences reproductive outcomes. The composition of the vaginal microbiota in pregnancy shows a higher and stable abundance of *Lactobacillus spp.* throughout pregnancy. In both pregnant and non-pregnant women, the vaginal microbiota can fluctuate and move from one CST to another. At the species level, hierarchical clustering analysis characterized the vaginal microbiota into five community state types (CST), four of which are characterized by relatively high abundance of specific *Lactobacillus species*: CST I – *Lactobacillus crispatus* (*L. crispatus*), CST II – *Lactobacillus gasseri (L. gasseri)*, CST III – *Lactobacillus iners (L. iners)*, CST IV – 'high diversity', CST V – *Lactobacillus jensenii (L. jensenii)*. CST IV ('high diversity') group was characterized by low levels of *Lactobacillus spp.* and the abundance of anaerobic bacteria such as Atopbium, Prevotella, Sneathia, Gardnerella and Mobiliuncus. There are various factors, such as ethnicity, hygiene, hormonal fluctuations, and contraception, that influence the structure and composition of the vaginal microbiota. Stability *of Lactobacillus spp*. in pregnancy increases reproductive fitness and protects against infection. This stability may also be due to the high levels of estrogen in pregnancy because the postnatal condition is characterized by a drastic change to lower numbers of *Lactobacillus spp*.[24,25]

Gupta et al. also stated that many studies have revealed "Lactobacillus" to be the most dominant member of the vaginal flora in most healthy reproductive age groups and pregnant women. Gupta et al. also stated that the vaginal microbiome in pregnancy plays an important role in preterm and spontaneous labor because there is evidence that a microbiome rich in *Lactobacillus spp*. decreased drastically and became more diverse in the postnatal period indicating a decrease in *Lactobacillus spp*. associated with spontaneous labor.[26,27]

Different results were found in vaginal swab cultures of PPI patients, wherwere only most patients' other types of microbiotas were found, namely Staphylococcus aureus (37%), Staphylococcus epidermidis (27%) and E. coli (15%) with *Lactobacillus spp*. only present in 12% of patients. It has been stated that depletion of *Lactobacillus spp*. is associated with an increase in other bacterial infections that lead to pregnancy complications such as premature rupture of membranes, chorioamnionitis and premature labor. Di Giulio et al. found an association between spontaneous preterm birth and deficiency of *Lactobacillus spp*. vagina during pregnancy after weekly sampling and analysis of the cervicovaginal flora during pregnancy with the help of 16S rRNA gene sequencing.[28]

During pregnancy, the vaginal microbial community (VMC) interacts with its host to provide protection against potential pathogens to the developing fetus. The breakdown of free glycogen by α-amylase provides a rich source of energy that can be converted into lactic acid thereby lowering the overall pH of the environment. Lactic acid production is almost entirely produced by species from the genus Lactobacillus. By maintaining an acidic pH below 4.5, Lactobacillus spp. limiting colonization by other taxa including potential pathogens.[29] If there is a depletion of *Lactobacillus spp.* then the growth of potential pathogens will occur.

The mechanism by which staphylococci causes PPI is the possibility of infection of the amniotic fluid by staphylococci, which causes premature birth. Bacterial lysates and, especially, *S.aureus* and *S.agalacticae* clearly trigger most of the inflammatory, anti-inflammatory, TH1/TH2 and chemokine cytokines. 60 Increased inflammations during these infections will cause contractions in the uterus due to the production of cortisol, oxytocin, and various prostaglandins. This situation will stimulate cervical ripening, uterine contractions and rupture of the decidua and membranes.[29]

Research by Farooqi et al. in 2022 stated that 16/68 (23.5%) of term placenta samples were positive for *S. aureus*; on the other hand, 4/16 (25%) preterm placenta samples confirmed culture growth of *S. aureus* and it was concluded that there was a significant presence of *S. aureus* in the placenta of mothers with term and preterm labor which may be responsible for preterm birth.[23] Apart from that, Vanesha et al. also found *Escherichia coli, Enterobacter cloacae* and *Staphylococcus aureus* bacteria in premature rupture of membranes, which also contributed to premature birth in patients. *Escherichia coli* is a non-pathogenic gram-negative microorganism that is often found in the human intestine. Gram-negative bacteria are the organisms most found in the maternal rectovaginal area. *Escherichia coli* is associated with premature birth, especially delivery before 34 weeks of gestation. Urinary tract infections by *Escherichia coli* are also associated with premature birth.[30]

Vulvovaginitis in pregnancy often occurs as evidenced by research conducted by Mu'arofah et al which stated that *Staphylococcus aureus* bacteria were found in 35% of samples and *Staphylococcus epidermidis* bacteria in 65% of samples when examining the vaginal secretions of pregnant women in Kediri City. In patients who are found to have these bacteria, there are no significant symptoms, such as smelly vaginal discharge, so treatment is often not carried out, which can cause further complications, such as premature birth.[31]

In this study, 3% and 6% of *Prevotella spp.* and *Gardnella vaginalis* were found respectively in the vaginal swabs of PPI pregnant women, whereas in the group of healthy pregnant women these types of germs were not found. Bacterial vaginosis resulting from infection with *Gardnella vaginalis* and *Prevotella sp.* has been associated with increased vaginal concentrations of the cytokine interleukin (IL)-1β and the pro-inflammatory chemokine IL-8, both of which are also increased in the amniotic fluid and cervical fluid of women with microbial invasion of the amniotic cavity and preterm labor.[32,33] In this study only a small proportion of patients had BV findings. This may occur because BV is symptomatic, characterized by smelly vaginal discharge, making it easier for pregnant women to detect and treat more quickly.

Research Limitations

This study only compared the vaginal microbiota found in PPI pregnancies with normal pregnancies and did not assess other flora such as fungi, viruses, and parasites which in previous studies were also found to be risk factors for PPI occurrence.

CONCLUSION

1. In patients with normal pregnancies and PPI, similar characteristics were found, where most were aged 20-35 years, had a high school education and had a normoweight BMI.
2. The microbiota most commonly found in normal pregnancies is *Lactobacilus spp.*, while in pregnancies with imminens premature parturition, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *E. coli are* more commonly found.
3. There is a significant difference between the vaginal microbiota of normal pregnant women and pregnancies with PPI (p=0.000).

Suggestions

1. The results of this study can be used as a source of new scientific reference regarding the comparison of microbiota in pregnancies with imminent preterm labor (PPI) and normal pregnancies.
2. Future research is expected to assess other vaginal flora, such as fungi, viruses and parasites, which also contribute to the incidence of PPI.
3. Based on the results of this study, it is necessary to carry out further research on probiotic research in patients with PPI.

DAFTAR PUSTAKA

1. Griggs KM, Hrelic DA, Williams N, McEwen-Campbell M, Cypher R. Preterm labor and birth: a clinical review. *MCN Am J Matern Nurs*. 2020;45(6):328-337.

2. Safari S, Hamrah MP. Epidemiology and related risk factors of preterm labor as an obstetrics emergency. *Emergency*. 2017;5(1).

3. Seedat F, Stinton C, Patterson J, et al. Adverse events in women and children who have received intrapartum antibiotic prophylaxis treatment: a systematic review. *BMC Pregnancy Childbirth*. 2017;17(1):1-14.

4. Nunez N, Réot L, Menu E. Neonatal immune system ontogeny: the role of maternal microbiota and associated factors. How might the non-human primate model enlighten the path? *Vaccines*. 2021;9(6):584.

5. Brown RG, Marchesi JR, Lee YS, et al. Vaginal dysbiosis increases risk of preterm fetal membrane rupture, neonatal sepsis and is exacerbated by erythromycin. *BMC Med*. 2018;16(1):1-15.

6. Singh A, Kanti V, Dayal S, Shukla SK, Mishra N. Prevalence and risk factors of bacterial vaginosis among women of reproductive age attending rural tertiary care institute of Western Uttar Pradesh. *J Evol Med Dent Sci*. 2016;5(43):2695-2702.

7. Lamont RF, Sobel JD, Akins RA, et al. The vaginal microbiome: new information about genital tract flora using molecular based techniques. *BJOG An Int J Obstet Gynaecol*. 2011;118(5):533-549.

8. Tamarelle J, Ma B, Gajer P, et al. Nonoptimal vaginal microbiota after azithromycin treatment for Chlamydia trachomatis infection. *J Infect Dis*. 2020;221(4):627-635.

9. Bayar E, Bennett PR, Chan D, Sykes L, MacIntyre DA. The pregnancy microbiome and preterm birth. In: *Seminars in Immunopathology*. Vol 42. Springer; 2020:487-499.

10. Kacerovsky M, Vrbacky F, Kutova R, et al. Cervical microbiota in women with preterm prelabor rupture of membranes. *PLoS One*. 2015;10(5):e0126884.

11. Kamgobe E, Grote S, Mushi MF, et al. Multi-drug resistant facultative pathogenic bacteria colonizing the vagina of pregnant women with premature rupture of membrane, Tanzania. *East Africa Sci*. 2020;2(1):29-35.

12. Masteryanto HM, Hardianto G, Joewono HT, Koendhori EB. Infeksi Saluran Kemih Sebagai Faktor Risiko Terjadinya Ancaman Persalinan Preterm. *Maj Obstet dan Ginekol*. 2015;23(2):75-81.

13. Giannella L, Grelloni et al. J. Microbiome change on pregnancy disorders. MDPI. 2023.

14. Hillier SL, Nugent RP, Eschenbach DA, et al. Association between bacterial vaginosis and preterm delivery of a low-birth-weight infant. *N Engl J Med*. 1995;333(26):1737-1742.

15. Aagaard K, Riehle K, Ma J, et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PLoS One*. 2012;7(6):e36466.

16. Hyman RW, Fukushima M, Jiang H, et al. Diversity of the vaginal microbiome correlates with preterm birth. *Reprod Sci*. 2014;21(1):32-40.

17. Kirana P, Islamy N, Yonata A. G4P3A0 hamil 30 minggu belum inpartu dengan partus prematurus imminens dan ketuban pecah dini. Medula. 2020; 10(3): 456-60.

18. Widiana I, Putra I, Budiana I, Manuaba I. Karakteristik pasien *partus prematurus imminens* di RSUP Sanglah Denpasar periode 1 April 2016-30 September 2017. E-Jurnal Medika. 2019 ; 8(3).

19. Widandi MN, Herdiyantini M, Sudiarta KE. Karakteristik Partus Prematurus Imminens Di RSPAL Dr Ramelan Surabaya Periode Juni 2019 – Juni 2020. HTMJ, 2022; 19(2): 193-207.

20. Yeni CM, Rismawati, Hasanuddin. Ancaman Persalinan Preterm: Mana yang menjadi Persalinan Preterm?. 2020; 8(4): 216-21.

21. Fuchs F, Monet B, Ducruet T, Chaillet N, Audibert F. Effect of maternal age on the risk of preterm birth: A large cohort study. PLoS ONE. 2018; 13(1): e0191002.

22. Suman V, Luther EE. Preterm Labor. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK536939/

23. Dudenhausen JW, Kunze M, Wittwer-Backofen U, et al. The relationship between maternal age, body mass index, and the rate of preterm birth. *J Turk Ger Gynecol Assoc*. 2018;19(4):182-186. doi:10.4274/jtgga.2018.0057

24. Grewal K, MacIntyre DA, Bennett PR. The reproductive tract microbiota in pregnancy. *Biosci Rep*. 2021;41(9):BSR20203908. doi:10.1042/BSR20203908,

25. Li D, Chi X, Zhang L, Chen R, Cao J, Sun X, et al. Vaginal microbiome analysis of healthy women during different periods of gestation. *Biosci Rep*. 2020; 40 (7): BSR20201766. doi: https://doi.org/10.1042/BSR20201766

26. Gupta P, Singh MP and Goyal K. Diversity of Vaginal Microbiome in Pregnancy: Deciphering the Obscurity. Front. Public Health. 2020; 8:326. doi: 10.3389/fpubh.2020.00326

27. Nunn KL, Witkin SS, Schneider GM, et al. Changes in the Vaginal Microbiome during the Pregnancy to Postpartum Transition. *Reprod Sci*. 2021;28(7):1996-2005. doi:10.1007/s43032-020-00438-6

28. DiGiulio DB, Callahan BJ, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci*. 2015;112(35):11060-11065.

29. Mohammadi D, Naghshineh E, Sarsangi A, Zare Sakhvidi MJ. Environmental extreme temperature

and daily preterm birth in Sabzevar, Iran: a time-series analysis. *Environ Health Prev Med*. 2019;24(1):1-13.

30. Vanesha V, Wantania JJE, Lengkong RA. Pola Mikroorganisme Vagina pada Ketuban Pecah Dini. Indones J Obstet Gynecol. 2021;9(4): 198-203.

31. Mu'arofah B, Minawa DVP. Deteksi Bakteri Staphylococcus spp. pada Sekret Vagina Ibu Hamil di RSIA Citra Keluarga Kota Kediri. J. Sintesis. 2021; 2(1): 32-8

32. Yang S, Reid G, Challis JRG, et al. E ff ect of Oral Probiotic Lactobacillus rhamnosus GR-1. Published online 2020:1-17.

33. Kervinen K, Holster T, Saqib S, et al. Parity and gestational age are associated with vaginal microbiota composition in term and late term pregnancies. *EBioMedicine*. 2022;81.