Correlation Between B Group Streptococcus Infection in Vagina and Maternal Serum Procalcitonin Level with Preterm Delivery

Tjokorda Gde Agung Suwardewa, I Nyoman Hariyasa Sanjaya, Anom Suardika, I Gede Ngurah Harry Wijaya Surya, Kadek Fajar Marta, and I Gede Suputra Indrawan

ABSTRACT

Preterm delivery is one of the biggest contributors to neonatal death because the organ systems in premature babies are not yet fully developed. The cause of preterm labor is multifactorial, one of which is complex inflammation due to Group B Streptococcus (GBS) bacterial infection. Maternal serum procalcitonin is a prohormone involved in the inflammatory process, and specifically as a marker of bacterial infection. This study time to determine the relationship between vaginal GBS bacterial infection and high maternal serum procalcitonin levels in preterm labor. This study used a cross-sectional method. Samples were 31 pregnant women who came to the Obstetrics ER at Sanglah Hospital Denpasar with a diagnosis of preterm delivery that met the inclusion criteria. Vaginal mucus samples were examined at the Clinical Microbiology Laboratory of Sanglah Hospital and procalcitonin samples were examined at the Clinical Pathology Laboratory of Sanglah Hospital. Descriptive and analytical analysis was performed on the data obtained. There were 7 positive GBS results (22.5%) and 24 negative GBS results (77.5%). The results of high procalcitonin obtained 12 (38.7%) while the normal 19 (61.3%). In the Chi-Square test, the PR value was 3.4 (95% CI: 1.61-7.30) with p value = 0.004. Therefore, it can be concluded that there is a relationship between GBS infection and serum procalcitonin levels, where positive GBS infection is a risk factor that increases the occurrence of high serum procalcitonin levels in preterm labor.

Keywords: Group B Streptococcus, procalcitonin, preterm labor.

T. G. A. Suwardewa*
Department of Obstetrics and Gynecology, Medical Faculty of Udayana University/Sanglah Hospital, Indonesia.
(e-mail suwardewa.tjok@gmail.com)

I. N. Hariyasa Sanjaya
Department of Obstetrics and Gynecology, Medical Faculty of Udayana University/Sanglah Hospital, Indonesia.

A. Suardika
Department of Obstetrics and Gynecology, Medical Faculty of Udayana University/Sanglah Hospital, Indonesia.

I. G. N. H. Wijaya Surya
Department of Obstetrics and Gynecology, Medical Faculty of Udayana University/Sanglah Hospital, Indonesia.

K. Fajar Marta
Department of Obstetrics and Gynecology, Medical Faculty of Udayana University/Sanglah Hospital, Indonesia.

I. G. S. Indrawan
Department of Obstetrics and Gynecology, Medical Faculty of Udayana University/Sanglah Hospital, Indonesia.

*Corresponding Author

I. INTRODUCTION

Preterm delivery is one of the causes and contributors' most significant neonatal mortality. Organ systems in babies premature do not yet develop ideally. Increasing medical education develops to enhance the continuity of life in babies premature. Complications that occur consequence of preterm birth will cause happening sequelae. This thing could cause a drop in quality-of-life generation successor nation. Preterm birth is delivery that occurs before the gestational age of 37 full weeks (259 days). Neonates born before 34 complete weeks of gestation are called early preterm, while neonates born between 34 and 36 full weeks of pregnancy are called late preterm [1].

Preterm births accounted for 10.6% of live births globally in 2014, equivalent to 14.84 million live newborns. Nearly 85% of preterm births occur in the late preterm period, and more than 80% occur in Asia and Sub-Saharan Africa [2]. In Indonesia, the incidence of infants born preterm have a significant mortality and morbidity rate. In 2010, Indonesia ranked fifth in the world for the number of preterm births, with a preterm birth rate of 15 per 1000 live births [3], [4].

Preterm labor is multifactorial, including genetic, maternal, fetal, and environmental factors. Gestational age, low body mass index, and multiple pregnancies are maternal

factors associated with the incidence of preterm delivery. The most common factor other factors include antepartum bleeding, mechanical elements such as the incompetent cervix, excessive uterine distension, hormonal changes, and bacterial infection and inflammation. Complex inflammation is one condition that plays a role when the infection is thought to be a major factor in preterm labor [5].

Early detection of infection is the most crucial thing in obstetrics. Failure to diagnose can lead to severe complications for the mother, fetus, and newborn. Usually, laboratory tests cannot predict infection during pregnancy, such as white blood count (WBC), neutrophil count, or vaginal bacterial culture. Procalcitonin (PCT) is a prohormone involved in the inflammatory process. PCT originates from almost all cell types during the inflammatory process, including monocytes and parenchyma tissue. PCT potential is more Specific as marker infection bacteria [6].

PCT increases 6 hours after infection and will reach the peak concentrations in 12 - 24 hours and has a half-life of up to 25-30 hours under normal conditions and 30-45 hours in severe renal impairment. PCT can is said to be stable as a diagnostic test. Studies by [7] uncover an increase in PCT of about 0.5 mg/dL/hour in the first 2-3 hours, then there is a rapid increase in PCT in the first 6-12 hours and persists for 48 hours. Procalcitonin was first reported as a marker of sepsis by Moya in 1975 [8].

PCT serum levels have been studied in women pregnant with amniotic fluid broken early premature (PPROM). However, only one publication with a small size sample evaluates PCT levels in women with preterm delivery vs in pregnant women healthy [9]. Other research by [10] reported that PCT levels in threatened preterm labor between 24 and 36 weeks of gestation were not statistically different from that of women with spontaneous preterm labor and term delivery. Moreover, this study revealed no difference between PCT levels at admission between 24 to 28 weeks of age, between 28 to 32 weeks, and more than 32 weeks.

Streptococcus Group B or starting now referred to as GBS, is a gram-positive diplococci bacterium that produces -hemolytic bacterial colonies on blood agar, and the primary strain is hemolytic. Most of the strains were resistant to bacitracin and showed positive results on examination with cyclic Adenosine Monophosphate (cAMP) [11].

GBS is a gram-positive beta-hemolytic bacteria that colonize the gastrointestinal tract; in 18% of women globally, GBS is a gastrointestinal and vaginal flora. GBS can develop into bacterial pathogens in susceptible populations such as newborns, pregnant women, and the elderly. In pregnancy, ascending GBS infection is associated with preterm delivery, intrauterine fetal death, and fetal injury. In infection by this GBS bacteria important to know toxin bacteria. The existence virulence of GBS bacteria is marked by the toxin hemolysin/cytolysin (from now on, it will be called hemolysin). This toxin plays a role in the characteristics of the -hemolytic zone seen in GBS and the type of hemolytic strain that will cause it—related to virulence. Hyper-hemolytic GBS is more pathogenic than non-hemolytic GBS and is more easily attenuated. The mechanism of ascending GBS infection and the immune response during this process have not been well identified. Studies using pregnant animals have shown that intrauterine inflammation caused by

bacterial infection triggers disruption of the placental membrane resulting in fetal injury and preterm delivery [12].

The fetus is still wrapped by the amniotic layer and is sufficiently protected from the mother's bacterial flora; the amniotic fluid has the function of inhibiting the growth of *E. coli* and other bacteria because it contains lysozyme, transferrin or immunoglobulins (IgA and IgG) which are thought to function as bacteriostatic. If there is damage to the amnion layer (intentionally or not, for example, in the amniocentesis procedure), the fetus is easily infected through amnionitis. Vaginal area infection is a significant risk factor. At the time of birth, bacterial invasion through ingested amniotic fluid and skin infection spreads through the soft tissues and sutures of the head or venous thrombosis. If the immune system cannot overcome the bacteremia, a systemic response will occur (Systemic Inflammatory Response Syndrome / SIRS) [13].

Bacterial infections such as GBS infection lead to increased PCT synthesis. This increase is associated with increased CALC-I gene expression, which results in increased PCT synthesis by various cells, tissues, and organs (liver, kidney, pancreas, adipose tissue, and leukocytes). The function of PCT as a marker of infection is produced in two ways method that is directly and indirectly. Production is directly related to inducing PCT synthesis via toxin production and bacterial liposaccharides. In contrast, indirect production is associated with increased production of inflammatory mediators on host cells, such as Interleukins-1b, Interleukins-6 and Tumor Necrosis Factor-alpha (TNF- α) [14].

II. Discussion

This study used a cross-sectional method. Samples were 31 pregnant women who came to the Obstetrics ER at Sanglah Hospital Denpasar (including Preterm labor with preterm delivery) with gestational age from 20 weeks to < 37 weeks who went to the Midwifery IGD Room at Sanglah Hospital Denpasar. Samples were taken by consecutive sampling from preterm pregnant women in the first stage of labor (including Threatened Preterm Delivery) in the Midwifery IGD Sanglah Hospital Denpasar, which has met the inclusion criteria and exclusion criteria. Vaginal mucus samples were examined at the Clinical Microbiology Laboratory of Sanglah Hospital, and procalcitonin samples were analyzed at the Clinical Pathology Laboratory of Sanglah Hospital. Descriptive and analytical analysis was performed on the data obtained.

Research has been done on 31 pregnant women aged 20 weeks pregnant until < 37 weeks to come with a diagnosis of preterm delivery (including Threatened Preterm Delivery). GBS examination from vaginal swab and levels of Procalcitonin from blood cubital vein. Obtained positive GBS result as many as 7 people (22.5%) and GBS negative 24 people (77.5%). Procalcitonin results are high, obtaining 12 (38.7%) while the normal 19 (61.3%).

The results showed that the age in the GBS (+) group with a mean of 26.4 years (SD: 8,3), while in the GBS group (-), the average was 28.7 years (SD: 7.1). The results of this study are different from the research which found maternal age > 36 years was associated with persistent colonization and showed higher rates of GBS colonization in women > 40

years [15].

The results showed that gestational age in the GBS (+) group was obtained with a mean of 34 weeks six days (SD: 1,1), while in the GBS group (-) it was found to mean of 32 weeks two days (SD: 0.7). These results are in line with the study that explain preterm labor was obtained at 28-32 weeks of gestation [16]. There is also study that found that patients experiencing preterm labor were between 28.5 weeks and 33 weeks of pregnancy [17].

The history of parity 0 (nullipara) in this study was found to be the most in the GBS (-) group, namely 12 samples (38.7%), while the lowest was in the GBS (+) group, namely parity 1, 2, and 3 (3.2%). The increased risk of spontaneous preterm birth <37 weeks in nulliparous women with an OR of 1.95 and women in their fifth pregnancy OR 1.26 compared with women in their second pregnancy. Similar results were seen for spontaneous premature births <32 and <28 weeks. These data show an independent association between nulliparas and spontaneous preterm birth at <37, <32 and <28 weeks. Increased risk of spontaneous preterm delivery in women in their fifth pregnancy with the highest risk for preterm delivery at early gestational age. Both nulliparous women and women in the fifth gestational age have the highest risk for all preterm birth outcomes [18].

TABLE I: CHARACTERISTICS OF SUBJECT

Characteristics	Group		P
	GBS(+)	GBS (-)	
Age (mean ± SD)	26.4 ± 8.3	28.7 ± 7.1	0.176 **
Gestational Age (mean ± SD)	34.6 ± 1.1	32.2±0.7	0.153 **
Parity (n, %)			
0	4 (12.9%)	12 (38.7%)	
1	1 (3.2%)	8 (25.8%)	0.770 *
2	1 (3.2%	2 (6.5%)	
3	1 (3.2%)	2 (6.5%)	
Body Mass Index (BMI) (n, %)			
Underweight (< 18 kg/m2)	0 (0%)	0 (0%)	
Normal (18-25.9 kg/m 2)	1 (3.2%)	8 (25.8%)	
Overweight (> 25.9 kg/m 2)	6 (19.4%)	12 (38.7%)	0.219 *
Obesity (> 30 kg/m2)	0 (0%)	4 (12.9%)	
Procalcitonin	0.5±0.2	0.1 ± 0.2	0.011 **

Based on BMI, it was found that overweight (> 25.9 kg/m 2) was mainly in the GBS group (-) as many as 12 samples (38.7 %) while normal BMI was the least in the GBS group (+) 1 sample (3.2 %). This result is slightly different from the study who found that about 18.4% of mothers with preterm delivery were overweight but found that overweight and obese women had an increased risk of preterm delivery. Birth regardless of weight in early pregnancy [19]. Pre-pregnancy weight was associated with a higher risk of preterm delivery, while overweight and obesity were associated with a higher risk of preterm delivery. Insufficient body weight during pregnancy is associated with a higher prevalence of preterm labor and premature rupture of membranes [20].

The normality test results of the data used the Shapiro Wilk test because the data was < 50 samples. The data is presented in Table II.

TABLE II: NORMALITY TEST RESULTS AND DATA HOMOGENITY

Variable	Normality	Homogeneity
Mother's Age	0.000	0.882
Gestational Age	0.001	0.011
Procalcitonin	0.000	0.156

Characteristics based on positive Group B Streptococcus (GBS) were 22.4% fewer than those of Group B Streptococcus (GBS) negative. The results of this study are in line with the study that found that in the early trimester, GBS was found to be only 5.7% and in the third trimester, 9.5%. GBS colonization at an early stage of Pregnancy is also associated with an increased risk of preterm delivery with an Odds Ratio of 2.42 and childbirth < 34 weeks with an OR of 14.22 [21].

Different studies also found an association between abnormal vaginal colonization detected in the 2nd trimester and preterm birth before 28 weeks. Klebsiella pneumonia has been identified as the causative microorganism preterm birth. Group B Streptococcus (GBS) is a Gram-positive bacterium and a commensal of the body that infects the lower gastrointestinal tract and urogenital in women as much as 20-30%. These bacteria also have an increased potential for intrauterine infection, transmission during the puerperium, and risk of severe disease in the newborn. GBS colonization of the vagina during pregnancy can trigger preterm birth and premature rupture of membranes, posing a danger to pregnant women [22].

Due to infection, the pathogenesis of preterm labor can occur with an early process, where bacterial invasion occurs in the decidual chorion. This bacterial invasion will trigger the release of endotoxins and exotoxins and activate the decidua and fetal membranes releasing various cytokines, such as TNF-a, IL-a, IL-1ß, IL-6, IL-8, and Granulocyte Colony-Stimulating Factor (GCSF). Cytokine levels This in amniotic fluid is associated with the presence of chorioamnion infection. Prostanoid production in the decidua, chorion, amnion and myometrial cells and endothelin production by amniotic and decidual cells is stimulated by high endotoxin concentrations as well as by IL-1 and TNF-a [23].

The second pathway of the pathogenesis of preterm labor occurs through this pathway. In patients with chorionic infection, there will be a decrease in prostaglandin dehydrogenase. Decreased prostaglandin dehydrogenase This increases the number of prostaglandins that reach the myometrium, which then triggers uterine contractions [23].

The third pathway in the pathogenesis of preterm labor involves the fetus itself. In infected fetuses, there is an increase in the production of CRH (Corticotropin-Releasing Hormone) by the fetal hypothalamus and placenta which causes an increase in fetal corticotropin secretion. The increase in corticotropin causes an increase in the production of cortisol by the fetal adrenals, which ends in an increase in the production of prostaglandins and causes uterine contractions. The infected fetus will increase the production of cytokines and the delivery time will be faster.

TABLE III: RELATIONSHIP OF GROUP B STREPTOCOCCUS WITH PROCALCITONIN IN PRETERM LABOR

Variable	Procalcitonin		RP	95% CI	P
	High (≥ 0.5 ng/L)	Normal (<0.5 ng/L)			
GBS positive (n,%)	6 (19.4%)	1 (3.2%)	3.4	1.61- 7.30	0.004
GBS negative f (n,%)	6 (19.4%)	18 (58.1%)			

In 88% of cases of infected fetuses will occur within 48-72 hours later. The time difference between the occurrence of infection with the incidence of preterm labor is not known for sure [23]. Procalcitonin obtained an average of 0.26 (SD: 0.29), with the category of high procalcitonin in 12 samples (38.7%) and normal in as many as 19 samples (61.3%). There is research found different results with a median value of 0.039 ng/ml, serum PCT not relevant for predicting preterm delivery at 7 or 14 days in women admitted with threatened preterm delivery. Between 24 and 36 weeks, and thus, it is not a suitable biologic marker to confirm the hypothesis of inflammatory processes associated with preterm birth [10]. Different results were also obtained involving 48 mothers with preterm delivery, and procalcitonin was obtained with a mean of 0.104. Different results were also obtained in a study involving 76 patients with preterm labor 19.73% developed chorioamnionitis. No significant differences could be detected in gravidity, parity, duration of hospitalization, and the gestational week at diagnosis. There was no detectable difference in procalcitonin levels either at the time of hospital admission or before delivery between the two groups, so it was concluded that procalcitonin was not found to be predictive of chorioamnionitis [17].

Procalcitonin is a new parameter for diagnosing infection, where its occurrence and mechanism in pregnancy are still unknown until now. PCT cut off point = 1.7 ng/mL and obtained a sensitivity of 52%, specificity 70%, NPP 57% and NPN 66% in predicting labor preterm [9].

Streptococcal relationship analysis Group B with procalcitonin in preterm labor using the Chi Square test and is presented in Table III.

The study results in Table III show that there is a relationship between GBS and procalcitonin with a p-value <0.05 and RP value > 1 means positive GBS is a risk factor for high serum procalcitonin. Research showed a significant relationship between maternal infection with histological chorioamnionitis and PCT inflammation index at delivery. There was no significant relationship between maternal infection and histological chorioamnionitis. Ascending intrauterine infection with Group B or Streptococcus bacteria Streptococcus agalactiae increases the risk of preterm premature rupture of membranes, injury/trauma to the fetus, sepsis, preterm birth, and meningitis in young infants younger than 90 days [24].

Ascending GBS infection and the immune response that occurs during this process have not been well identified. Studies using pregnant animals show that intrauterine inflammation caused by bacterial infection triggers disruption of the placental membrane resulting in injury to the fetus and preterm birth [24].

Bacterial infections such as GBS infection led to increased PCT synthesis. This increase is associated with increased expression of the CALC-I gene, which results in increased synthesis of PCT by various cells, tissues, and organs (e.g. liver, kidney, pancreas, adipose tissue, and leukocytes). The function of PCT as a marker of infection is produced in two ways: direct and indirect. Production is directly related to the induction of PCT synthesis through the production of toxins and bacterial liposaccharides, while indirect production is associated with increased production of inflammatory mediators on host cells, such as Interleukins-1b, Interleukins-6 and Tumor Necrosis Factor-alpha (TNF-a) [14].

The weakness in this study is that it was not explicitly examined to exclude other infectious diseases (intragenital and extragenital) but to minimize this, history has been carried out (history of infection in pregnant women who have been diagnosed previously), and view the data in the ANC book. Factors other than infection were examined with ultrasound support (cervical checking length, amount of amniotic fluid, fetal biometry). This is done to get rid of confounding variables.

Limitations source studies others researching GBS infection and serum procalcitonin Mother with preterm delivery, and not yet existence studies about connection GBS infection and serum procalcitonin levels in preterm labor, especially in Indonesia, are capable novelty add knowledge in the field health so that could be made consideration for to do screening or inspection early on mother pregnant during ANC. Taking sample study this done in 3 months so the sample research obtained was limited. The connection between GBS infection and increased rate of procalcitonin with preterm delivery will be strengthened with total more samples a lot.

III. CONCLUSION

Based on the results of research and discussion, conclusions can be drawn such as Group B Streptococcus Infection in the vagina in preterm labor, there are Maternal Serum Procalcitonin levels that are high in preterm labor and Group B Streptococcus infection of the vagina, and the rate of High Maternal Serum Procalcitonin is a factor risk of preterm delivery (Ratio Prevalence 3.4).

REFERENCES

[1] Chatterjee J, Gullam J, Vatish M, Thornton S. The management of preterm labour. *Arch Dis Child Fetal Neonatal Ed.* 2007; 92(2): F88-F93.

[2] De Costa A, Moller AB, Blencowe H, et al. Study protocol for WHO and UNICEF estimates of global, regional, and national preterm birth rates for 2010 to 2019. *PLoS One.* 2021; 16(10): e0258751.

[3] Beal T, Tumilowicz A, Sutrisna A, Izwardy D, Neufeld LM. A review of child stunting determinants in Indonesia. *Matern Child Nutr.* 2018; 14(4): e12617.

[4] Chawanpaiboon S, Vogel JP, Moller AB, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health.* 2019; 7(1): e37-e46.

[5] Sungkar A, Fattah ANA, Surya R, Santoso BI, Zalud I. High Preterm Birth at Cipto Mangunkusumo Hospital as a National Referral Hospital in Indonesia. *Medical Journal of Indonesia.* 2017; 26(3): 198-203.

[6] Lisonkova S, Potts J, Muraca GM, Razaz N, Sabr Y, Chan WS, et al. Maternal age and severe maternal morbidity: A population-based retrospective cohort study. *PLoS Med.* 2017; 14(5): e1002307.

[7] Leonard SA, Main EK, Carmichael SL. The contribution of maternal characteristics and cesarean delivery to an increasing trend of severe maternal morbidity. *BMC Pregnancy Childbirth.* 2019; 19(1): 16.

[8] Barrett PM, McCarthy FP, Evans M, et al. Risk of long-term renal disease in women with a history of preterm delivery: a population-based cohort study. *BMC Med.* 2020; 18(1): 66.

[9] Torbé A, Czajka R. Proinflammatory cytokines and other indications of inflammation in cervico-vaginal secretions and preterm delivery. Int *J Gynaecol Obstet.* 2004; 87(2): 125-130.

[10] Ducarme G, Desroys du Roure F, Le Thuaut A, Grange J, Vital M, Dimet J. Efficacy of serum procalcitonin to predict spontaneous preterm birth in women with threatened preterm labour: a prospective observational study. *BMC Pregnancy Childbirth*. 2018; 18(1): 65.

[11] Burman LG, Christensen P, Christensen K, et al. Prevention of excess neonatal morbidity associated with group B streptococci by vaginal chlorhexidine disinfection during labour. The Swedish Chlorhexidine Study Group. *Lancet*. 1992; 340(8811): 65-69.

[12] Whidbey C, Vornhagen J, Gendrin C, Boldenow E, Samson JM, Doering K, et al. A streptococcal lipid toxin induces membrane permeabilization and pyroptosis leading to fetal injury. *EMBO Mol Med.* 2015; 7(4): 488-505.

[13] Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. *Lancet*. 2017; 390(10104): 1770–1780.

[14] Angeletti S, Spoto S, Fogolari M, Cortigiani M, Fioravanti M, De Florio L, et al. Diagnostic and prognostic role of procalcitonin (PCT) and MR-pro-Adrenomedullin (MR-proADM) in bacterial infections. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica.* 2015; 123(9): 740–748.

[15] Patras KA, Nizet V. Group B Streptococcal Maternal Colonization and Neonatal Disease: Molecular Mechanisms and Preventative Approaches. *Front Pediatr.* 2018; 6: 27.

[16] Howman RA, Charles AK, Jacques A, Doherty DA, Simmer K, Strunk T, et al. Inflammatory and haematological markers in the maternal, umbilical cord and infant circulation in histological chorioamnionitis. *PLoS One.* 2012; 7(12): e51836.

[17] Asadi N, Faraji A, Keshavarzi A, Akbarzadeh-Jahromi M, Yoosefi S. Predictive value of procalcitonin, C-reactive protein, and white blood cells for chorioamnionitis among women with preterm premature rupture of membranes. *Int J Gynaecol Obstet.* 2019; 147(1): 83-88.

[18] Koullali B, van Zijl MD, Kazemier BM, Oudjik MA, Mol BW, Pajkrt E, et al. The association between parity and spontaneous preterm birth: a population based study. *BMC Pregnancy Childbirth.* 2020; 20(1): 233.

[19] Su XJ, Huang SJ, Li X, Du QL. Prepregnancy Overweight and Obesity Are Associated with an Increased Risk of Preterm Birth in Chinese Women. *Obes Facts.* 2020; 13(2): 237-244.

[20] Pigatti Silva F, Souza RT, Cecatti JG, Passini Jr R, Tedesco RP, Lajos GJ, et al. Role of Body Mass Index and gestational weight gain on preterm birth and adverse perinatal outcomes. *Sci Rep.* 2019; 9(1): 13093.

[21] Tano S, Ueno T, Mayama M, Yamada T, Takeda T, Uno K, et al. Relationship between vaginal group B streptococcus colonization in the early stage of pregnancy and preterm birth: a retrospective cohort study. *BMC Pregnancy Childbirth.* 2021; 21(1): 141.

[22] Son KA, Kim M, Kim YM, Kim SH, Choi SJ, Oh S, et al. Prevalence of vaginal microorganisms among pregnant women according to trimester and association with preterm birth. *Obstet Gynecol Sci.* 2018; 61(1): 38-47.

[23] Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet.* 2008; 371(9606): 75-84.

[24] Whidbey C, Vornhagen J, Gendrin C, Boldenow E, Samson JM, Doering K, et al. A streptococcal lipid toxin induces membrane permeabilization and pyroptosis leading to fetal injury. *EMBO Mol Med.* 2015; 7(4): 488-505.