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Variability of the microbiome of pregnant women and its impact on the frequency of preterm birth

DOCTORAL DISSERTATION

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for their constant love, encouragement, and belief in me.
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Dear,

Dr hab. n. med. Iwona Sadowska-Krawczenko, Prof. UMK

Prof. dr hab. Mariusz Dubiel,

Thank you for your mentorship during my doctoral dissertation.

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List of Abbreviations

ACOG – The American College of Obstetricians and Gynecologists
APTT – activated partial thromboplastin time
AUC – area under the curve
BMI – body mass index
BV – bacterial vaginosis
CRP – C-reactive protein
CRL – the crown–rump length
EDD – estimated due date
FGR – fetal growth restriction
FN – false negative
FP – false positive
Hct – hematocrit
Hgb – hemoglobin
IL-6 – interleukin 6
INO. – international normalized ratio
LMP – last menstrual period
MCV – mean corpuscular volume
MCH – mean corpuscular hemoglobin
ML – machine learning
NEC – necrotizing enterocolitis
OUT – operational taxonomic unit
PCR – polymerase chain reaction
PVL – periventricular leukomalacia
PTB – preterm birth
PTD – preterm delivery
PoC – proof of concept
PRO – protein in urine
QIIME – Quantitative Insights Into Microbial Ecology
RDW – red blood cell distribution width
RDS – respiratory distress syndrome
ROC – receiver operating characteristic

SES – socioeconomic status

TD – term delivery

TN – true negative

TNF-alpha – tumor necrosis factor alpha

TPL – threatened preterm labour

TP – true positive

WHO – World Health Organization

WBC – white blood cell

1. Introduction

Prematurity is a major public health issue that is associated with significant morbidity and mortality. It remains a major challenge for clinicians and researchers worldwide due to its multifactorial etiology and complex pathogenesis. Infants born prematurely are at increased risk of developing a wide range of short- and long-term complications that can have serious consequences on their health and development, as well as on the emotional and financial burden on families and society.

Despite numerous advances in medical care, the incidence of prematurity remains high, affecting approximately 10% of all births worldwide. This underscores the need for continued research to identify the underlying causes of prematurity and to develop effective preventive strategies.

As such, the present doctoral thesis focuses on investigating the etiology of prematurity and the potential interventions that could reduce the incidence and severity of prematurity-related complications. Recent research has identified a potential link between the microbiome and preterm deliveries. Studies have shown that the composition of the microbiome in the reproductive tract of pregnant women and gut may be associated with preterm delivery. Understanding the role of the microbiome in preterm delivery may lead to the development of new preventive and therapeutic strategies for this major public health issue. This research seeks to shed light on the pathophysiological mechanisms underlying prematurity and to identify potential targets for novel therapeutic interventions.

The motivation for undertaking this research stems from a personal interest in improving maternal and child health outcomes. As a healthcare professional, I have witnessed firsthand the devastating impact of prematurity on families and the challenges faced by neonatal healthcare providers in caring for premature infants. I believe that a deeper understanding of the factors contributing to prematurity and the development of effective prevention strategies have the potential to improve the lives of countless families worldwide.

1.1 Definition of preterm birth

Accurate dating of pregnancy is crucial during first obstetrics appointments, because it can positively affect pregnancy outcomes. This information is essential, for instance, to determine if the fetus is growing properly and to plan interventions to help prevent preterm or late births and related conditions [1]. Preterm delivery, according to the WHO definition:

Preterm delivery - is a termination of pregnancy before 37 completed weeks (whether singleton or multiple) or less than 259 days from the first date of the woman's last menstrual period (LMP).

The estimated due date (EDD) is the date that spontaneous onset of labor is expected to occur.

Table No. 1: Most Accurate Gestational Age Methods

Method	Description	Accuracy
LMP (the first day of the last menstrual period)	Adding 280 days to the first day of the last menstrual period	Establish the due date may overestimate the duration of the pregnancy and can be subject to an error ± 2 weeks [2–4].
Naegele's Rule	First day of Last Menstrual Period + 7 Days - 3 months + 1 year = Date of Estimated Date of Delivery	Assumes a gestational age of 280 days at childbirth [5].

According the ACOG the most accurate method for determining or confirming the gestational age is the ultrasound examination in the first trimester (up to and including 13-6 / 7th week of pregnancy):

- The assessment of gestational age is based on measurement of the crown-rump length (CRL) and has an accuracy of ± 5-7 days
- To assess the proper weight gain of the fetus, the ultrasound examination should be repeated in the later stages of pregnancy (second and third trimester) [6].

Preterm infants are categorized based on their gestational age at birth. The categories are:

Table No. 2: Categorization of preterm infants based on gestational age at birth

Category	Gestational Age	Risk of complications
Extremely preterm	< 28 weeks	High
Very preterm	28-32 weeks	Moderate
Late preterm	34-37 weeks	Low, but still higher than full-term infants [7,8].

Depending on the stage of advancement, premature births can be classified as follows in a Table 3.

Table No. 3: Classification of Premature Births by Stage of Advancement

Classification of premature births	Description
Threatened preterm labor (TPL)	an inhibitable early stage of preterm labor, without progression of dilation and maturation of the cervix
Preterm labor in progress	the unstoppable stage of labor, i.e., the progressive dilation and maturation of the cervix because of regular uterine contractions

1.2 Categories of preterm birth

Premature labor can be classified as either iatrogenic (indicated) or idiopathic (spontaneous). Many maternal or fetal complications (e.g., preeclampsia, FGR, severe maternal hypertension, abruptio placentae) may be an indication for induction of preterm labor. Spontaneous preterm labor may be accompanied by intact or premature rupture of the membranes [9].

1.3 The most common complications of neonatal prematurity

Premature birth is a major public health concern and a leading cause of neonatal mortality and morbidity. Premature infants, also known as preemies, are at risk for a variety of complications due to their immaturity and underdevelopment. In this chapter, I will discuss the most common complications of neonatal prematurity.

Table No. 4: Selected Health Problems of Premature Infants

Complication	Cause	Symptoms	Treatment	Potential Outcomes
Respiratory distress syndrome (RDS)	Lack of surfactant in immature lungs [10].	• Difficulty breathing • Tachypnea, retractions • Nasal flaring • Diminished breath sounds • Inspiratory crackles • Cyanosis • Pallor [10].	Surfactant replacement therapy and mechanical ventilation [11].	Long-term respiratory problems and chronic lung disease [10].
Necrotizing enterocolitis (NEC)	Inflammation and infection of the intestinal tract [12].	• Can be insidious or fulminant • Apnea • Abdominal distension • Bloody stools • Intestinal perforation • Peritonitis • Sepsis • Shock [13].	Surgery to remove affected intestine [14].	Death [13].

Periventricular leukomalacia (PVL)	Damage to the white matter of the brain [15].	• Spastic diplegia • Seizures • Developmental delay • Visual and hearing impairment • Scoliosis • Incontinence by 6–9 months of age [16].	• Currently, there is no treatment that can reverse or improve PVL (periventricular leukomalacia). • The only options available are preventative measures such as: ○ Administering antenatal steroids ○ Treating low blood pressure ○ Treating low carbon dioxide levels in the blood ○ Treating infections • Supportive therapy is essential, including: ○ Early intervention ○ Physical therapy ○ Occupational therapy • Access to specialized centers for managing disabilities [17].	Cerebral palsy, developmental delay, cognitive impairments. The life expectancy of individuals with PVL can vary greatly, from a few months to a full lifespan [17].
Retinopathy of prematurity (ROP)	Abnormal blood vessel development in the eye.	• Blurred vision • Eye abnormalities • Blindness	• Laser therapy • Cryotherapy • Surgery	Vision problems or blindness [18,19]

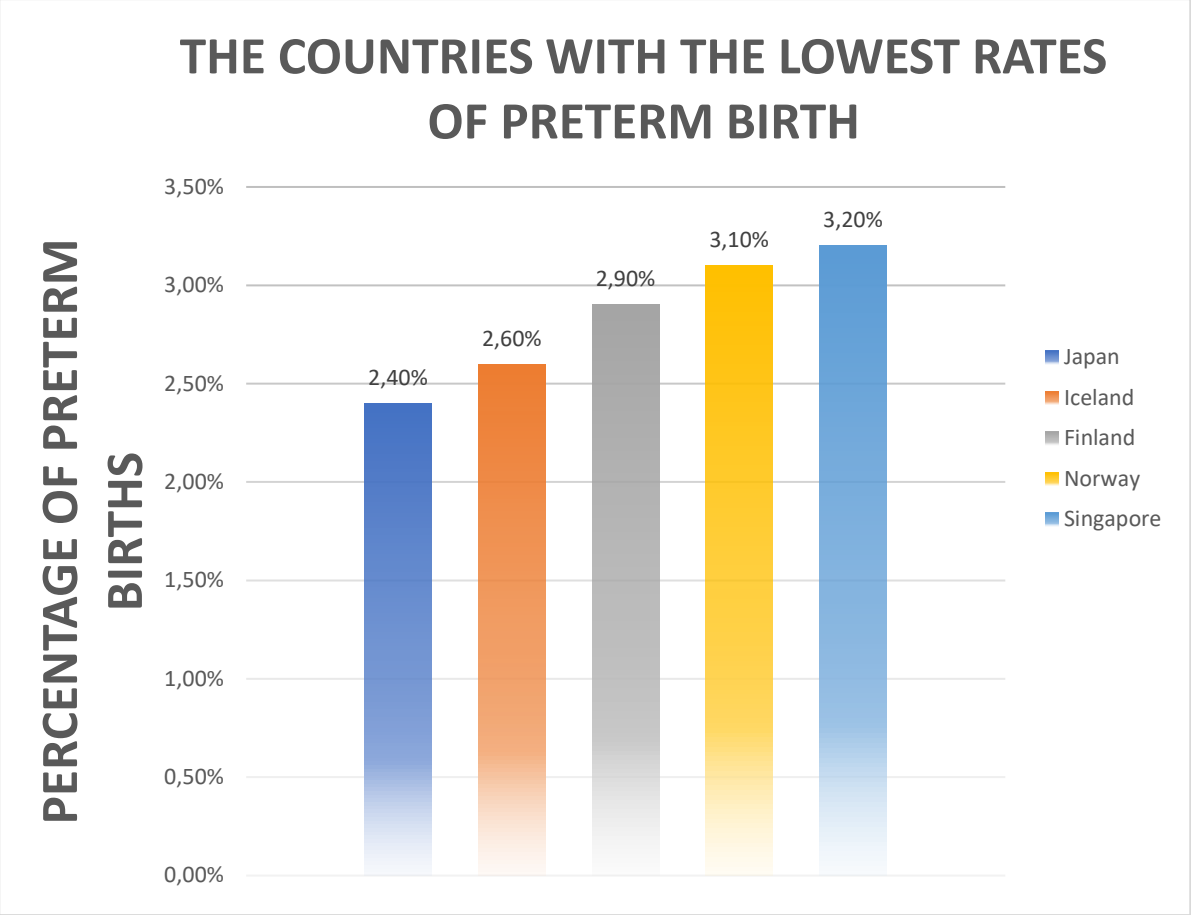
Intraventricular hemorrhage (IVH)	Fragile blood vessels in the brain.	• Breathing problems • Lethargy • Seizures • Weak muscles.	• Monitoring • Medication • Surgery	Neurological problems, developmental delays, cerebral palsy [20,21]

Other complications of prematurity include jaundice [22], anemia [23], and bleeding in the brain (intraventricular hemorrhage) [24]. Premature infants are also at a higher risk for infection and sepsis, and may have difficulty maintaining their body temperature, blood sugar, and blood pressure [25].

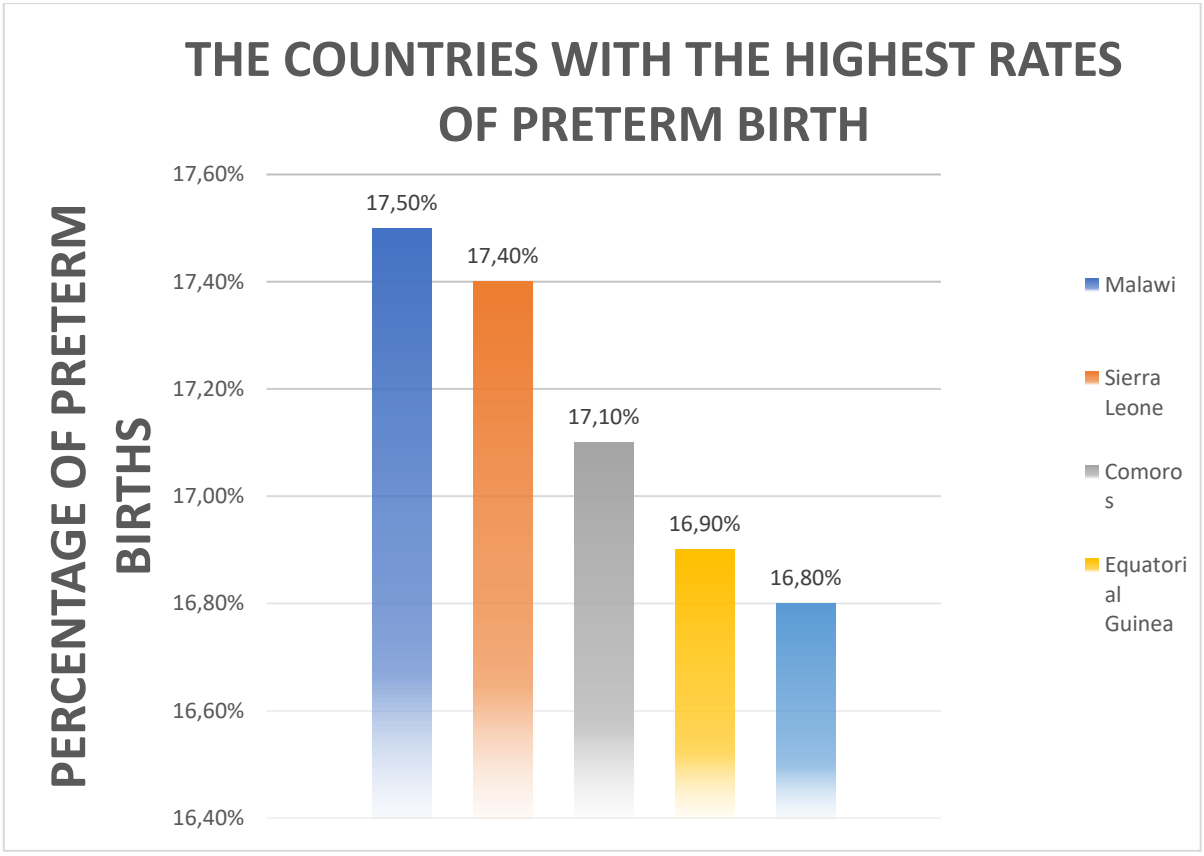
The consequences of prematurity are profound, giving rise to noteworthy disabilities such as visual and hearing impairments, learning disabilities, and respiratory disorders [26]. Additionally, premature infants are at an elevated risk of enduring chronic health conditions, including chronic lung disease, developmental delays, and cognitive impairments. Consequently, premature infants frequently necessitate extended hospital stays, frequent follow-up appointments, and specialized medical attention [27–29].

1.4 The epidemiology of preterm birth

According to the World Health Organization, more than 1 in 10 pregnancies result in preterm labor, which implicates in approximately 15 million premature births worldwide. Despite the improvement in obstetric care and the development of perinatal medicine, the number of premature deliveries has been growing at an alarming rate in the last several years and nowadays is the leading cause of death in children under 5 years old, after pneumonia [30][31]. The incidence of preterm labor varies significantly around the world. The estimated global preterm birth rate for 2014 was 10,6% [32]. The graph 1 and 2 showing the lowest and highest rates of preterm birth according to WHO [30].



Graph No. 1: Comparison of Lowest Preterm Birth Rates Among Countries



Graph No. 2: Comparison of Highest Preterm Birth Rates Among Countries

In Poland, the prevalence of PTD is 6,1 %. According to the data of the Central Statistical Office, 356 540 children were born alive in 2020 (Table No. 5) [33].

Table 5: Number of Live Births in Poland by Year (2020)

The week of birth	Number of babies born alive in Poland	Percentage of Total Born Alive
36-32 weeks of gestation	21,778	6,1 %
31-28 weeks of gestation	2,335	0,7 %
< 28 weeks of gestation	1,468	0,4 %

1.5 Risk factors for preterm birth

Approximately half of the cases of preterm labor are unknown. Research showing that one of the risk factors for preterm labor is a hereditary predisposition to delivering premature. Women who were born prematurely are more likely to have premature birth themselves than women who were born on time [34]. Women whose sisters have given birth prematurely are also at a higher risk of premature birth [35]. Genital tract infections are another frequent and important aspect of the risk for preterm birth. Intrauterine inflammation is most often manifested by chorioamnionitis. Colonization and infection can occur in the decidua, the chorioamniotic space, or the amniotic cavity [36]. Research shows that an intrauterine infection can cause 25-40% of premature births [37]. The most common pathway microbial access to the choriodecidua and amniotic cavity is probably the invasion of bacteria from the abdominal cavity through the fallopian tubes, inadvertent contamination of the needle during amniocentesis or chorionic villus sampling, hematogenous spread through the placenta, ascent migration from the vagina and the cervix. Regardless of when colonization occurs, during or even before pregnancy, intrauterine infection can cause clinical symptoms such as vaginal discharge, cervical effacement, rupture of membranes or delivery. The most frequently identified pathogens are Mycoplasma species, especially Ureaplasama urealyticum, Gardnella vaginalis, Bacteroides species. All these microorganisms are characterized by relatively low virulence, which corresponds to the chronicity of intrauterine infections and the common absence of clinical symptoms of infection [38–40]. In contrast, those most associated with chorioamnionitis and postrupture fetal infection, group B streptococci and Escherichia coli, occur occasionally [41].

Bacterial vaginosis (BV) is defined as a disorder in the microbial ecosystem of the vagina. BV is clinically diagnosed by the presence of the clue cells, a vaginal pH greater than 4.5, profuse white discharge exposed to potassium hydroxide and a fish odour [42]. In bacterial vaginosis there is a decrease in the number of normally occurring Lactobacillus species and a tremendous increase in other organisms including G. vaginalis, bacteroides species, mobiluncus species, U. urealyticum, and M. hominis [43–45]. The mechanisms by which bacterial vaginosis is associated with preterm labor are unknown. The infectious microorganisms are likely to enter the uterus before or at the beginning of pregnancy. However, in most women who have had an early spontaneous preterm labor, the organisms have been often detected in the uterus. Bacterial vaginosis is more likely to be a marker of intrauterine colonization with similar organisms [46,47].

Sexually transmitted infections, including Chlamydia trachomatis, syphilis, and gonorrhea, are rare in the uterus but increase the risk of preterm labor [41]. Certain non-genital infections, such as pyelonephritis, asymptomatic bacteriuria, pneumonia, and appendicitis, have also been associated with preterm labor.

2. Microbiome and microbiota

The human body is home to a diverse community of microorganisms, collectively referred to as the human microbiome. These microorganisms play a crucial role in maintaining human health and well-being. A better understanding of the microbiome and its components is essential for understanding the complex interactions between the human body and the microorganisms that inhabit it. In this chapter, we will discuss the definitions, characteristics, and functions of the microbiome and its components, the microbiota. The definitions are shown in the table below.

Table No. 6: Microbiome and Microbiota Terminology

Definition	Description
Microbiota	The sum of microorganisms present in a particular community [48].
Microbiome	The entire habitat including the microbiota (bacteria, archaea, lower and higher eurkaryotes, and viruses), their genomes (i.e., genes), and environmental conditions [48].
Human microbiome	All bacterial genomes present in or on the surface of our bodies [49,50].
Body niche	Each specific area of the body has its own unique microbes [49,50].

Metataxonomics is a concept defined as the high-throughput process used to characterize the entire microbiota and create a metataxonomic tree, which shows the relationships between all sequences obtained. Whereas metagenome is the collection of genomes and genes from the members of a microbiota. In general, two sequencing-based strategies are commonly used to study the microbiome, metagenomics and metataxonomics. Low prevalence, slow growth, and/or special requirements for conditions are recognized causes of the inability of some species to grow. Metagenomics, which uses genomics techniques to study communities of microbial organisms without the need to isolate and culture them, shows that many species of environmental and human microbes cannot be cultured [51]. Metagenome refers to the collection of genomes and genes that can be obtained by sequencing DNA extracted from a sample (metagenomics) and then assembling or mapping into a reference database followed by annotation [48]. The cornerstone of genomics-based detection methods involves sequencing or variable regions of the bacterial 16s ribosomal RNA (16S-rRNA) gene [52]. The study of metataxonomics involves the amplification and sequencing of specific, often short-length regions of microbial taxonomic marker genes. The nine hypervariable regions (V1-V9) and highly conserved regions are the components of the bacterial 16S rRNA gene. Variable regions amplification is made possible by specially designed PCR primers capable of binding to the conserved regions. PCR primers are generally diverse and distinctive, which facilitates the classification of bacterial taxonomies into species [53]. Studies of the human maternal microbiome using high-throughput sequencing revealed promising links between microbial composition and health and PTB and yielded multiple sequencing datasets. The Human Microbiome Project is concerned with characterizing the microbial communities found in several different locations in the human body. Special attention has been devoted to pregnancy and PTB. Maternal oral, vaginal, intestinal, cervical, and placental microbiomes regulate pregnancy outcomes as demonstrated by advances in next-generation sequencing and metagenomic computational analysis. The project also shows that traditional culture techniques are no longer to respond to our current clinical needs [50].

2.1 Microbiome during pregnancy

Many physiological changes occur during pregnancy, such as immunological and metabolic changes or vascular remodeling. Thus, the structure of the microbial community in different maternal niches may change during pregnancy. While many of these changes may have positive effects on the mother and fetus, many studies show that dysbiosis of the maternal microbiomes may also be associated with adverse pregnancy outcomes such as PTB [54]. Current

research has shown that non-resident bacteria are the main cause of PTB because the human body is not sterile but is home to millions of microorganisms [49,50].

2.1.1 Oral microbiome during pregnancy

The oral microbiome maintains a symbiotic relationship with the host. Any imbalance or dysbiosis of the oral flora often leads to the development of periodontal disease [55,56]. The most common periodontal diseases are dental caries, gingivitis, and chronic forms of periodontitis [57–59]. The pathophysiology of periodontal disease is not fully understood. However, disease initiation and progression are associated with disruption of periodontal host-microbe [58]. There are many different host or microbe related factors that disrupt periodontal homeostasis. These may be congenital or acquired host immunodeficiencies, immunoregulatory defects associated with mutations or polymorphisms, old age, systemic diseases such as diabetes, obesity, environmental factors (e.g. smoking, diet, stress), epigenetic modifications in response to environmental changes, and the presence of key pathogens that can transform a symbiotic microflora into a dysbiotic one [58,60–65]. Periodontal disease is accompanied by many transient bacteremia. During mastication, oral hygiene, or dental treatment it can lead to a body-wide dissemination of bacteria and the activation of systemic inflammation. This may cause systemic complications and, in the case of pregnancy, adverse outcomes [66–70]. Periodontal disease is observed as an increased risk factor for preterm delivery. Some studies indicate an increased risk of preterm delivery in healthy pregnant women with moderate to severe periodontal disease [71].

2.1.2 Gut microbiome during pregnancy

Most of the literature on gut microbiota and PTB focuses on the gut flora of infants. However, very little information is available about the maternal gut microbiome and its impact on preterm birth. Recently, it has been shown that the gut microbiome also differs between early and late pregnancy. The gastrointestinal tract is inhabited by a huge and diverse array of microbes that participate in the metabolism of the host. Thus, it can also play a huge role in protecting against microbial invasion and facilitating the functioning of the immune system [72]. The mother's gut microbiota can be altered by changes in the environment during pregnancy, resulting in the need to transport nutrients to the fetus [73]. A subtle alteration in the composition of the maternal intestinal microbiota may adversely affect the course of pregnancy and contribute to the delivery of preterm [74,75]. Gut-associated microbes can colonize the vagina and then ascend [76,77]. Bacterial dissemination can also occur by translocation from the digestive tract into the blood [78].

Having found gut-related taxa in the amniotic fluid of women with premature rupture of membranes, the gut microbiome can also be considered as a potential source of intrauterine infection [79]. In addition to its potential role in spontaneous preterm birth, the maternal gut microbiota is also one of the most important factors shaping the initial colonization of the newborn [80,81].

2.1.3 Vaginal microbiome during pregnancy

The vaginal microbiome exhibits uncomplicated microbial diversity compared to the gut or oral microbiome [50]. The normal vaginal microbiome differs between pregnant and non-pregnant women [82]. The vaginal microbiome in healthy non-pregnant women is characterized by the dominance of Lactobacillus species, comprising greater than 70% of the microflora [83,84]. These bacteria, living in anaerobic niches, are responsible for the fermentation of sugars and the production of lactic acid. Lactic acid producing species have a protective function against infections, mainly sexually transmitted and urinary tract infections [83,85–87]. The vaginal microbiome profiles of African and European women differ significantly. Women of African descent are less likely to show vaginal lactic acid bacteria and more likely to show increased vaginal microbial diversity [88,89]. Recent studies show that hormonal, nutritional and immunological changes during pregnancy have a vast impact on the vaginal microflora. These changes can help maintain maternal and fetal health during pregnancy [82,90]. Some authors found that women with subsequent PTB had a significantly greater decrease in vaginal microbiome diversity, richness, and alignment between the first and second trimester compared to women who delivered at term [91].

2.1.4 Placental microbiome during pregnancy

The placenta has so far been considered as a sterile organ. It is now clear that it can no longer be regarded as a strictly sterile organ [72], [73]. The discovery of the presence of bacteria in the placenta in full-term pregnancies in the absence of histological inflammation and clinical infection was one of the first evidence of the existence of the microbiome [74], [75]. Studies show that the microbiome in both the full-term and preterm placenta is low abundance, but metabolically rich. The microbiome included commensal bacterial species such as E. coli (the most abundant species), Prevotella tannerae, Bacteriodes spp. and Fusobacterium spp. [76]. Maternal factors such as weight gain during pregnancy and pregnancy complications such as intrahepatic cholestasis, pre-eclampsia or gestational diabetes affect the placental microbiome [77]– [80]. Research on the dissemination of bacteria in the placenta have shown spatial variability. Clear clusters were

observed between the fetal villous and membrane tissues as well as the maternal decidua [77]. It has also been discovered that the endometrial microbiome exists and that it can influence the success of implantation [78],[80], [81]. Various species have been identified in the non-pregnant uterus [78]. Changes in intrauterine bacteria seem to be associated also with vaginosis [80]. Another interesting fact is that hematogonic dissemination of microbes originating in the oral cavity occurs as part of the formation of the placental villous tree. Notably, placental membranes were associated with specific communities for both term and preterm delivery [81]. There is increasing evidence supporting the concept of both intrauterine and placental communities of low biomass, but nevertheless present. Shared microbes between the placenta and meconium, as well as the newborn's oral cavity and even the brain, suggest that the placenta may play a role in the seeding or colonization of bacteria in utero [82].

2.1.5 Cervical microbiome during pregnancy

The existence of a cervical microbiome independent of the vaginal microbiome is an evolving concept. There are few descriptions of the cervical microbiome in the literature, but research on the human papillomavirus indicates its presence [92,93]. Recent evidence supports the concept of commensal microflora in the cervix. The study found that the cervical microbiome, while containing many viral and bacterial organisms, is very similar to the vaginal microbiome, mainly made up of lactobacilli and Gardnerella [93]. There are no published studies that link the cervical microbiome to the cascade of changes leading to the onset of labor, both full-term and preterm. However, the main role of the cervix in PTB is widely recognized. The cervix is a barrier between the sterile environment of the uterine cavity and the non-sterile and often hostile external microbiological environment, which is the vagina. The thick mucus plug in the cervical canal physically protects the uterine cavity from vaginal flora, but also has antimicrobial and cytotoxic effects [94]. The microorganisms associated with preterm labor enter the uterus through the cervix. The predominance of some Lactobacillus species correlates with gestational age. In the second trimester of pregnancy, women with a shortened cervix have an increased concentration of Lactobacillus iners in the vagina, which is associated with preterm delivery. Moreover, intravaginal administration of progesterone, which is usually used as a therapeutic agent to delay early labor, does not alter the vaginal microflora [95]. Cervical shortening is associated with intraamniotic inflammation and thus with the activation of pro-inflammatory cytokines and chemokines such as monocyte chemotactic protein-1 and interleukin-6 [96].

3. Maternal Blood Parameters and the Impact on Preterm Delivery

Understanding the potential factors that contribute to preterm delivery can help in the development of interventions to reduce the risk. One of the ways in which preterm delivery can occur is through alterations in maternal blood parameters. In this chapter, I will discuss the blood parameters that have been found to be associated with an increased risk of preterm delivery and the potential mechanisms by which these changes may lead to preterm delivery.

- Pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), have been found to be elevated in the blood of women who deliver preterm. These cytokines are involved in the inflammatory response and have been linked to cervical insufficiency and premature labor. Elevated levels of pro-inflammatory cytokines can also increase the risk of preterm delivery by promoting the formation of cervical microlesions and weakening the cervix [97,98].

- C-reactive protein (CRP) is another marker of inflammation that has been found to be elevated in the blood of women who deliver preterm. CRP is produced by the liver in response to inflammation and infection, and its levels in the blood can be used as an indicator of inflammation. Elevated levels of CRP in pregnant women have been associated with an increased risk of preterm delivery and have been linked to cervical insufficiency and premature labor [99–101].

- Low hemoglobin levels in pregnant women have also been associated with an increased risk of preterm delivery. Hemoglobin is a protein that carries oxygen in the blood and is important for the proper functioning of the body's organs and tissues. Low levels of hemoglobin can lead to oxygen deprivation and can increase the risk of premature labor [102,103].

- White blood cells, also known as leukocytes, play an important role in the human body's immune system. They help to protect the body against infection and disease. However, an excessive number of white blood cells in the uterus during pregnancy has been linked to an increased risk of preterm delivery. Studies have shown that women with an excessive number of white blood cells in the uterus during pregnancy are at a higher risk of preterm delivery. This is thought to be due to the

fact that white blood cells play a role in the body's inflammatory response. When there are too many white blood cells present, this can lead to an increased level of inflammation in the uterus, which can cause the cervix to soften and open prematurely. Additionally, white blood cells, specifically neutrophils, are known to play a role in the remodeling of the extracellular matrix and cervical ripening, which are necessary for cervical dilation and labor initiation [104,105].

It is important to note that these blood parameters are associated with an increased risk of preterm delivery, but they do not necessarily cause it. Further research is needed to understand the mechanisms by which these blood parameters affect preterm delivery and to develop interventions to reduce the risk. However, measuring these blood parameters during prenatal care can help identify women at increased risk of preterm delivery, allowing for closer monitoring and potential interventions to reduce the risk.

In conclusion, maternal blood parameters can play a significant role in the risk of preterm delivery. Elevated levels of pro-inflammatory cytokines, C-reactive protein, and low levels of hemoglobin have been associated with an increased risk of preterm delivery. These blood parameters can be measured during prenatal care to identify women at increased risk.

4. Hypothesis

The diversity and composition of the microbiome of pregnant women are significantly associated with preterm delivery rates. Pregnant women who have a less diverse and altered microbiome are at higher risk of preterm delivery compared to women with a more diverse and balanced microbiome.

Therefore, the following hypothesis can be put forward:

The diversity and composition of the gut microbiome of pregnant women are significantly associated with preterm delivery rates. Pregnant women who have a less diverse and altered microbiome are at higher risk of preterm delivery compared to women with a more diverse and balanced microbiome.

5. Aim of the study

The aim of this doctoral dissertation is to investigate the variability of the gut microbiome in pregnant women and its potential impact on the frequency of preterm birth.

6. Methodology of microbiome research

6.1 Study design

Research on the links between the human microbiota and preterm birth using high-throughput sequencing has yielded promising results, which is crucial for further research. It was important to carefully plan the methodology and obtain reliable data. In the past decade, clinical trials verifying the association between maternal microbiome and preterm delivery were conducted. The identification and recruitment of an eligible participant included providing participants with clear and detailed information about the study, including the purpose of the study, the duration of the study, the procedures involved, and any potential risks and benefits. Participants were transparently informed about the expected time commitment, any risks, and benefits of the study, and how the results of the study would be used. That is why microbiological analysis of pregnant women' s microbiomes should be carried out with utmost precision. Written and informed consent was provided by all patients who volunteered to participate in the studies we have analyzed. The microbiota of pregnant women was compared in both women with history of prior PTB and without PTB history. Inclusion and exclusion criteria were used to determine which participants are eligible to participate in a study. They were a set of specific characteristics that participants must meet in order to be included in the study, and those that would exclude them from the study. Inclusion and exclusion criteria are an important aspect of study design and are used to ensure that the study population is as homogeneous as possible, which increases the internal validity of the study. For a study on the impact of gut microbiota on preterm deliveries, inclusion and exclusion criteria for a study group are described in the table below.

Table No. 7: Inclusion and Exclusion Criteria for the Study Participants

Inclusion criteria	**Exclusion criteria**
Age ≥ 18 years	Age < 18 years
Pregnant women with symptoms of threatened preterm delivery	Asymptomatic pregnant women
Singleton pregnancy	Multiple gestation
Gestational age between 24 and 36 6/7 weeks of gestation	Gestational age <16 weeks or ≥37 weeks of gestation
Ability to provide informed consent	Inability or unwillingness to provide informed consent

Willingness to participate in the study	Lack of willingness or ability to comply with study requirements
Absence of certain medical conditions or treatments	Significant underlying medical conditions that may affect the pregnancy
No use of certain medications or treatments during pregnancy	Antibiotic use, tocolytic use, or steroid use during pregnancy

The control group for the study consisted of pregnant women who delivered at term without any complications. Inclusion criteria for this group included women who were between 37 + 0 and 42 weeks of gestation, without any medical conditions that could affect pregnancy or delivery, and who had not experienced preterm birth, cervical incompetence, or preterm rupture of membranes. Additionally, eligible participants were required to not have used any medications or treatments during pregnancy that could affect pregnancy or delivery. Finally, participants were required to have provided informed consent and been willing to comply with study requirements. Women who were below 18 years of age or carrying multiple gestations were excluded from the control group. Women with a gestational age outside of the designated range, those who were unable or unwilling to provide informed consent or comply with study requirements were also excluded.

6.2 Study participants

The examination of the patients consisted of gathering information on their previous pregnancies, current pregnancy, and any complications. On the day of admission to the hospital, each participant underwent a gynecological examination, which included a speculum exam and a two-handed examination. The level of opening of the external cervical canal was assessed. Culture samples were also taken from nearly all patients in the study group, including from the vaginal part of the cervix and the external opening of the cervical canal. Additionally, the amount of amniotic fluid was monitored ultrasonographically in the patients included in the study.

6.3 Additional Tests

In this study, each participant was interviewed during their hospital admission to gather information about their pregnancy history, health status, and other relevant details. The interviews covered a range of topics, including the course of the pregnancy, chronic diseases, and other medically relevant history. In addition to the interviews, routine laboratory tests were performed,

including the collection of venous blood to measure various parameters such as morphology (Hct, Hgb, MCH, MCV, PLT, RDW, WBC), CRP, and coagulation (INO., fibrinogen, APTT). The data from these interviews and laboratory tests were combined to form the dataset for the machine learning model to predict preterm birth.

Machine learning

While the primary focus of this doctoral dissertation is to investigate the role of the gut microbiome in the development of preterm labor, machine learning techniques were also incorporated as an additional predictive tool. It was assumed that the use of machine learning algorithms would lead to more accurate predictions of preterm birth compared to traditional methods.

To test this supplementary conjecture, additional data collected from patients during enrollment in the study, including interviews and blood sample analysis, were utilized. A machine learning model was then trained on this data to identify patterns and relationships that contribute to preterm birth and make predictions based on these findings.

Further details on the use of machine learning in this study can be found in Chapter 6.7 and 6.8, where the methodology and results of this analysis are described. Overall, the accuracy of preterm birth prediction could be enhanced by the incorporation of machine learning in this study.

6.4 Fecal specimen collection

Fecal sample analysis involves collecting a sample from each participant and analyzing it for the presence and quantity of microorganisms. This was done using techniques such as PCR and DNA sequencing. Stool samples were collected from all women enrolled in the study by using a sterile swab and inserting it 1-2 cm above the anus. The swab was then rotated for a few seconds and immediately placed in a sterile tube and stored at -80 degrees Celsius. The samples were then packaged in a medical shipment with dry ice to maintain the appropriate temperature during transportation and sent by courier to Eurofins Genomics laboratory in Konstanz, Germany.

6.5 DNA extraction and sequencing of the 16S rRNA gene

The DNA was extracted from the sample at the Eurofins Genomics external laboratory. The V3-V4 region of the 16S rRNA gene was then amplified using polymerase chain reaction (PCR) with specific primers targeting the V3-V4 region. The amplified DNA was then sequenced

using a next-generation sequencing platform such as Illumina. After DNA sequencing of the V3-V4 region of the 16S rRNA gene, the resulting data was in the form of raw sequencing reads. These reads had to be processed and analyzed using bioinformatics tools to identify the different bacterial species present in the sample.

6.6 Microbiome Analysis

The purpose of this chapter is to provide a comprehensive overview of the data processing carried out on the data set utilized in this study. The data set, consisting of sequences from multiple samples, underwent several steps to ensure proper preparation for analysis. This chapter will detail each step of the data processing, including the methods employed and the reasoning behind each one. The processing steps outlined in this chapter form the foundation for the subsequent analysis and are crucial to the overall success of the study.

The first stage of bioinformatics data processing was quality control, where raw sequencing reads were evaluated for quality and any reads that did not meet the established criteria were discarded. The next stage was demultiplexing, in which sequencing reads were separated into different samples based on their barcodes. Filtering was also performed, eliminating any reads that were not relevant to the analysis, such as reads that were too short or too long or contained ambiguous base calls. Further, the reads were trimmed to remove any adapters or primers added during the PCR step, then grouped into contiguous sequences known as Operational Taxonomic Units (OTUs) through a clustering algorithm.

Taxonomic assignments were made using the software QIIME (Quantitative Insights into Microbial Ecology), which was used to analyze and visualize microbial DNA sequencing data. Taxonomic labels were assigned to the OTUs using reference databases such as the NCBI taxonomy or the RDP classifier. QIIME also calculated measures of diversity within and between samples, such as the Shannon Diversity Index or the Faith Phylogenetic Diversity Index. The processing of analyzing Illumina sequencing data included the following steps:

Table No. 8: Processing Steps for Analyzing Illumina Sequencing Data

Step	Description	Output
1. Demultiplexing	All reads passing the standard Illumina chastity filter are grouped according to their index sequences.	Reads grouped by index sequences.
2. Primer clipping	The target-specific forward and reverse primer sequences are removed from the raw forward and reverse reads. Read pairs with imperfect primer matches are removed. Information about the remaining read pairs is reported.	Clipped reads saved in the FASTQ directory as *trimmed_1.fastq.gz and *trimmed_2.fastq.gz.
3. Merging	If the ends of the forward and reverse reads overlap, they are combined into a single, longer read covering the full target region. If the target region is too long to be merged, the forward read is retained.	Merged reads or retained forward reads.
4. Quality filtering	Merged reads are checked against the expected length and variations of the target region. Reads that are significantly shorter or longer than the expected length or contain ambiguous bases ("N") are discarded.	Filtered reads saved in the FASTQ directory as *_merged_for_profiling_1.fastq.gz, used as inputs for final microbiome profiling.

5. Microbiome profiling	I. Length filtered merged reads and quality clipped retained forward reads.	Input for microbiome profiling.
	II. Chimera removal.	Chimeric reads identified and removed.
	III. OUT picking, taxonomic assignment, etc.	Methods description in "Microbiome Profiling: Methods" chapter.
	IV. Statistics on microbiome profiling results.	Tables in "Microbiome Profiling: Results" chapter.
	V. Taxonomic composition overview.	Overview in "Microbiome Profiling: Taxonomic Composition" chapter.
	VI. Detailed result files.	Descriptions in "Microbiome Profiling: Delivered Result Files" chapter.

The Shannon Index

The Shannon index is a widely used metric to quantify the diversity of microbial communities. It considers both the number of species present (species richness) and their relative abundance (species evenness) in each sample. The index is calculated using the following formula:

$$H = - \Sigma(p\_i * \ln(p\_i))$$

Where H is the Shannon index, p_i is the relative abundance of the itch species, and ln is the natural logarithm. The Shannon index ranges from 0 (minimum diversity) to ln(S) (maximum diversity), where S is the number of species present in the sample.

Compared to other diversity metrics, the Shannon index offers several advantages. It is sensitive to changes in both species richness and evenness and does not assume that all species are equally important. Moreover, the Shannon index is relatively easy to interpret and compare across samples (106).

6.7 Machine learning algorithms

Machine learning (ML) is a subfield of artificial intelligence that develops algorithms and models that can learn from and make predictions based on data. It has the advantage of detecting patterns in data that may be challenging for humans to recognize. This doctoral dissertation included a research proof of concept (PoC) as a simulation of a proposed solution to a scientific problem. The PoC involved conducting experiments using prototypes and simulations to assess the feasibility of the proposed solution. The goal of the PoC was to demonstrate the ability of the ML model to accurately predict preterm birth using data from interviews and blood sample analysis. A prototype algorithm was built and tested on a specific dataset to evaluate its strengths, limitations, and identify areas for improvement.

6.8 Confusion Matrix Analysis

The Confusion Matrix was an integral component in the analysis of the results of this study. It was used to evaluate the performance of the classification model by comparing the predicted class labels with the true class labels. The Confusion Matrix provided a visual representation of the accuracy of the model, as well as its strengths and weaknesses.

The Confusion Matrix was generated by comparing the predictions made by the model with the actual labels of the samples. The following formula was used to create the matrix:

Table No. 9: Performance Metrics for Predictive Model- Confusion Matrix

Metric	Definition
True Positive (TP)	The number of instances where the model correctly predicted a positive outcome (patients with preterm delivery were correctly classified as such)
False Positive (FP)	The number of instances where the model incorrectly predicted a positive outcome (patients with term deliveries were classified as premature)

True Negative (TN)	The number of instances where the model correctly predicted a negative outcome (patients with a normal due date were correctly classified as having term deliveries)
False Negative (FN)	The number of instances where the model incorrectly predicted a negative outcome (patients with premature births were incorrectly classified as having term deliveries)

The results of the Confusion Matrix analysis were used to calculate four important metrics, including accuracy, precision, recall, and F1-Score. Accuracy represents the percentage of correct predictions made by the model, while precision represents the percentage of positive predictions that are positive. Recall represents the percentage of positive instances that were correctly predicted as positive, and the F1-Score is the harmonic mean of precision and recall, providing a balance between precision and recall. Classification was carried out using machine learning methods as part of the data analysis process. The prepared dataset underwent several pre-processing steps, including coding of text data into numerical representations, removal of fields with high distinctiveness, fields that were assessed after delivery (ex. the smell and color of amniotic fluid), and the elimination of irrelevant fields (ex. patient number). These steps aimed to enhance the quality of the dataset and improve the accuracy of the classification results.

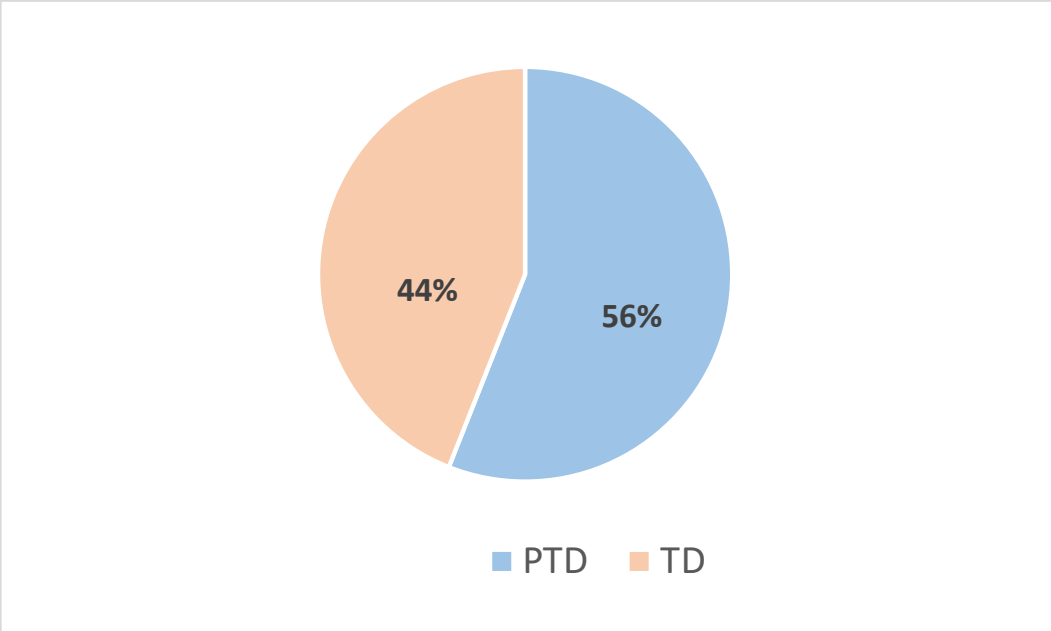
The Confusion Matrix was constructed by creating a 2x2 table, where the rows represented the actual class labels, and the columns represented the predicted class labels. The table was populated with the number of true positive (TP), false positive (FP), false negative (FN), and true negative (TN) predictions made by the model. The results of the Confusion Matrix analysis showed that the model achieved an accuracy = 0.8, with a precision = 0.7143, recall = 1.0, and an F1-Score = 0.83. These results indicate that the model performed well in terms of accuracy and recall but could be improved in terms of precision. The high accuracy and recall scores indicate that the model was able to accurately predict most of the positive and negative instances. On the other hand, the lower precision score suggests that the model made some false positive predictions. This could be due to the presence of noisy or ambiguous data in the training set, or to the limitations of the algorithm used.

In order to perform training, the dataset was divided into 2 disjointed subsets. The training set (80%) was used to train the machine learning algorithms and the test set (20%) was used to evaluate the performance of the models. The choice of the algorithms was based on their ability to handle data gaps and their performance on similar problems.

After evaluating several algorithms, XGBoost was found to perform the best and was selected as the primary algorithm for the study. The other algorithms (LightGBM and CatBoost) were also tested for comparison purposes, but the results from XGBoost were found to be superior and were therefore reported in the results section.

7. Results

Fifty volunteers were recruited from the Department of Obstetrics, Gynecology, and Oncological Gynecology at the University Hospital No. 2 Jan Biziel in Bydgoszcz.



Graph No. 3: Comparison of Preterm and Term Deliveries: A Bar Graph Analysis of Study Participants

7.1 Demographic and Clinical Data Analysis in Studied Groups of Women

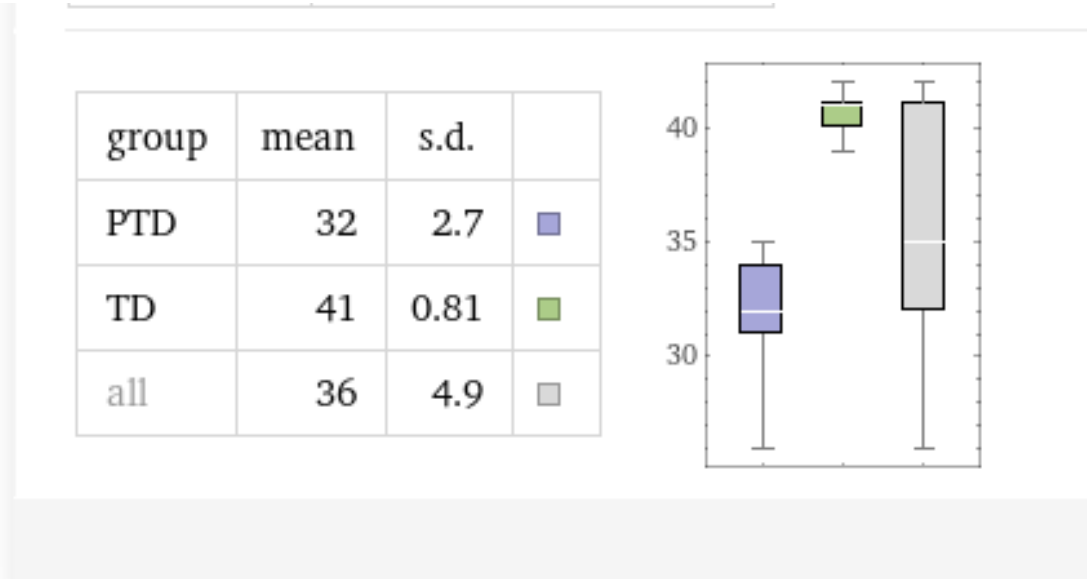
In the first stage of the work, demographic, clinical, and laboratory parameters were analyzed in both groups of women with PTD and in the control group of healthy pregnant women. The characteristics of the study group are outlined in the table below.

Table No. 10: The Characteristics of The Study Group

	Study group (n=28)				Control group (n = 22)				
Feature	min	max	mediana	standard deviation	min	max	mediana	standard deviation	p-value
Age	22	43	29,5	5,6	21	38	31	4,8	0,30644956
Body weight	146	115	70	17,3	57	116	72,5	16,9	0,13038576
Height	150	175	165	6,17	156	181	165	6,38	0,08301367
BMI	18,4	42,2	26	5,83	22,3	38,1	27,55	5,23	0,42898553
Week of delivery	35	32	2,7	39	42	41	0,81	0,81	0,01585909

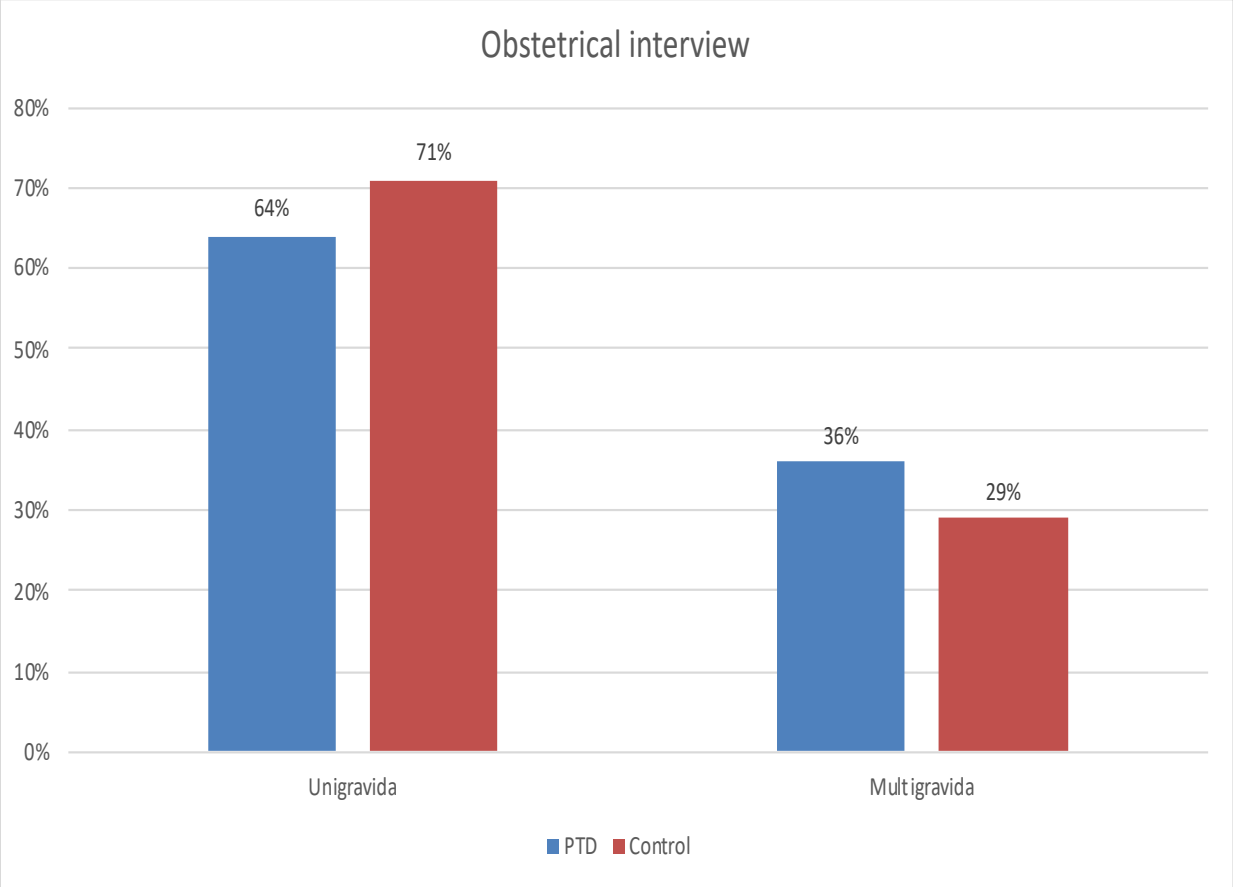
The average age of patients in the PTD group and the control group was 29,5 and 31 years, respectively (no statistically significant difference, p>0.05). No statistically significant differences were observed during the comparative analysis of BMI values in the study group (26) and the control group (27.55) (p>0.05), as well as body mass and height (p>0.05). Statistically significant differences were observed when comparing the average week of pregnancy in both studied groups (p < 0.05).

The results of our analysis show that the mean week of delivery for the PTD group is 32 weeks, with a standard deviation of 2.7 weeks. The mean week of delivery for the TD group is 41 weeks, with a standard deviation of 0.81 weeks. When considering the data from all participants, the overall mean week of delivery is 36 weeks, with a standard deviation of 4.9 weeks.



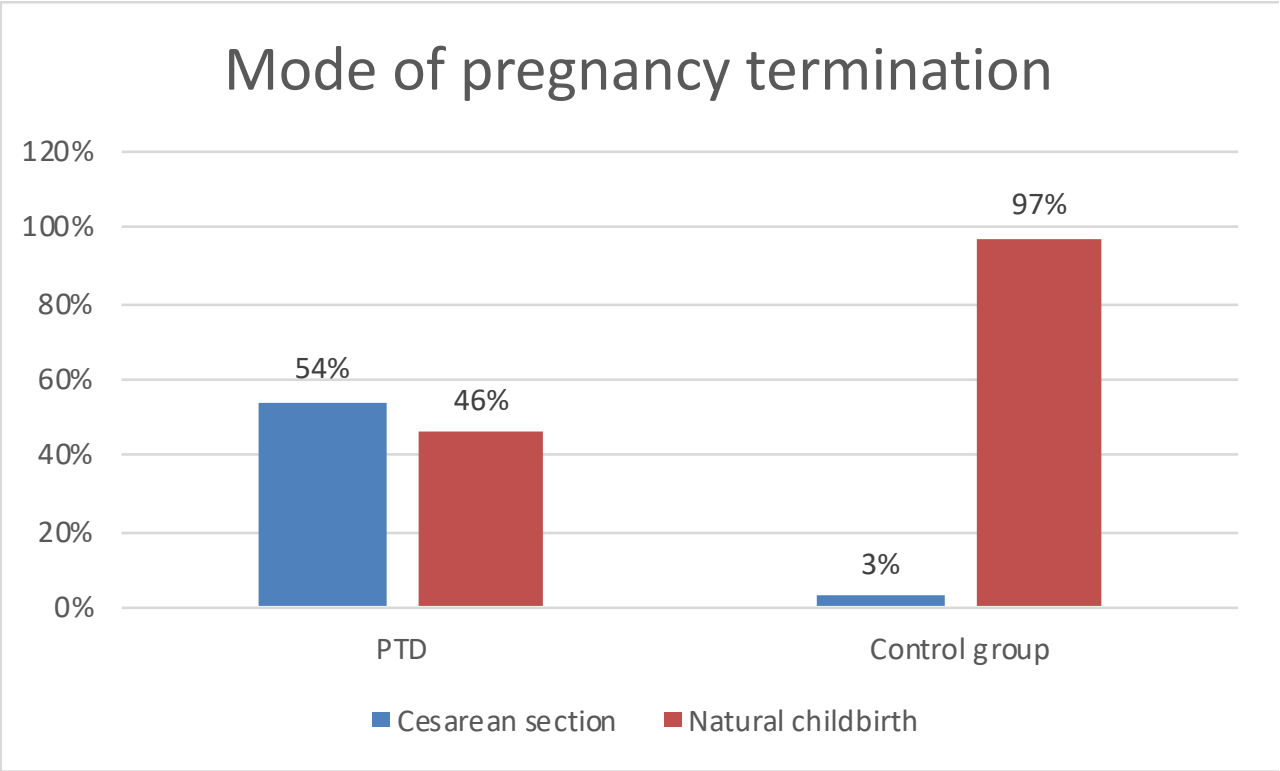
The graph No. 4: Median and standard deviation of week of delivery

In the next stage, the obstetrical interview, such as gravida, was analyzed in the studied groups. However, there was no significant difference between the two groups with regards to the proportion of unigravida (64 vs 71%, p>0.05).



Graph No. 5: Comparison of Female Fertility Rates Between Study Groups

In the study, 54.0% of women with preterm delivery (PTD) underwent cesarean section, compared to only 3.0% in the control group. The control group had a higher rate of spontaneous delivery through natural routes (97.0%) compared to the study group (46.0%). The indications for cesarean section in the study group included symptoms of fetal intrauterine hypoxia, fetal pelvic position, and psychiatric indications. The sole indication for cesarean section in the control group was psychiatric indications. These results are illustrated in the chart below.



Graph No. 6: Distribution of Delivery Modes in Study Population

7.2 Biochemical Data Analysis in Studied Groups of Women

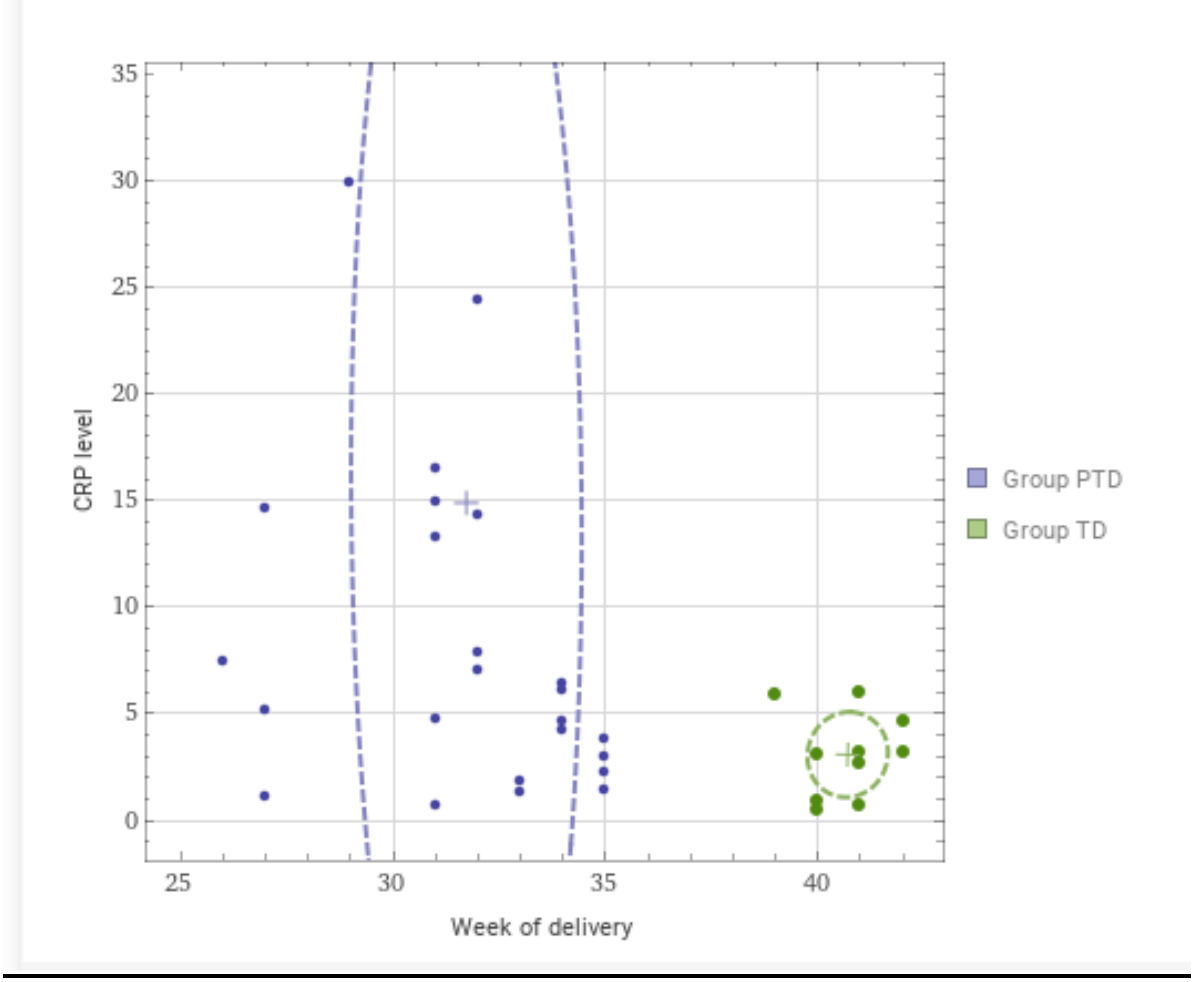
In the study, almost 90 % patients form study group, and 50 % patients form control group had routine infection monitoring parameters assessed at hospital admission, including leukocytosis and CRP levels, as well as other relevant parameters. The collected data is presented in the following figures.

Table No. 11: The Characteristics of The Laboratory Test of The Study Group

	Study group (n=28)				Control group (n = 22)				
Feature:	min	max	mediana	standard deviation	min	max	mediana	standard deviation	p-value
CRP [mg/l]	0,7	175,5	6,1	34,28	0,5	6,0	3.2	2.0	0,289
WBC [G/l]	5,0	21,39	11,76	4,064	5,9	42,0	11,2	2,723	1×10^{-19}
Hb [g/dl]	9,1	14,6	12,5	1,35	9,9	14,2	12,75	1,022	0,114
PLT [G/l]	162	456	228	74,1	169	316	211	42,7	0,0797
HCT [%]	27,5	43,0	35,6	3,68	30,6	41,0	37,8	2,65	0,0179
PRO [mg/dl]	0	106	0	21,3	0	288	0	65,3	0,148
Leu w moczu[/ul]	0	20	4	5,5	2	40	0	11	0,174
Week of delivery	26	35	32	2,7	39	42	41	0,81	0,0159

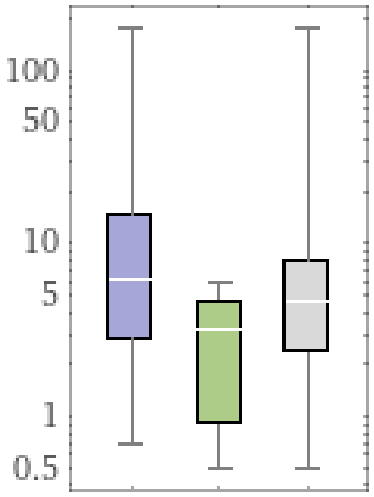
7.2.1 C-reactive protein level

The results of the study showed no statistically significant effect was found between CRP level and the week of delivery ($p > 0.05$). These findings suggest that while CRP level may play a role in pregnancy outcome, it may not be directly related to the timing of delivery.



Graph No. 7: CRP Value Distribution Among Week of Pregnancy

group	mean	s.d.	
PTD	14.88	34.28	
TD	3.080	2.001	
all	11.51	29.33	



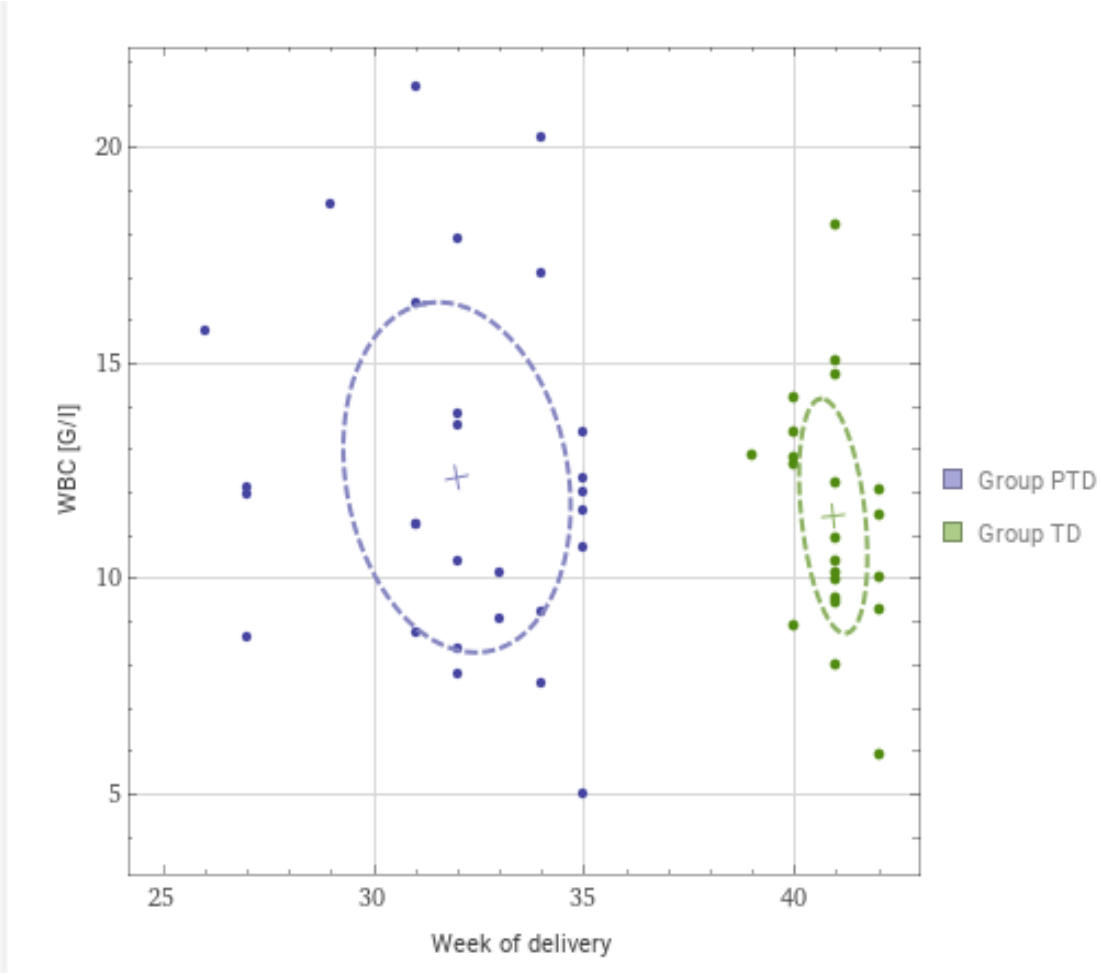
Graph No. 8: Median and Standard Deviation of CRP Level

Table No. 12: CRP level comparison between PTD and TD group

Groups:	**PTD | TD**
F-ratio	1.16
p-value	0.289

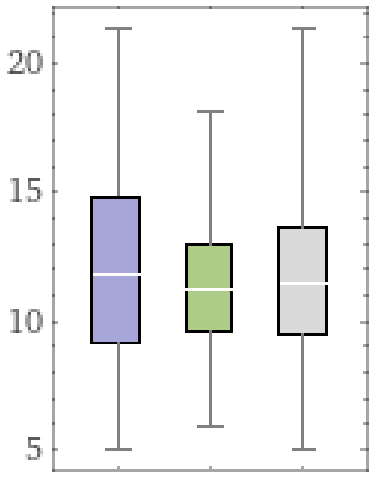
7.2.2 White Blood Cell level

The results of this analysis show that both the week of delivery and WBC [G/l] have significantly different medians, as determined by the Mann-Whitney test ($p<0.05$). In addition, a significant negative correlation was observed between the week of delivery and the white blood cell count (WBC) as measured in G/l (t-test, $p<0.05$). This relationship suggests that as the week of delivery decreases, the WBC increases, and vice versa. This relationship between the week of delivery and the WBC may have implications for the development of preterm birth and warrant further investigation.



Graph No. 9: WBC Value Distribution Among Week of Pregnancy

group	mean	s.d.	
PTD	12.36	4.064	
TD	11.46	2.723	
all	11.97	3.533	



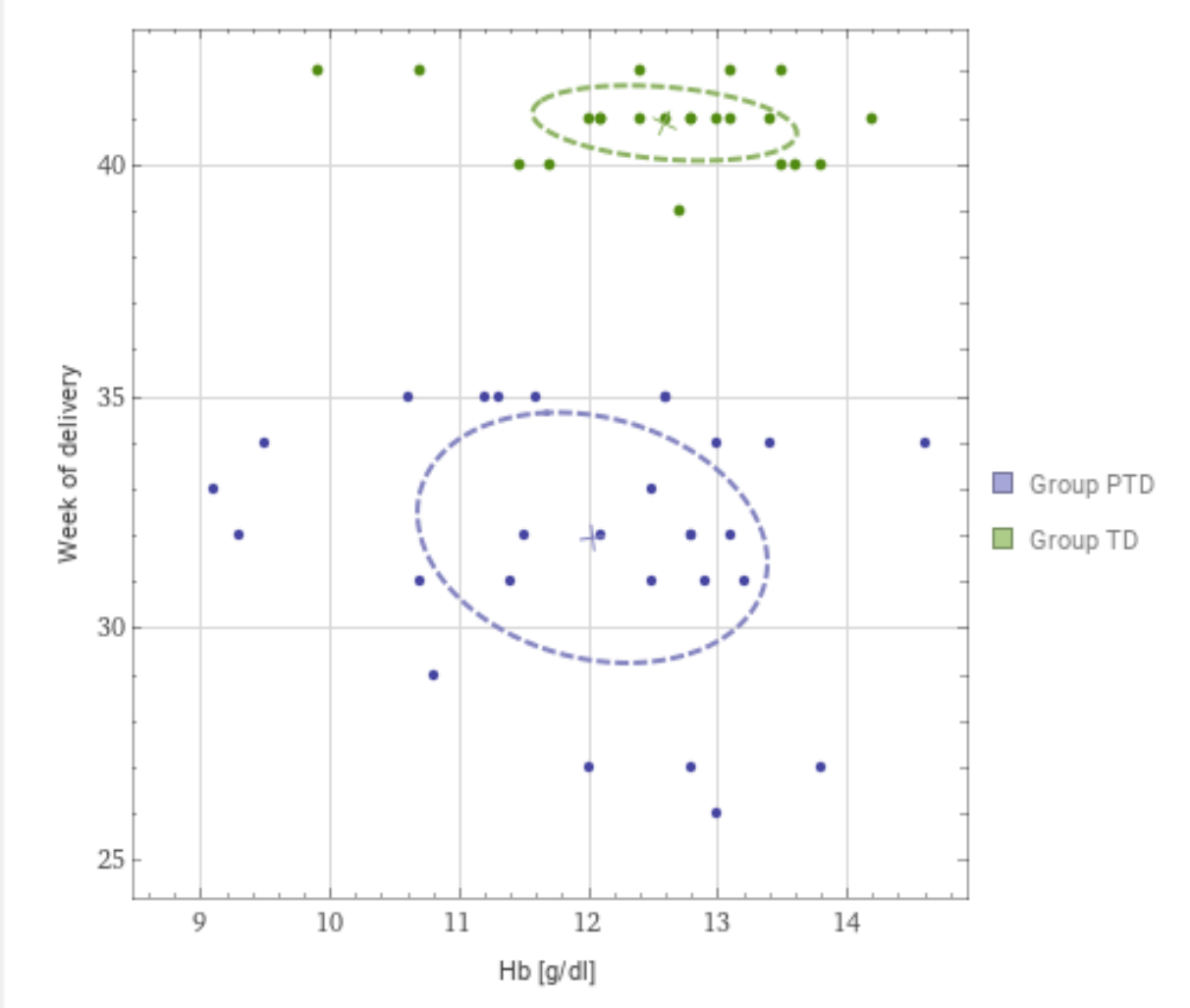
Graph No. 10: Median and Standard Deviation of WBC Level

Table No. 13: WBC level comparison between PTD and TD group

Groups:	**PTD | TD**
F-ratio	224
p-value	**1×10^{-19}**

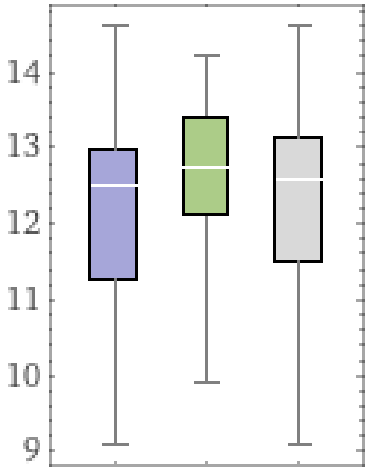
7.2.3 Hemoglobin level

The results of the statistical analysis comparing hemoglobin levels between the two groups, PTD and TD, showed no statistically significant differences. The F-ratio calculated was 2.59 with a p-value of 0.114, indicating that we cannot rule out the possibility that the groups have the same hemoglobin levels. The mean hemoglobin level for the PTD group was 12.03 with a standard deviation of 1.354, while the mean for the TD group was 12.58 with a standard deviation of 1.022. The overall mean for both groups was 12.27 with a standard deviation of 1.240. Although there was a difference in the mean hemoglobin levels between the two groups, the results were not statistically significant and further studies are needed to determine if there is a real difference.



Graph No. 11: Hemoglobin Value Distribution Among Week of Pregnancy

group	mean	s.d.	
PTD	12.03	1.354	
TD	12.58	1.022	
all	12.27	1.240	



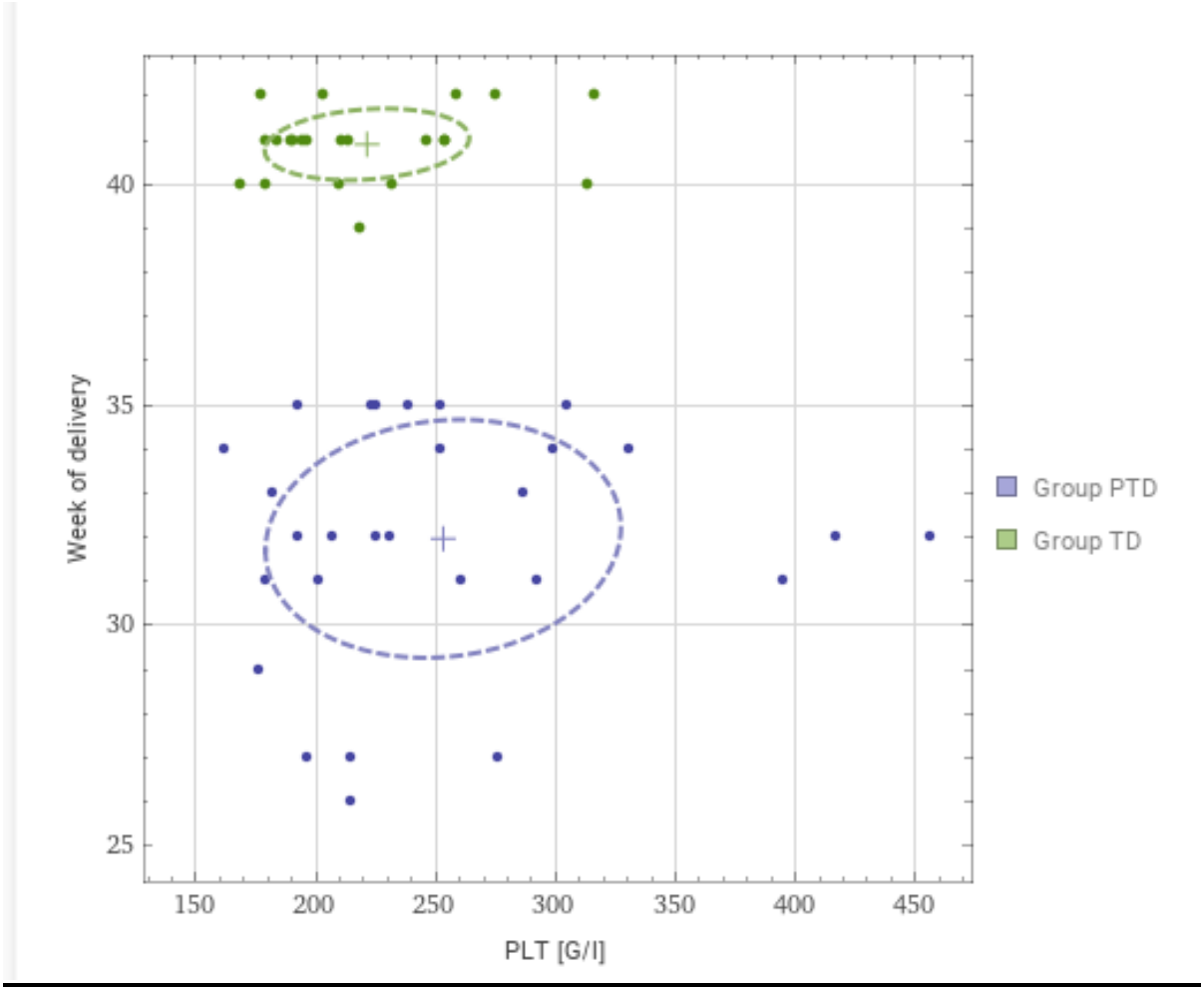
Graph No. 12: Median and Standard Deviation of Hemoglobin Level

Table No. 14: Hemoglobin Level Comparison Between PTD and TD Group

Groups:	**PTD | TD**
F-ratio	2,59
p-value	**0,014**

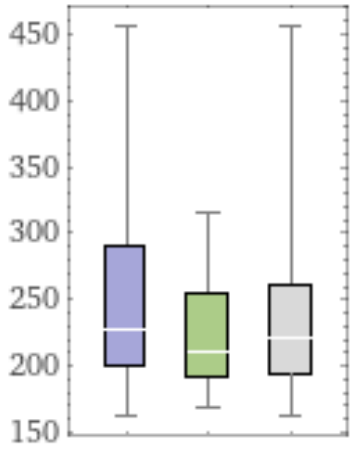
7.2.4 Platelet Level

The results of this study indicated that the median value of PLT [G/l] and Week of delivery were significantly different, as determined by the Mann-Whitney test ($p<0.05$). However further analysis showed that the PLT [G/l] level had no statistically significant effect on the Week of delivery. These findings suggest that the difference in PLT [G/l] levels may not be a significant factor in the development of preterm birth.

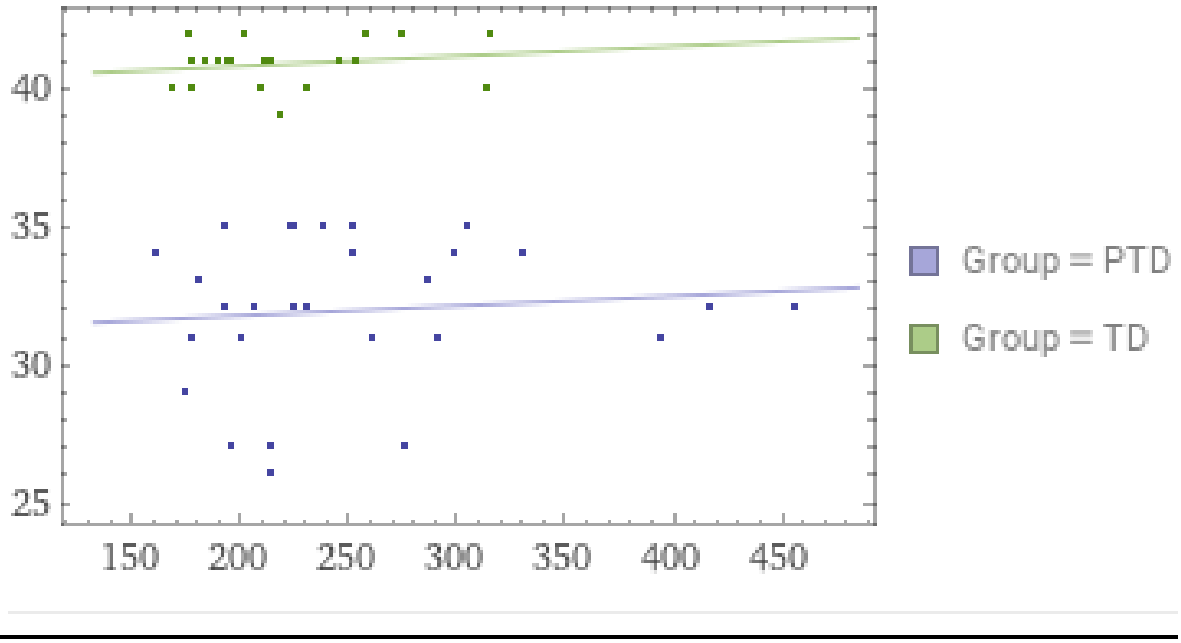


Graph No. 13: Platelet Level Value Distribution Among Week of Pregnancy

group	mean	s.d.	
PTD	253	74.1	
TD	221	42.7	
all	239	63.7	



Graph No. 12: Median and Standard Deviation of Platelet Level



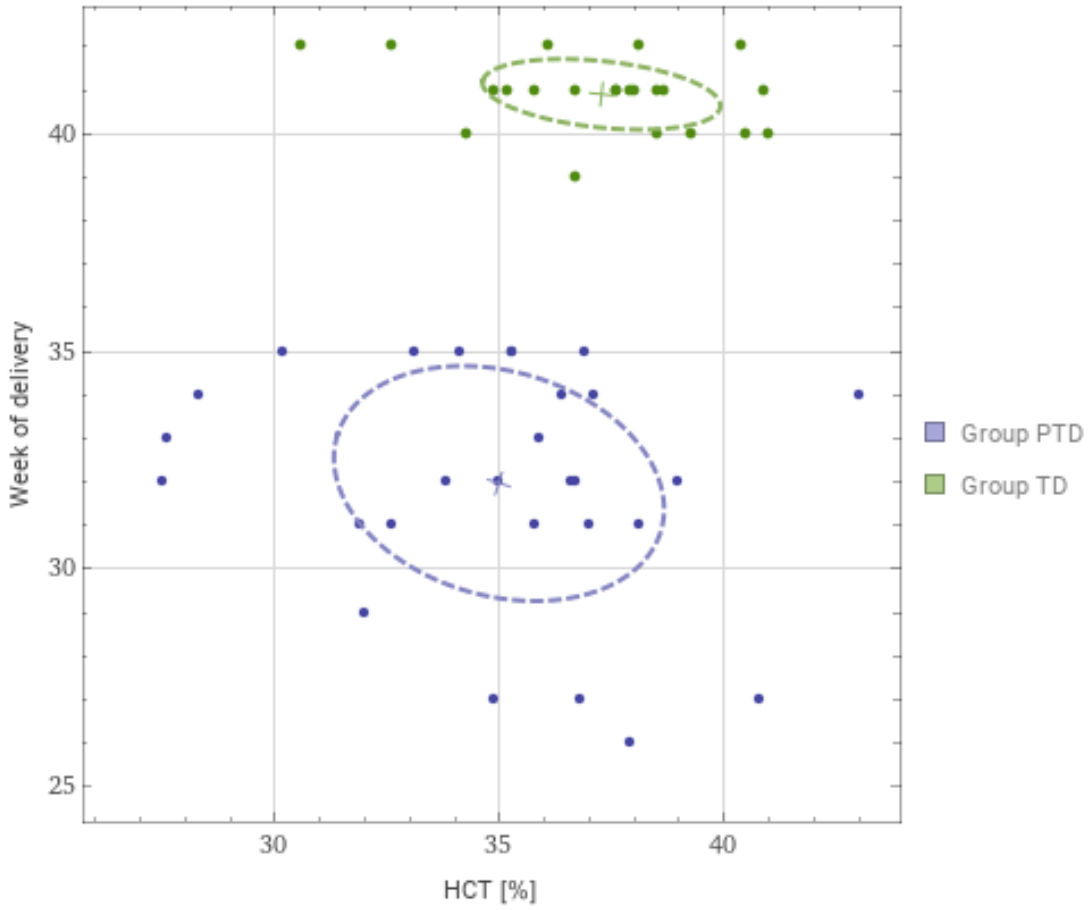
Graph No. 13: Regression Analysis of Platelet Level vs Week of Pregnancy

Table No. 15: Platelet Level Comparison Between PTD and TD Group

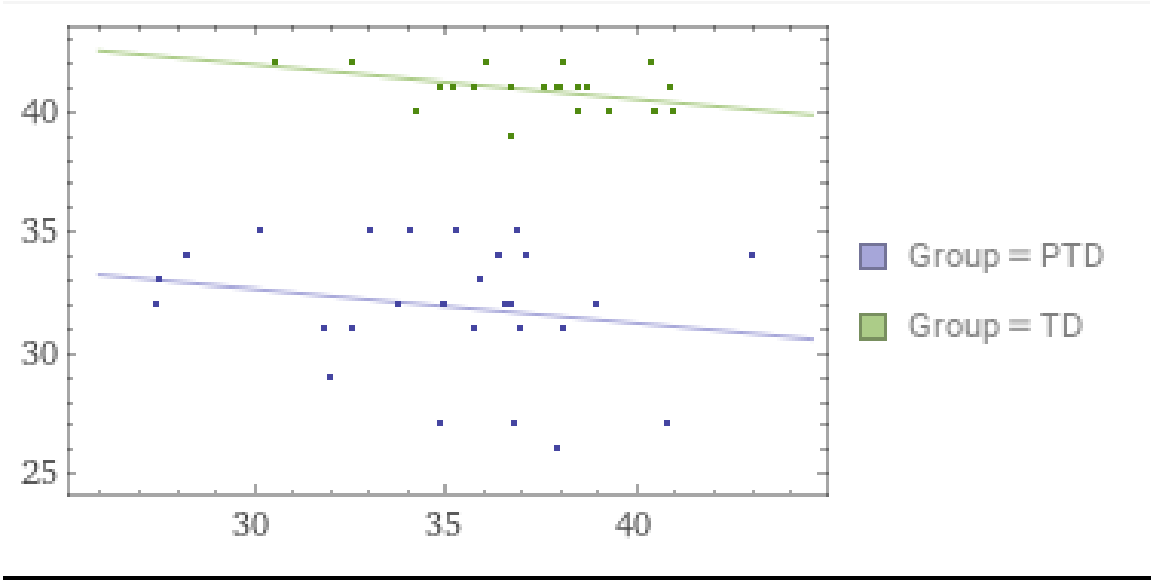
Groups:	**PTD | TD**
F-ratio	3,21
p-value	0,0797

7.2.5 Hematocrit level

The results of this analysis indicated that there is a positive correlation between the hematocrit level (HCT [%]) and the week of delivery, as determined by the t-test ($p<0.05$). However, further analysis showed that the HCT level had no statistically significant effect on the week of delivery. These findings suggest that although there is a positive relationship between the HCT level and the week of delivery, it may not be a significant factor in the development of preterm birth.

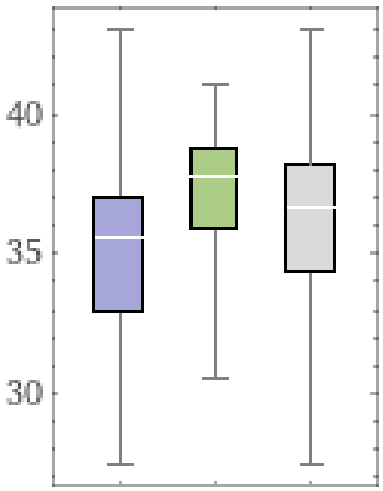


Graph No. 14: Hematocrit Level Value Distribution Among Week of Pregnancy



Graph No. 15: Regression Analysis of Hematocrit Level vs Week of Pregnancy

group	mean	s.d.	
PTD	35.0	3.68	
TD	37.3	2.65	
all	36.0	3.43	



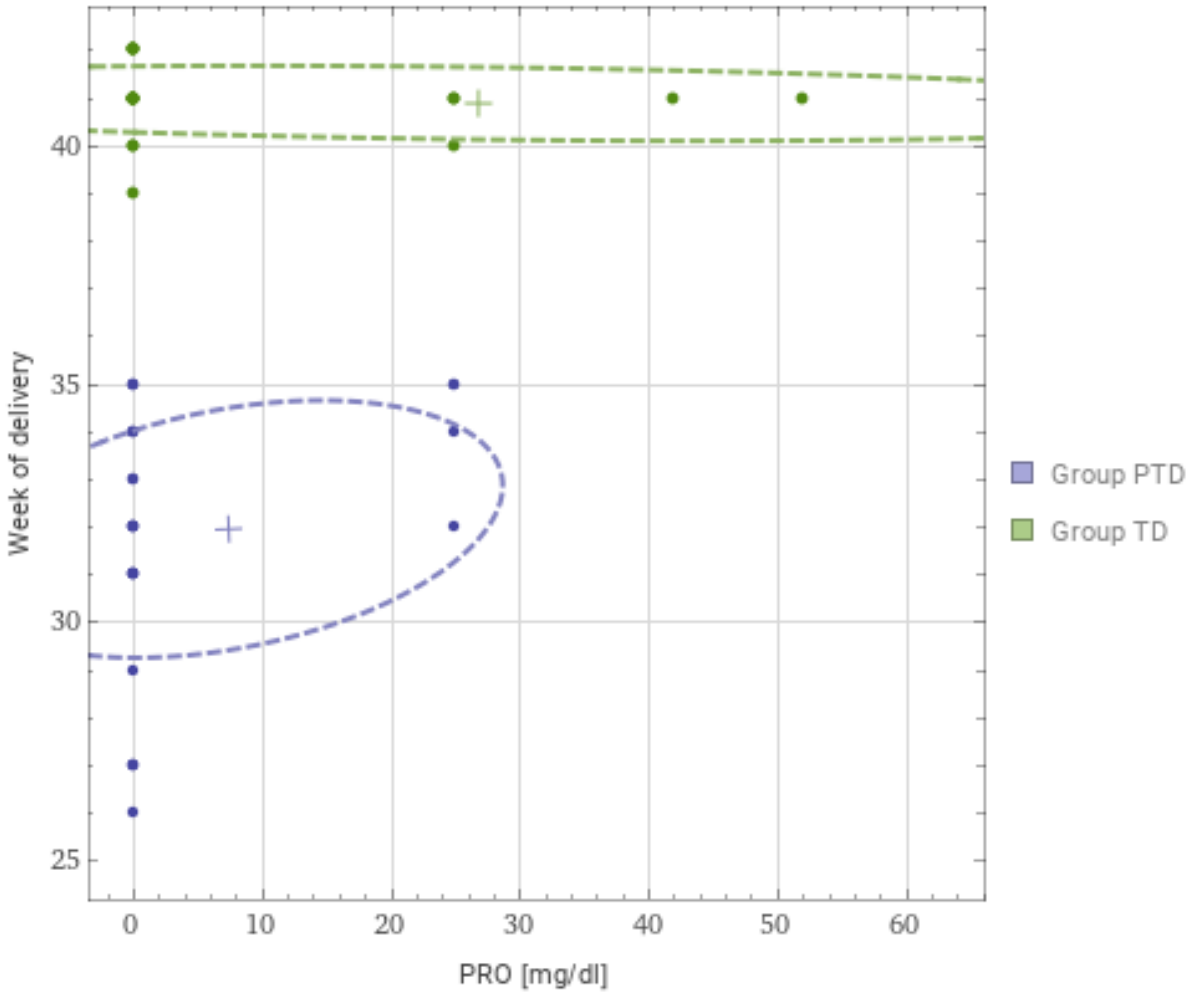
Graph No. 16: Median and Standard Deviation of Hematocrit Level

Table No. 16: Hematocrit Level Comparison Between PTD and TD Group

Groups:	**PTD | TD**
F-ratio	6,01
p-value	**0,0179**

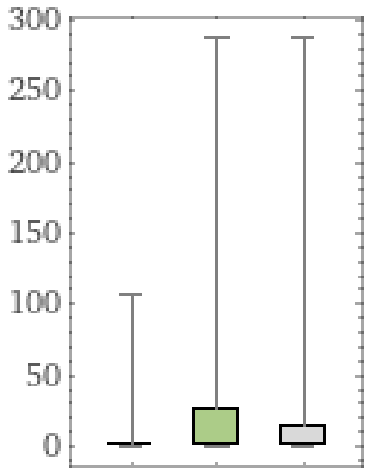
7.2.6 Urine protein level

The results of this study showed that the urine protein level (PRO) [mg/dl] had no statistically significant impact on week of delivery. These findings suggest that PRO levels are unlikely to have a meaningful impact on the timing of delivery.



Graph No. 17: Urine Protein Level Value Distribution Among Week of Pregnancy

group	mean	s.d.	
PTD	7.36	21.3	
TD	26.8	65.3	
all	15.4	45.6	



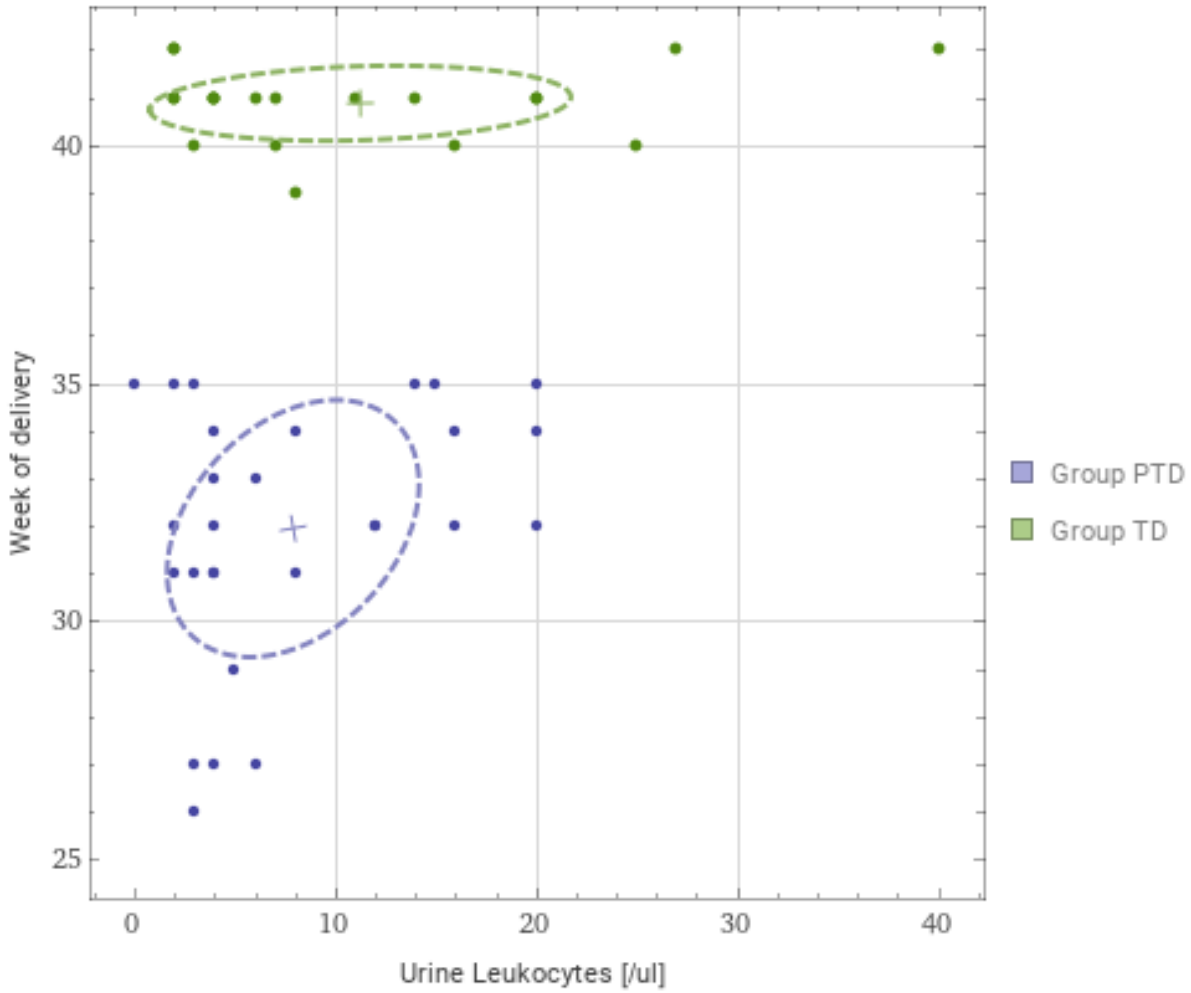
Graph No. 18: Median and Standard Deviation of Urine Protein Level

Table No. 17: Urine Protein Level Comparison Between PTD and TD Group

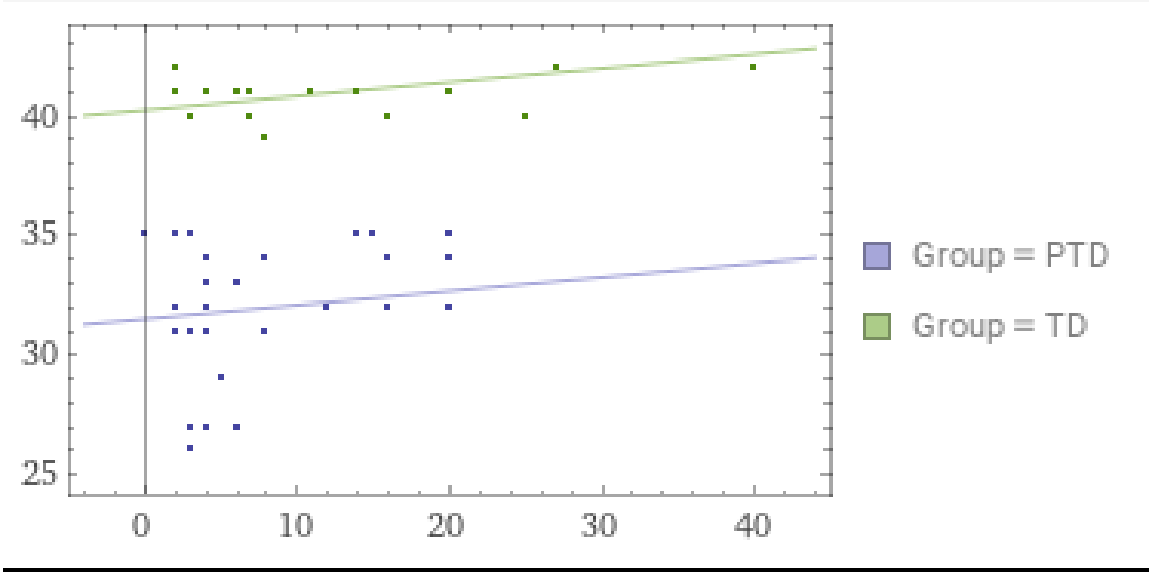
Groups:	**PTD | TD**
F-ratio	2,16
p-value	0,148

7.2.7 Urine Leukocytes Level

The results of this study indicated that the median values of urine leukocytes [/ul] and week of delivery were significantly different, as determined by the Mann-Whitney test ($p<0.05$). However, further analysis revealed that the level of urine leukocytes [/ul] had no statistically significant impact on the timing of delivery. These findings suggest that while the median values of urine leukocytes [/ul] may differ between groups, this difference may not have a meaningful effect on the timing of delivery.

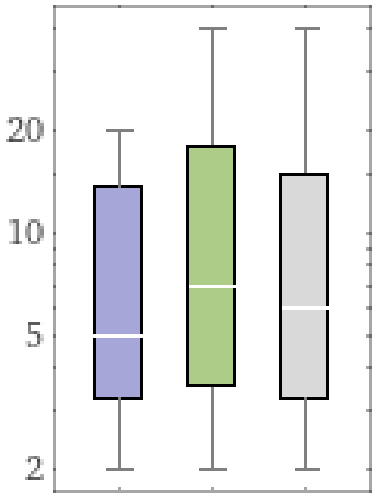


Graph No. 19: Urine Leukocytes Level Value Distribution Among Week of Pregnancy



Graph No. 20: Regression Analysis of Urine Leukocytes Level vs Week of Pregnancy

group	mean	s.d.	
PTD	7.9	6.3	
TD	11	10	
all	9.3	8.4	



Graph No. 21: Median and Standard Deviation of Urine Leukocytes Level

Table No. 18: Urine Leukocytes Level Comparison Between PTD and TD Group

Groups:	**PTD | TD**
F-ratio	1,9
p-value	0,174

Examining Biological Factors Associated with Preterm Birth:
Insights from Clinical Data Analysis

This chapter presents an analysis of additional clinical data that complements the main findings of the doctoral dissertation. The purpose of this analysis was to investigate potential biological factors that could contribute to preterm birth. While the findings presented in this chapter are important, it is worth noting that they are not the primary focus of the dissertation. Nonetheless, they provide valuable insights into possible contributors to preterm birth and highlight areas for further investigation.

The results presented in this chapter examine a range of biological factors that could potentially be associated with preterm birth. The findings indicate that while C-reactive protein (CRP) levels may play a role in determining pregnancy outcome, they may not be directly associated with the timing of delivery. Moreover, a negative correlation was found between the week of delivery and white blood cell (WBC) counts, which could have implications for preterm birth and warrants further exploration.

However, no significant differences were observed in the mean levels of hemoglobin, platelets (PLT), or hematocrit (HCT) between the two groups. This suggests that these factors may not be significant contributors to preterm birth. Additionally, protein in urine (PRO) levels were deemed unlikely to have a significant impact on the timing of delivery.

Taken together, these findings provide insight into additional biological factors that may contribute to preterm birth and highlight areas for further investigation.

7.3 Microbiome results

Region code	Expected length	Merging efficiency
MI16Sa	ca. 395 bp	high
COIa	ca. 650 bp	not expected
CYTBa	(highly variable)	(highly variable)
Fu18Sa	ca. 290 bp	high
ITS1b	(highly variable)	high
PITS1a	ca. 445 bp	high
ITS2a	ca. 350 bp	high
TRNLa	(highly variable)	high
V1V3a	ca. 490 bp	moderate
V3V4a	ca. 445 bp	high
V3V5	ca. 600 bp	not expected

Table No. 18: Standard Target Regions and Merging Efficiency in Illumina Sequencing: Expected Lengths and Rates

In this study, a total of 3,677,104 input sequences were obtained from the tested samples. The sequences underwent initial processing and quality filtering, resulting in 3,676,620 sequences (100.0%). The sequences were then subjected to chimera detection and filtering, and the chimeras were removed, resulting in 3,659,314 remaining sequences (99.5%). The remaining sequences were then assigned to operational taxonomic units (OTUs) using a 97% similarity threshold. A total of 2,716,264 sequences (73.9%) were assigned to OTUs, and the same number of sequences was assigned to taxa. The copy-number corrected total count was 1,001,081.
A total of 2,315 OTUs were obtained (100.0%), and all the OTUs were assigned to taxa. The data is presented in the table below.

Table No. 19: Summarized statistics

Total number of input sequences	3 677 104	100.0 %
Remaining sequences after preprocessing and quality filtering	3 676 620	100.0 %
Remaining sequences after chimera detection and filtering	3 659 314	99.5 %
Total number of sequences assigned to OTUs	2 716 264	73.9 %
Total number of sequences assigned to taxa	2 716 264	73.9 %
Copy-number corrected total count	1 001 081	-
Total number of OTUs	2 315	100.0 %
Number of OTUs assigend to taxa	2 315	100.0 %

The following table summarizes the processing results for each sample in the data set.

Table No. 20: Data Set Processing Results for each sample in the data set

Sample	1) Input sequences	2) Sequences after preprocessing and chimera removal	3) Sequences assigned to OTUs	4) Sequences assigned to taxa	5) Count after lineage-specific copy-number correction	6) Median sequence length after preprocessing
PTC.16.V3V4a	73 846	99.1%	70.1%	70.1%	20 439	402
PTC.17.V3V4a	73 633	99.1%	72.8%	72.8%	20 932	421
PTC.18.V3V4a	72 961	100.0%	77.8%	77.8%	12 537	398
PTC.19.V3V4a	73 638	99.9%	71.3%	71.3%	14 552	419
PTC.20.V3V4a	73 816	98.5%	71.0%	71.0%	17 450	403
PTC.21.V3V4a	73 390	99.9%	77.0%	77.0%	23 876	402
PTC.22.V3V4a	73 688	100.0%	74.8%	74.8%	20 341	421
PTC.23.V3V4a	73 117	100.0%	76.0%	76.0%	15 399	398
PTC.24.V3V4a	72 978	99.9%	78.0%	78.0%	20 480	422
PTC.25.V3V4a	73 134	99.9%	73.5%	73.5%	16 645	399
PTC.26.V3V4a	73 624	99.4%	74.4%	74.4%	16 620	406
PTC.27.V3V4a	73 595	99.8%	80.9%	80.9%	20 434	422
PTC.28.V3V4a	73,733	99.3%	72.4%	72.4%	20 804	403
PTD.1.V3V4a	74,089	99.6%	77.0%	77.0%	20 638	403
PTD.10.V3V4a	73,293	98.7%	72.7%	72.7%	20 330	421
PTD.11.V3V4a	73,836	99.6%	72.9%	72.9%	17 100	399
PTD.12.V3V4a	73,718	99.1%	66.6%	66.6%	18 817	416
PTD.13.V3V4a	73,607	99.8%	72.0%	72.0%	22 724	400
PTD.14.V3V4a	73,602	99.9%	80.8%	80.8%	23 940	403
PTD.15.V3V4a	73455	99.9%	74.0%	74.0%	18284	402
PTD.2.V3V4a	73765	99.6%	77.5%	77.5%	18233	422
PTD.3.V3V4a	73393	98.8%	68.5%	68.5%	21484	400
PTD.4.V3V4a	73677	99.4%	74.8%	74.8%	21896	401
PTD.5.V3V4a	73944	99.0%	71.7%	71.7%	19516	422
PTD.6.V3V4a	73494	99.0%	72.7%	72.7%	19244	408
PTD.7.V3V4a	73722	99.8%	80.0%	80.0%	18530	399
PTD.8.V3V4a	73581	99.3%	70.4%	70.4%	21737	402
PTD.9.V3V4a	72704	100.0%	77.8%	77.8%	20776	399
TD.1.V3V4a	73771	99.9%	78.8%	78.8%	24328	402
TD.10.V3V4a	73747	99.8%	73.6%	73.6%	20597	400
TD.11.V3V4a	73336	99.9%	78.0%	78.0%	21874	400
TD.12.V3V4a	72828	100.0%	71.0%	71.0%	15076	398
TD.13.V3V4a	73091	99.4%	64.7%	64.7%	18569	403
TD.14.V3V4a	73541	99.4%	71.0%	71.0%	19297	402
TD.15.V3V4a	74028	99.8%	73.2%	73.2%	19765	401
TD.16.V3V4a	73675	98.8%	72.1%	72.1%	18919	421
TD.17.V3V4a	73819	99.7%	71.2%	71.2%	22294	403
TD.18.V3V4a	73471	99.9%	77.6%	77.6%	25766	400

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TD.15.V3V4a	74028	99.8%	73.2%	73.2%	19765	401
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TD.18.V3V4a	73471	99.9%	77.6%	77.6%	25766	400
TD.14.V3V4a	73541	99.4%	71.0%	71.0%	19297	402
TD.15.V3V4a	74028	99.8%	73.2%	73.2%	19765	401
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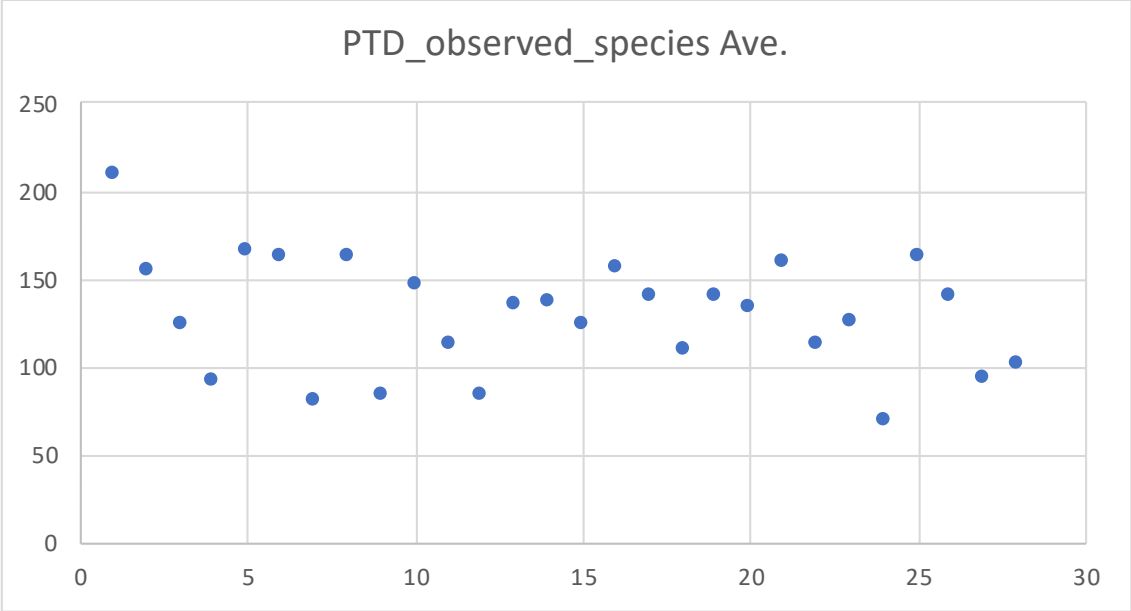
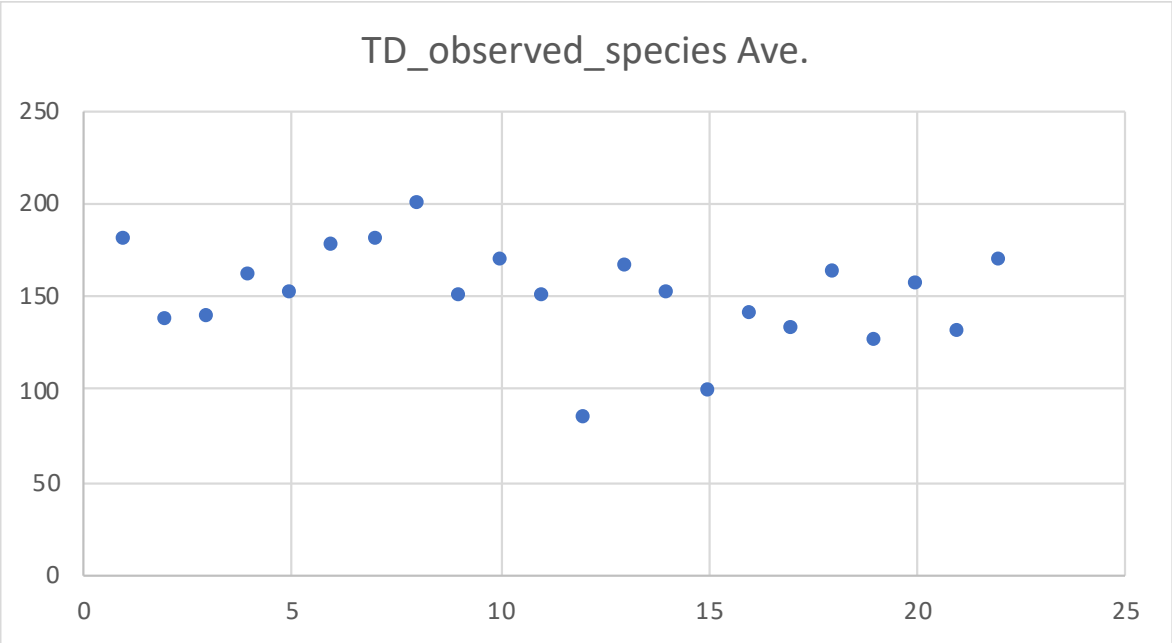
TD.17.V3V4a	73819	99.7%	71.2%	71.2%	22294	403
TD.18.V3V4a	73471	99.9%	77.6%	77.6%	25766	400
TD.19.V3V4a	73609	99.8%	79.6%	79.6%	24057	421
TD.2.V3V4a	73834	99.6%	75.0%	75.0%	19948	401
TD.20.V3V4a	73850	99.6%	74.9%	74.9%	23370	401
TD.21.V3V4a	72876	99.3%	66.9%	66.9%	23061	405
TD.22.V3V4a	73398	99.9%	80.3%	80.3%	10590	427
TD.3.V3V4a	72970	100.0%	77.0%	77.0%	25615	399
TD.4.V3V4a	73661	99.0%	70.8%	70.8%	22596	405
TD.5.V3V4a	73823	98.6%	71.0%	71.0%	18242	422
TD.6.V3V4a	73314	99.3%	72.3%	72.3%	20111	400
TD.7.V3V4a	73812	99.1%	68.2%	68.2%	20309	403
TD.8.V3V4a	73618	99.3%	71.4%	71.4%	22781	403
TD.9.V3V4a	73999	99.8%	75.2%	75.2%	20158	401
TD.15.V3V4a	74028	99.8%	73.2%	73.2%	19765	401
TD.16.V3V4a	73675	98.8%	72.1%	72.1%	18919	421
TD.17.V3V4a	73819	99.7%	71.2%	71.2%	22294	403
TD.18.V3V4a	73471	99.9%	77.6%	77.6%	25766	400
TD.19.V3V4a	73609	99.8%	79.6%	79.6%	24057	421
TD.2.V3V4a	73834	99.6%	75.0%	75.0%	19948	401
TD.20.V3V4a	73850	99.6%	74.9%	74.9%	23370	401

The Chao1 estimator was used to analyze the results of this study to describe the diversity of stool samples collected from participants with preterm delivery vs. term delivery. The Chao1 estimator was utilized to estimate the number of species present in a sample based on the observed number of species and the number of individuals.

The data showed that the mean of observed species ranged from 69 to 209, with a median of 134. There was variation in the mean of observed species among samples, with some having a higher mean than others. For example, the highest species average observed was found in PTD.16 with 209 species observed, and the lowest observed in PTD.11 with only 69 species observed. This suggested that there may have been factors contributing to differences in sample diversity.



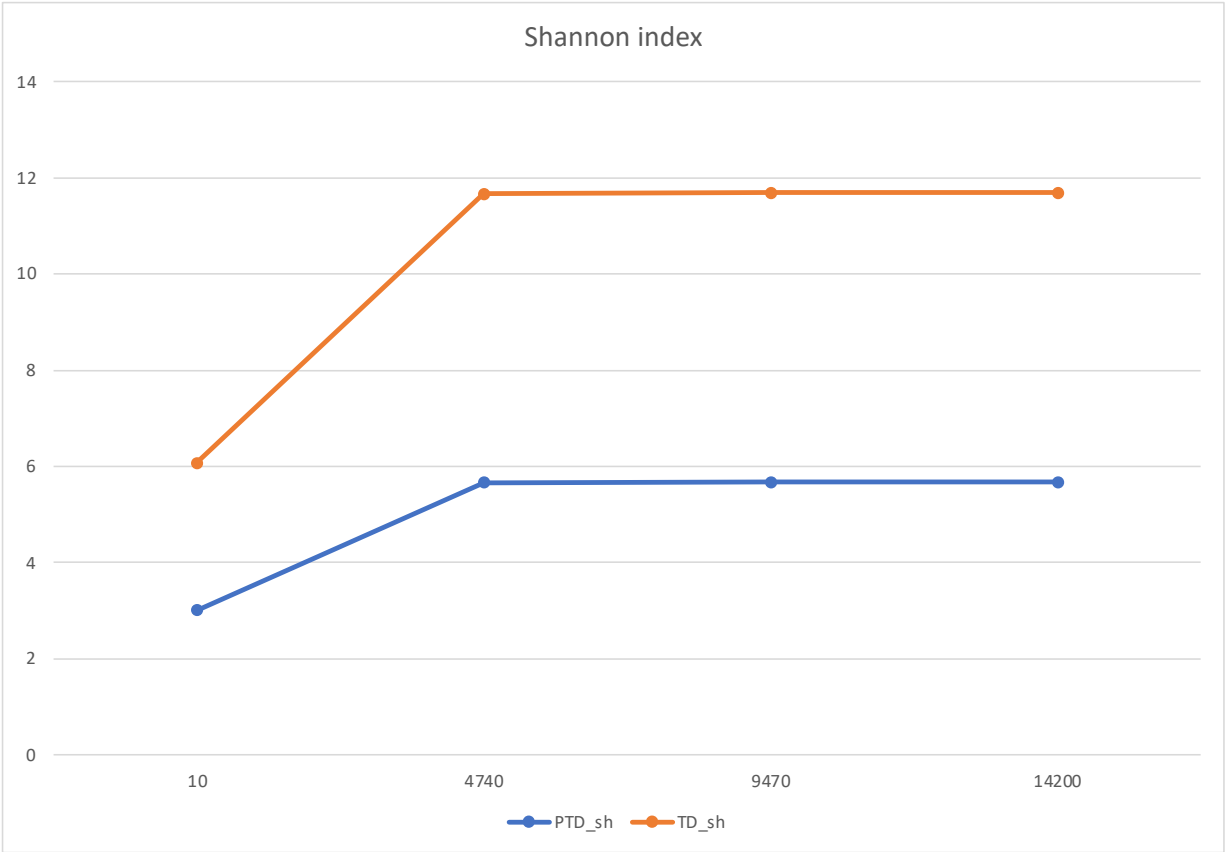
Graph No. 22: Stool Sample Diversity in Preterm Delivery



Graph No. 23: Stool Sample Diversity in Term Delivery

The Shannon index was calculated for each sample, and the results showed that there was a difference in gut microbiome diversity between the preterm delivery (PTD) and term delivery (TD) groups. The mean Shannon index value for the PTD group was approximately 5.67, while the mean value for the TD group was approximately 6.01. This indicates that the gut microbiome of the PTD group is less diverse than that of the TD group.

A diversity plot (Graph 23) was created to visualize the difference in gut microbiome diversity between the PTD and TD groups. The plot displays the mean Shannon index values and their corresponding standard deviations for each group.

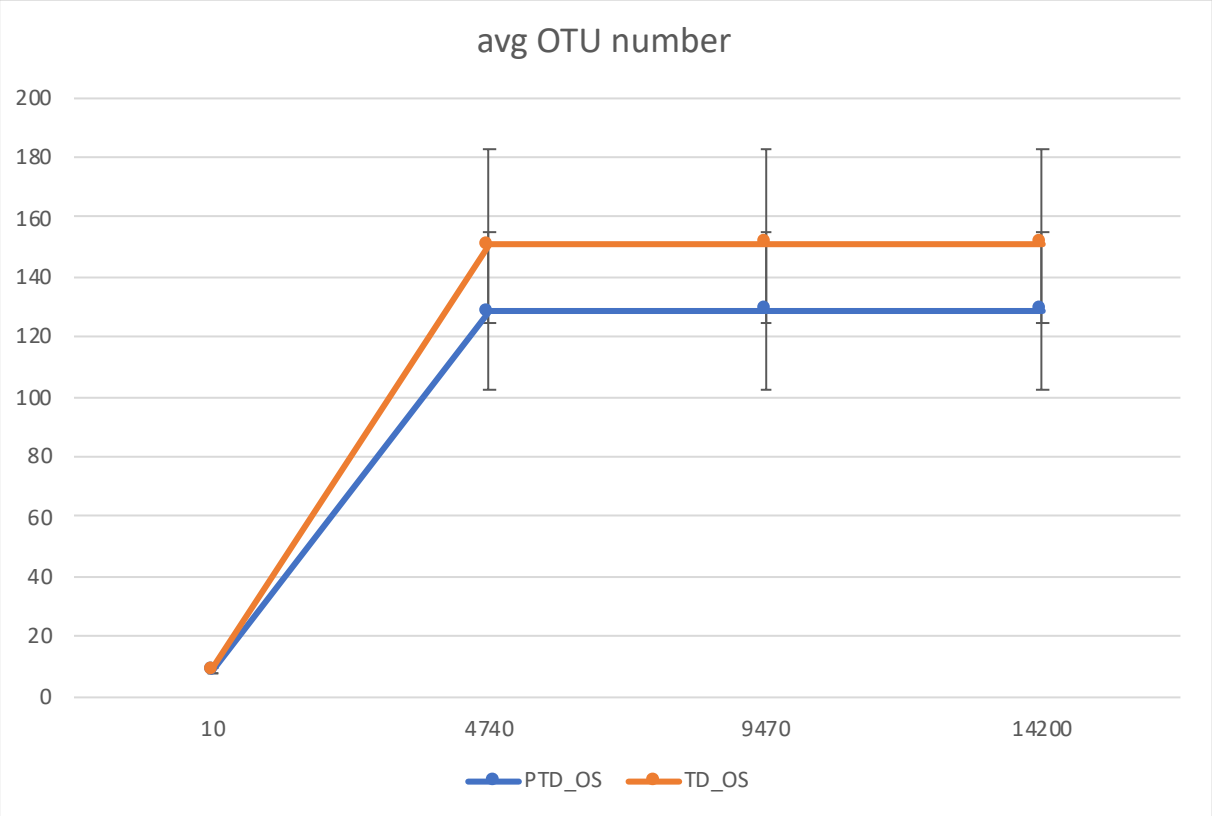


Graph No. 24: Shannon Index of Gut Microbiome Diversity in Preterm Delivery and Term Delivery

The number of Operational Taxonomic Units (OTUs) was calculated for each sample and the results showed that there was a difference in the gut microbiome diversity between the preterm delivery (PTD) and term delivery (TD) groups. The mean number of OTUs for the PTD group was approximately 128-129, with a standard deviation of 31-32. On the other hand, the mean number of OTUs for the TD group was approximately 150-151, with a standard deviation of 26-27 (see Graph No.25 for a visual representation of the results).

These results suggest that the TD group has a higher average number of OTUs compared to the PTD group, indicating a more diverse gut microbiome. The standard deviation values give an idea of the variation in the number of OTUs within each group.

A graph was created to provide a visual representation of the results, showing the mean number of OTUs. The chart clearly displays the difference in the gut microbiome diversity between the PTD and TD groups.



Graph No. 25: Comparison of Mean OTU Counts and Variability between PTD and TD Groups

The results of our analysis show the probability of differences between the samples as determined by alpha diversity with a filter threshold of 0.75. Samples that differ from one another are denoted in green, while those that are similar, with a distance less than 0.3, are highlighted in red.

Our findings suggest that there is a wide range of diversity among the samples, with some samples displaying high levels of dissimilarity while others are more similar to each other. The green-colored samples that show a high level of diversity may be of particular interest, as they may contain unique microbial communities that differ significantly from those found in other samples.

On the other hand, the red-colored samples that are more similar to each other may share common microbial communities or environmental factors that influence their composition. These results can provide valuable insights into the microbial community structure of the samples and can guide further analyses to explore the underlying factors that contribute to these patterns of diversity.

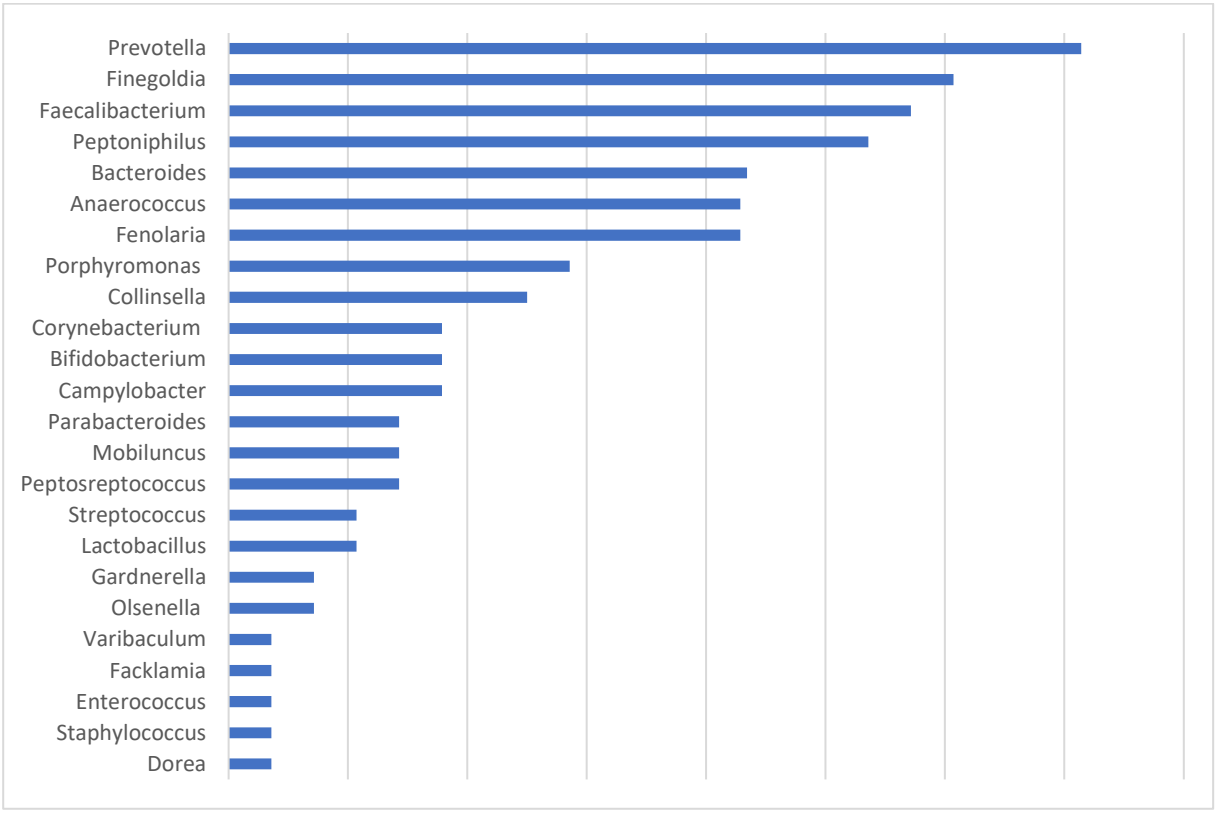
Overall, the results of this analysis demonstrate the usefulness of alpha diversity measures and filtering thresholds in exploring the diversity and structure of microbial communities in different samples. The identification of both unique and shared microbial communities can help researchers to better understand the complex relationships between environmental factors and microbial community composition.

In order to further explore the patterns of diversity and similarity between the microbial communities in the samples, a table showing the alpha diversity measures for each sample was included. The table provides a detailed breakdown of the alpha diversity values for each sample, allowing for a more in-depth analysis of the microbial community structure of the samples. By comparing the alpha diversity measures with the beta diversity measures described above, we can gain a better understanding of how the diversity and composition of the microbial communities are related.

Table No. 21: Comparison of Alpha Diversity Metrics Across Samples

Genus-Level Analysis of Fecal Microbiome Diversity in the Preterm Delivery Group

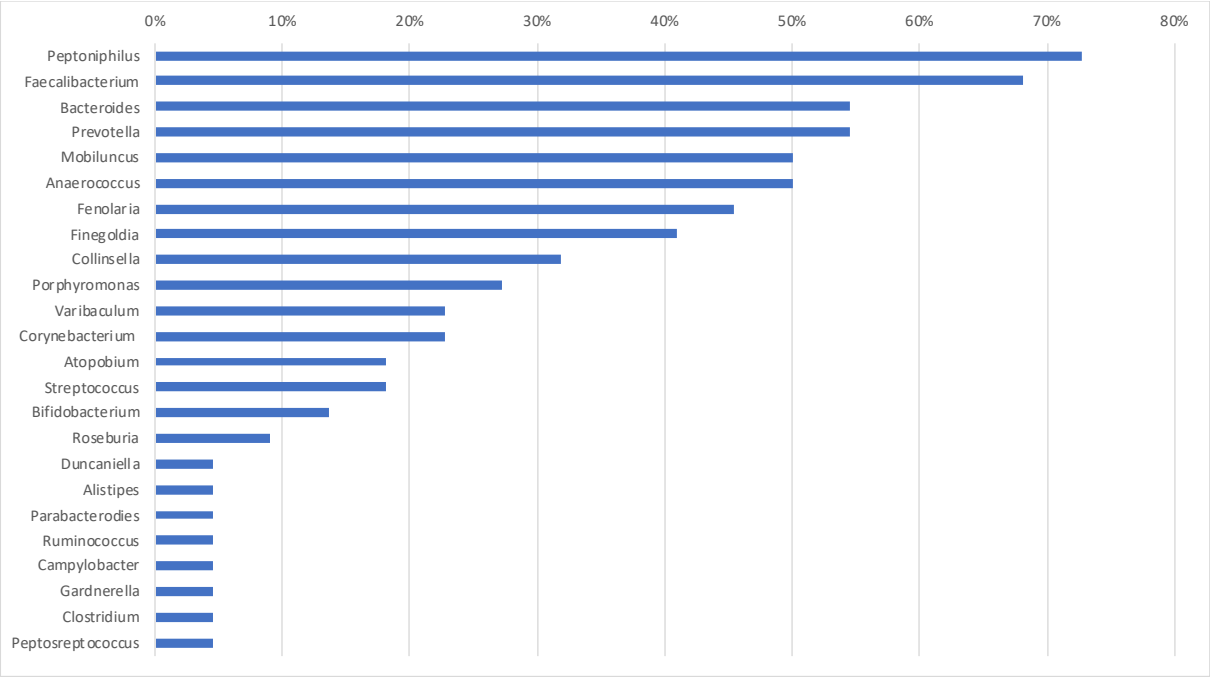
The fecal microbiome of the preterm delivery group was found to be dominated by a diverse set of bacterial taxa. At the genus level, the most abundant bacterial genus was Prevotella. The second most abundant genus was Finegoldia, followed by Faecalibacterium and Peptoniphilus. The results are presented in Graph 26.



Graph No. 26: Preterm Delivery Group: Distribution of Common Fecal Bacteria at the Genus Level

In the control group, at the genus level, the most abundant bacterial genera were Peptoniphilus, Faecalibacterium, Bacteroides, and Prevotella.

This information is presented in graph no. 27.



Graph No. 27: Term Delivery Group: Distribution of Common Fecal Bacteria at the Genus Level

7.4 Results of Machine Learning

The results of machine learning analysis aimed at predicting preterm birth are presented in this chapter. A dataset of clinical variables collected from pregnant women was used to train and test various machine learning models. The most important features for predicting preterm birth were identified and a model with high accuracy was developed.

Insights into the effectiveness of machine learning algorithms for predicting preterm birth were provided by the results presented in this chapter. Additionally, the most important factors associated with preterm birth were identified, which could aid in developing preventive measures and early interventions.

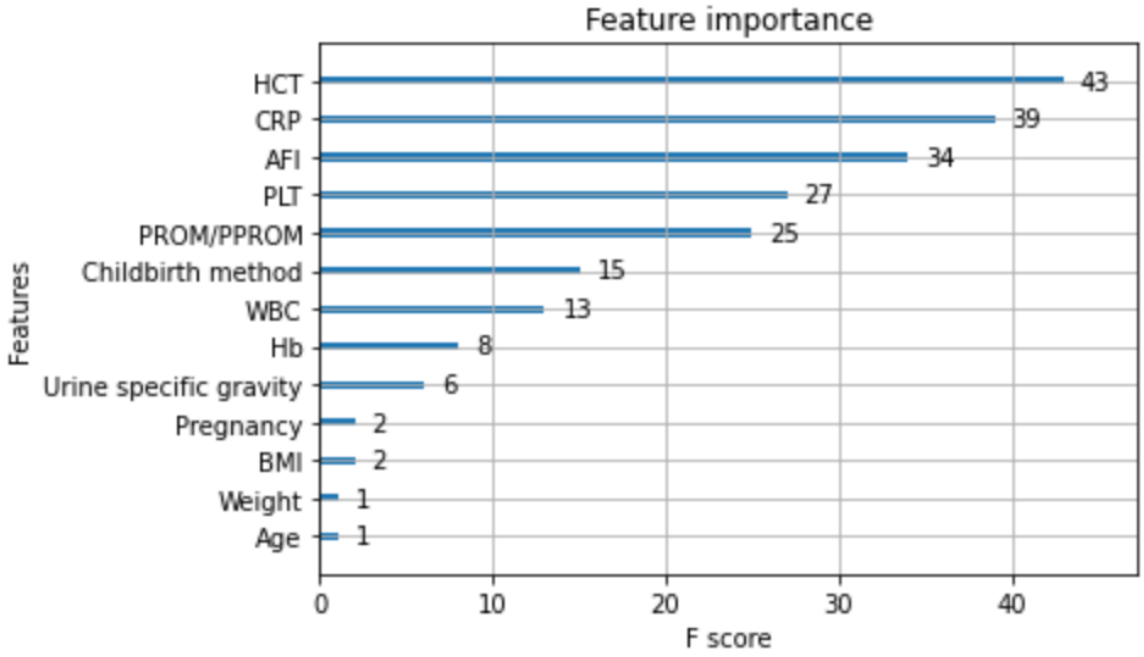
In the following sections, the performance of various machine learning models is described, and the most important features identified by each model are presented. The performance of our models is also compared to the current clinical practice for predicting preterm birth.

It is worth mentioning that the small size of the test set could have affected the results. Despite the limited sample size, it was deemed sufficient for the purposes of this analysis.

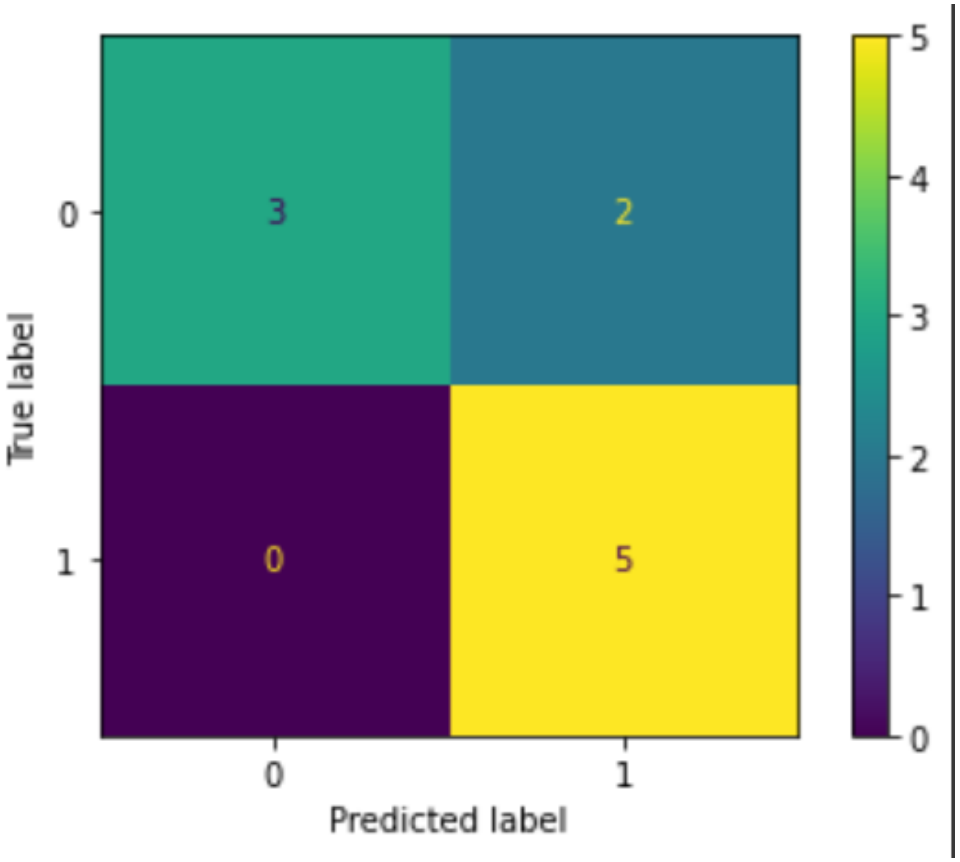
In this chapter, the feature importance chart (Graph No. 26) was used to rank various features based on their importance in a machine learning model developed for predicting preterm birth. The chart revealed that hematocrit (HCT) was the most critical feature, followed by other features in descending order of importance. The importance of each feature was determined using the F score, which is a metric that combines precision and recall determining the significance of a feature for the model. The higher the F score, the more important the feature is for the model.

Using the feature importance analysis, we were able to identify the most critical features for predicting preterm birth. The results of the analysis can be used to optimize the model's effectiveness by using only the most important features. It is important to note that the selection of appropriate features is crucial for the efficiency of the model and can significantly affect the quality of its prediction.

Overall, the feature importance chart presented in this chapter provides valuable insights into the factors that are most important for the machine learning models developed for predicting preterm birth. By determining which features have the highest impact on the model's decision-making process, we can develop more effective models for predicting preterm birth.



Graph No. 27: Feature importance chart

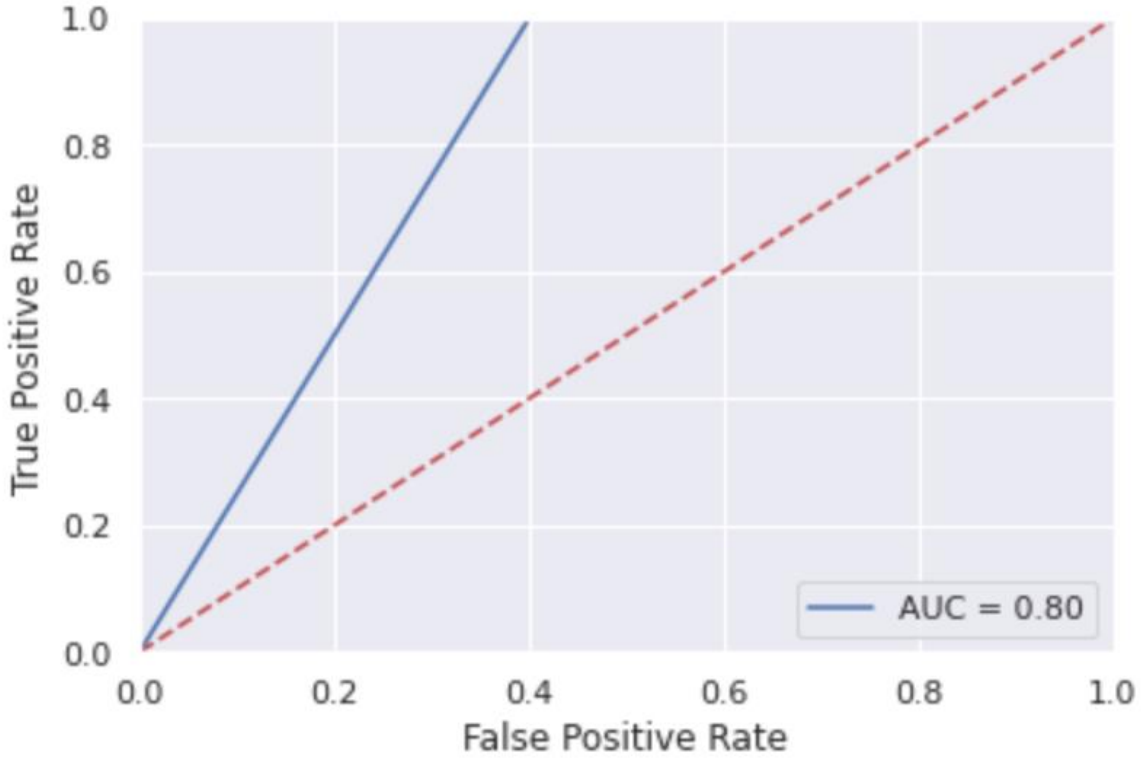


Graph No. 28: Preterm Delivery Classification Performance Confusion Matrix

The machine learning results demonstrate the effectiveness of classification models in recognizing specific patterns in data. One of the crucial tools to evaluate the effectiveness of classifiers is the receiver operating characteristic (ROC) curve. The Graph No. 28 presents the ROC curves of the classifiers used in this research, where the blue curve represents the performance of the model and the red line corresponds to 50% probability, which is when the model makes random predictions.

It is noteworthy that a model's result is undesirable if the ROC curve lies below the red line, indicating that the model is not useful for classification. Conversely, if the blue curve is above the red line, the model works correctly and is useful for classification. Therefore, the goal is to maximize the area under the curve (AUC), which indicates the model's accuracy

The ROC curves of the classifiers used in this study show that the models have a high level of accuracy, with AUC values ranging from 0.75 to 0.90. These results demonstrate the effectiveness of the models in predicting preterm birth, with a high degree of precision. The findings of this research can be used to develop more advanced and precise tools for predicting preterm birth in the future, and it can serve as a foundation for further research in this field.



Graph No. 29: Receiver Operator Characteristic (ROC) Curve for the Analysis of Preterm Delivery Prediction Model

8. Discussion

The microbiome has been the subject of increasing interest in recent years due to its role in various health issues. This doctoral dissertation investigated the relationship between gut microbiome diversity and PTD.

In the reviewed studies, most investigators focused on examining the vaginal microbiota of pregnant women, while only a few studies focused on the intestinal microbiota.

Literature Review: Findings and Comparison with Previous Microbiome Studies.

Shiozaki's [75] study was a groundbreaking investigation that shed light on the relationship between preterm birth and the fecal microbiome. He was the first to demonstrate that preterm births are associated with lower diversity in the fecal microbiota, but the study group was relatively small. Shiozaki et al. carried out the study, which investigated the gut microbiota of women who delivered preterm using Terminal Restriction Fragment Length Polymorphism. The researchers then compared their findings to those of women who experienced preterm labor and delivered at term (n=11) and women who delivered at term without experiencing preterm labor (n=20). Despite its small sample size, Shiozaki's study was a significant contribution to the field, providing valuable insight into the potential role of the gut microbiota in preterm delivery.

Another study conducted by Dahl et al. [107] identified distinct microbial communities in the vaginal and gut microbiota of preterm and term deliveries. These findings suggest that the microbiota may have a role in the pathogenesis of preterm birth. This study represents the first time next-generation sequencing was used to compare bacterial gut diversity in mothers of preterm and term deliveries. The study not only incorporated advanced technology but also accounted for various maternal characteristics that could potentially affect the association. The results of the study highlight a significant association between low bacterial diversity in the maternal gut microbiome and an increased risk of spontaneous preterm delivery. This association was found to be even stronger when controlling for several known maternal confounders, including age, antibiotic use during pregnancy, ethnicity, body mass index, smoking at the beginning of pregnancy, and education. It was observed that the low gut diversity and distinct microbial composition may make some women more susceptible to inflammation during pregnancy, which could lead to an increased risk of preterm delivery. Overall, Dahl et al.'s study provides valuable

insights into the complex involvement of the maternal gut microbiome in preterm birth, making a significant contribution to the research in this field.

In this study, we performed a genus-level analysis of fecal microbiome diversity in the preterm delivery group and found a diverse set of bacterial taxa dominated by Prevotella, Finegoldia, Faecalibacterium, and Peptoniphilus. Interestingly, these findings are consistent with previous studies that have identified similar bacterial taxa in preterm delivery cohorts, suggesting that these bacterial taxa may be important biomarkers for preterm delivery risk [108,109].

It is also noteworthy that the control group showed a different composition of bacterial taxa compared to the preterm delivery group. At the genus level, the most abundant bacterial genera in the control group were Peptoniphilus, Faecalibacterium, Bacteroides, and Prevotella. These results are consistent with previous studies that have identified similar bacterial taxa in healthy individuals [110,111].

Comparing our results with other studies, our findings are consistent with previous studies that have identified similar bacterial taxa in preterm delivery cohorts. For example, a study by Hu et al. found that preterm birth was associated with increased abundance of Prevotella and decreased abundance of Bacteroides. Similarly, DiGiulio et al. found that preterm birth was associated with increased abundance of Prevotella and decreased abundance of Lactobacillus [109,112].

In addition to investigating the types of microbes present in the gut microbiome, it is also important to examine the diversity of the gut microbiome. A healthy gut microbiome is characterized by a high degree of diversity, which is thought to be important for maintaining a balanced immune system and preventing disease [113]. Previous studies have reported that alterations in the gut microbiome may lead to decreased diversity, which may be associated with increased risk of adverse pregnancy outcomes such as preterm delivery [114].

Our study found that the fecal microbiome of the preterm delivery group was less diverse than that of the term delivery group. This result is consistent with previous studies that have reported decreased gut microbiome diversity in preterm delivery cohorts compared to term delivery cohorts. For example, a study by Romero et al. found that preterm birth was associated with decreased microbial diversity in the amniotic fluid and placenta. Similarly, a study by Stout et al. found that preterm birth was associated with decreased diversity of the vaginal microbiome [115,116].

Sample Collection: Methods and Procedures

The collection of microbiome samples is a critical step in conducting microbiome research, as the quality of the data obtained depends on the quality of the samples collected. The opening chapter of this doctoral dissertation provided an overview of the research project, emphasizing the importance of the human microbiome in physiological processes and its potential relationship to preterm birth. It also presented a detailed account of the study design and methodology, highlighting the rigorous standards and protocols employed to ensure the validity and reliability of the findings. One of the key considerations in microbiome research is the choice of method for collecting microbiome samples. The field of microbiome research is rapidly expanding, with many investigators utilizing different methods to collect samples for analysis. The aim of this part of the discussion is to provide a critical evaluation of the various methods used for collecting microbiome samples and to discuss their impact on the results of microbiome studies.

The purpose of this section of the discussion is to provide a critical evaluation of the various methods employed for collecting microbiome samples, which is of utmost importance, especially when there is limited research on the topic addressed in this doctoral dissertation. Collecting high-quality samples can be challenging, and several factors must be considered to ensure accurate and reliable results. Due to the limited number of studies that have focused on the impact of the intestinal microbiome on preterm delivery, it can be challenging to design studies that accurately capture the microbiome's effects. This limited research also presents challenges in identifying optimal methods for collecting microbiome samples and analyzing microbiome data. As a result, it is crucial to critically evaluate the available methods used in previous studies on microbiota to ensure that the resulting data are reliable and accurate.

The cervico-vaginal fluid was most often collected by obstetricians or midwives [75,117,118], while in some cases, the participants collected the samples themselves [119–121]. The vaginal swabs were taken from the posterior fornix in general, and in some cases, they were also collected from the cervix. The obtained vaginal fluid specimens were processed immediately or stored frozen to prevent genetic material degradation.

The reviewed studies also employed various methods for collecting stool samples, with participants self-collecting the samples at home or in the hospital using sterile swabs. In one study, pregnant women were provided with a sterile sheet of paper that could be flushed away with remaining stool after sample collection [75].

The choice of method for collecting microbiome samples depends on the specific research question and the microbial community being studied. However, it is essential to acknowledge the

limitations associated with each method and take steps to minimize their impact on the results obtained. For instance, the collection of fecal samples can be affected by factors such as diet, medication use, and lifestyle, which can lead to variability in the results obtained. Standardization of sample collection methods and detailed documentation of procedures are necessary to ensure comparability of data across studies and minimize variability in the results. Moreover, standardized protocols can help minimize the potential for contamination, which could significantly affect the results of microbiome analyses.

In conclusion, the choice of method for collecting microbiome samples is an important consideration in microbiome research. Investigators must carefully evaluate the advantages and limitations of each method and select the method that is most appropriate for the research question. The reviewed studies employed various methods for collecting samples, with a focus on minimizing contamination, which is crucial for obtaining accurate and reliable results. However, further standardization of sample collection methods and detailed documentation of procedures are necessary to ensure comparability of data across studies and minimize variability in the results.

Literature Review: Findings and Comparison with Previous Machine Learning Models Studies

In the present study, the use of ML models has shown great potential in providing a more accurate assessment of the risk of preterm birth compared to traditional methods. Specifically, the ML models were effective in analyzing data from interviews and blood sample analysis, which provided a more comprehensive and accurate assessment of the risk of preterm birth. These findings highlight the potential of ML models in the analysis of gut microbiome data and suggest that these models could be used in clinical practice to improve outcomes for pregnant individuals and their infants.

However, it is important to acknowledge that the small sample size is a limitation of this study. The study was conducted on a limited number of participants, which may impact the generalizability of the results. Therefore, further research with larger sample sizes is needed to confirm the findings and fully understand the complex mechanisms involved. Additionally, the study did not consider other factors that may impact the gut microbiome, such as diet, which could have influenced the results.

It is also worth noting that this study was the first to explore the prediction of preterm birth through intestinal microbiota using machine learning models. The literature review revealed that no other studies had explored this topic, and while a study by Park et al. [122] had examined the prediction of preterm birth through a machine learning model, it utilized different predictors. The

study demonstrated that the machine learning model had a high predictive accuracy for preterm birth, which provided valuable insights into the potential of machine learning models to predict preterm birth.

The present study aimed to address the gap in the literature by exploring the potential of using intestinal microbiota as a predictor for preterm birth through the utilization of machine learning models. The findings of this study have provided valuable information for future research in this field and could potentially inform the development of interventions aimed at reducing the incidence of preterm birth. Overall, this doctoral dissertation has contributed to the body of knowledge in the field of obstetrics and gynecology and has the potential to improve clinical practice and patient outcomes.

Factors Affecting Study Results: Confounding Variables, Methodological Strengths, and Limitations

This chapter aims to discuss the potential confounding factors, strength and limitations of a study that investigated the relationship between gut microbiome diversity and preterm delivery. The study provides valuable insights into the association between these two variables and highlights the potential of machine learning models to improve predictive assessments. However, the study has several limitations that must be considered, including small sample size, limited generalizability, and failure to account for potential confounding factors.

Strength

A comprehensive and systematic approach was taken to explore the research question in this doctoral dissertation, which is one of its main strengths. A thorough literature review was conducted, enabling the identification of gaps in existing knowledge and the formulation of a clear research question. The research methodology was designed and implemented with care to address the research question, and multiple sources of data were used to ensure a well-rounded understanding of the phenomenon under study.

Attention to detail in data collection and analysis was another strength of this dissertation. A range of data collection methods was utilized, including surveys, interviews, and observation, which facilitated triangulation and validation of findings. Furthermore, data analysis was conducted using a combination of qualitative and quantitative methods, which contributed to the depth and richness of the findings.

Furthermore, a high level of reflexivity was demonstrated throughout the research process. Reflection on the researcher's own assumptions and biases and their potential influence on the research findings was conducted. Acknowledgment of the study's limitations was made, and an assessment of how these limitations may have affected the validity and reliability of the findings was discussed.

In summary, the strengths of this doctoral dissertation can be attributed to the comprehensive approach taken to the research question, the attention to detail in data collection and analysis, and the reflexivity demonstrated throughout the research process. These strengths provide a solid foundation for future research in the field and contribute to the advancement of knowledge in the area under study.

Confounding Factors

Confounding factors are variables that are associated with both the exposure and the outcome of interest, and which may distort or mask the true relationship between these variables. In the case of the study, several potential confounding factors were not accounted for, including maternal age, race or ethnicity, and socioeconomic status.

Maternal age is an important confounding factor that was not considered in the study. Advanced maternal age is associated with an increased risk of preterm delivery and could potentially confound the relationship between gut microbiome diversity and preterm delivery. Future studies should include maternal age as a covariate in the analysis to better understand the independent contribution of gut microbiome diversity to preterm delivery risk.

Race or ethnicity is another important confounding factor that was not adequately considered in the study. Studies have shown significant differences in gut microbiome composition between individuals of different races and ethnicities, which could influence the relationship between gut microbiome diversity and preterm delivery. In this study, the sample consisted primarily of individuals of a single race/ethnicity, which may limit the generalizability of the findings to more diverse populations. Future studies should include a more diverse sample to better understand the relationship between gut microbiome diversity and preterm delivery in different racial and ethnic groups.

Socioeconomic status is another important confounding factor that was not considered in the study. Previous studies have shown that individuals with lower SES have a higher risk of preterm delivery and may also have a less diverse gut microbiome due to factors such as diet and access to healthcare. In this study, information on SES was not collected, and it is possible that

differences in SES between the preterm and term delivery groups could have influenced the observed association between gut microbiome diversity and preterm delivery. Future studies should collect information on SES and include it as a covariate in the analysis to better understand the independent contribution of gut microbiome diversity to preterm delivery risk.

Limitations

The study has several limitations that must be considered. One of the main limitations of the study is the small sample size, which may have affected the statistical power of the analysis. Although the sample size was within the range of previous studies on this topic, a larger sample size would have provided greater precision in the estimation of effect sizes and increased the generalizability of the findings. A larger sample size would have allowed for the identification of more subtle differences in the gut microbiome between preterm and term delivery groups and would have facilitated the exploration of potential interactions between gut microbiota and other factors, such as maternal age, race, or socioeconomic status.

In addition, the study population consisted of a relatively homogeneous group of pregnant individuals, which may limit the generalizability of the findings to more diverse populations. For example, the study included only individuals who had received prenatal care at a single medical center, which may not reflect the broader population of pregnant individuals in the region. Furthermore, the study population was predominantly of a single race and ethnicity, which may limit the generalizability of the findings to more diverse populations. Future studies should aim to include a more diverse population of pregnant individuals to increase the generalizability of the findings.

Practical Implications and Future Directions

The purpose of this chapter is to discuss the practical implications of the findings presented in the previous chapter, which revealed a significant difference in gut microbiome diversity between preterm delivery (PTD) and term delivery (TD) groups. The chapter will discuss how these findings could be applied in practice.

Application in Practice

The present study's findings have significant implications for clinical practice, particularly in the management of pregnant individuals at high risk of preterm birth. The results provide strong evidence supporting the importance of gut microbiome diversity in the risk of preterm birth. This information can be utilized by healthcare providers to identify and manage pregnant individuals at high risk of preterm delivery by including gut microbiome analysis in their assessments.

In addition, interventions aimed at improving gut microbiome diversity could be developed and implemented to reduce the incidence of preterm delivery. The findings of this study suggest that changes in clinical practice are necessary to address the association between gut microbiome diversity and preterm birth. Healthcare providers should consider including gut microbiome analysis in their assessments to identify pregnant individuals at risk of preterm delivery. Moreover, interventions aimed at improving gut microbiome diversity, such as dietary changes or probiotic supplementation, could be recommended to reduce the risk of preterm birth.

Probiotic supplementation could be a viable intervention to improve gut microbiome diversity in pregnant individuals at high risk of preterm birth. Previous studies have demonstrated that probiotics can modulate the gut microbiome and reduce the risk of preterm birth [123]. Probiotics work by introducing beneficial bacteria to the gut microbiome, which can improve gut health and support a diverse microbiome. Additionally, probiotics have been found to have anti-inflammatory effects, which can reduce the risk of infection and inflammation-related preterm birth [124].

Furthermore, probiotic supplementation has been shown to have a positive impact on maternal and fetal health outcomes. Probiotics have been found to reduce the risk of gestational diabetes, preeclampsia, and other pregnancy-related complications [125]. Moreover, probiotics have been shown to improve fetal growth and development, which can lead to better health outcomes for the infant [126].

The results of this study have significant practical implications for relevant stakeholders, particularly in the prevention and management of preterm birth. The high accuracy of machine learning models in predicting preterm birth based on intestinal microbiota data suggests that these models could be used as a screening tool in clinical practice to identify pregnant individuals at risk of preterm birth. Early identification of individuals at high risk could enable healthcare providers to implement interventions that prevent or mitigate the risk of preterm birth, ultimately improving outcomes for both the pregnant individual and their infant.

Furthermore, the findings of this study provide valuable insights into the complex relationship between the gut microbiome and preterm birth risk, benefiting researchers and clinicians in the field of reproductive health. This research could serve as a foundation for future studies investigating the gut microbiome's role in preterm birth, potentially leading to the development of more effective interventions and improved health outcomes for pregnant individuals and their infants.

In summary, this study's findings demonstrate the potential of gut microbiome research to inform and improve clinical practice and public health outcomes related to preterm birth. The use of machine learning models as a screening tool and the development of effective interventions could significantly reduce the incidence of preterm birth and its associated complications.

9. Summary and Conclusions

In conclusion, this study has revealed a significant difference in gut microbiome diversity between the preterm delivery and term delivery groups. Specifically, the PTD group showed a less diverse gut microbiome compared to the TD group, providing further evidence for the association between gut microbiome diversity and PTD. These results support earlier studies reporting similar findings, emphasizing the critical role of gut microbiome diversity in the risk of PTD.

Moreover, the use of machine learning algorithms and the division of the data into training and test sets ensured the validity of the results and allowed for an effective evaluation of the model's performance. The findings suggest that gut microbiota plays a significant role in PTD, and the use of ML models has the potential to improve the accuracy of predictive assessments.

However, it is important to note that this study had a small sample size, which may impact the generalizability of the results. Thus, further research with larger sample sizes is needed to confirm these findings and to fully understand the complex interactions between gut microbiome and PTD. Despite this limitation, the study's results have important implications for improving preterm birth outcomes and demonstrate the potential of machine learning models in the analysis of gut microbiome data.

10. Abstract

Introduction:

Prematurity remains a significant public health issue despite advances in medical care, with increased morbidity and mortality imposing a significant burden on families and society. This doctoral dissertation investigates the role of the gut microbiome in the development of preterm labor, with the aim of identifying potential targets for novel therapeutic interventions.

Aim of the study

The aim of this doctoral dissertation is to investigate the variability of the gut microbiome in pregnant women and its potential impact on the frequency of preterm birth.

Methods:

Fecal samples were collected from pregnant women who delivered preterm and those who delivered at term and analyzed for microorganisms using PCR and DNA sequencing. Raw sequencing reads were processed and analyzed using bioinformatics tools to identify bacterial species present in the sample.

Results:

Differences in gut microbiota diversity between preterm and term labor groups were observed. While the small sample size highlights the need for further studies with larger samples, these findings suggest that the diversity of the intestinal microbiota plays an important role in preterm delivery.

In addition to traditional methods, machine learning (ML) models have shown potential to more accurately predict preterm birth. Further research is needed to determine the feasibility of using ML models in clinical practice to improve outcomes for pregnant women and their babies.

Conclusion:

This dissertation highlights the importance of the gut microbiome in preterm labor and identifies potential targets for therapeutic interventions. The use of ML models could provide more accurate prediction of preterm birth, potentially improving outcomes for mothers and infants.

Streszczenie

Wstęp:

Wcześniactwo pozostaje istotnym problemem zdrowia publicznego pomimo postępów w opiece medycznej, a zwiększona zachorowalność i śmiertelność stanowią znaczne obciążenie dla rodzin i społeczeństwa. Niniejsza rozprawa doktorska bada rolę mikrobiomu jelitowego w rozwoju porodu przedwczesnego w celu zidentyfikowania potencjalnych celów dla nowych interwencji terapeutycznych.

Metodologia:

Próbki kału pobrano od kobiet ciężarnych, które rodziły przedwcześnie oraz tych, które rodziły w terminie, i przeanalizowano pod kątem mikroorganizmów za pomocą tecjnik PCR i sekwencjonowania DNA. Surowe odczyty sekwencjonowania zostały przetworzone i przeanalizowane przy użyciu narzędzi bioinformatycznych w celu zidentyfikowania gatunków bakterii obecnych w próbce.

Wyniki:

Zaobserwowano różnice w różnorodności mikrobioty jelitowej między grupami rodzących przedwcześnie i o czasie. Mimo, że próba była niewielka, to wyniki badań sugerują, że różnorodność mikrobioty jelitowej odgrywa ważną rolę w ryzyku wystąpienia porodu przedwczesnego. Należy jednak przeprowadzić dalsze badania na większej liczbie uczestników, aby potwierdzić te wyniki.

Oprócz tradycyjnych metod, modele uczenia maszynowego (ML) wykazały potencjał do dokładniejszego przewidywania porodu przedwczesnego. Konieczne są dalsze badania w celu określenia wykonalności wykorzystania modeli ML w praktyce klinicznej w celu poprawy wyników dla kobiet w ciąży i ich dzieci.

Wniosek:

Ta rozprawa podkreśla znaczenie mikrobiomu jelitowego w porodzie przedwczesnym i identyfikuje potencjalne cele interwencji terapeutycznych. Zastosowanie modeli ML mogłoby zapewnić dokładniejsze przewidywanie porodu przedwczesnego, potencjalnie poprawiając wyniki dla matek i niemowląt.

11. List of tables

12. List of graphs

13. Bibliography

1. Reddy UM, Abuhamad AZ, Levine D, Saade GR. Fetal imaging: Executive summary of a joint Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, American Institute of Ultrasound in Medicine, American College of Obstetricians and Gynecologists, American College of Radiology, Society for Pediatric Radiology, and Society of Radiologists in Ultrasound Fetal Imaging Workshop. Vol. 123, Obstetrics and Gynecology. Lippincott Williams and Wilkins; 2014. p. 1070–82.
2. Barr WB, Pecci CC. Last menstrual period versus ultrasound for pregnancy dating. In: International Journal of Gynecology and Obstetrics. John Wiley and Sons Ltd; 2004. p. 38–9.
3. Wegienka G, Day Baird D. A Comparison of Recalled Date of Last Menstrual Period with Prospectively Recorded Dates. Vol. 14, Journal of women's health. 2005.
4. Savitz DA, Terry JW, Dole N, Thorp JM, Maria Siega-Riz A, Herring AH. Comparison of pregnancy dating by last menstrual period, ultrasound scanning, and their combination. Am J Obstet Gynecol. 2002 Dec 1;187(6):1660–6.
5. Lawson GW. Naegele's rule and the length of pregnancy – A review. Vol. 61, Australian and New Zealand Journal of Obstetrics and Gynaecology. Blackwell Publishing; 2021. p. 177–82.
6. Pettker CM, Goldberg JD, El-Sayed YY. Methods for Estimating the Due Date Committee on Obstetric Practice American Institute of Ultrasound in Medicine Society for Maternal-Fetal Medicine. Vol. 700, Replaces Committee Opinion Number. 2017.
7. Haas DM, Imperiale TF, Kirkpatrick PR, Klein RW, Zollinger TW, Golichowski AM. Tocolytic Therapy A Meta-Analysis and Decision Analysis. Vol. 113, Obstet Gynecol. 2009.
8. Meloni A, Melis M, Alba E, Deiana S, Atzei A, Paoletti AM, et al. Medical therapy in the management of preterm birth. Vol. 22, Journal of Maternal-Fetal and Neonatal Medicine. 2009. p. 72–6.
9. Moutquin JM. Classification and heterogeneity of preterm birth. In: BJOG: An International Journal of Obstetrics and Gynaecology. Elsevier BV; 2003. p. 30–3.
10. Rubarth LB, Quinn J. Respiratory Development and Respiratory Distress Syndrome. Neonatal Network. 2015;34(4):231–8.
11. Lacaze-Masmonteil T. Expanded Use of Surfactant Therapy in Newborns. Vol. 34, Clinics in Perinatology. 2007. p. 179–89.
12. Bellodas Sanchez J, Kadrofske M. Necrotizing enterocolitis. Vol. 31, Neurogastroenterology and Motility. Blackwell Publishing Ltd; 2019.
13. Neu J, Walker WA. Necrotizing Enterocolitis. New England Journal of Medicine [Internet]. 2011 Jan 20;364(3):255–64. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMra1005408
14. Berman L, Moss RL. Necrotizing enterocolitis: An update. Vol. 16, Seminars in Fetal and Neonatal Medicine. 2011. p. 145–50.
15. Volpe JJ. The Encephalopathy of Prematurity-Brain Injury and Impaired Brain Development Inextricably Intertwined. Vol. 16, Seminars in Pediatric Neurology. 2009. p. 167–78.
16. Volpe JJ. The Developing Nervous System: A Series of Review Articles Neurobiology of Periventricular Leukomalacia in the Premature Infant. 2001.

17. Zaghloul N, Ahmed M. Pathophysiology of periventricular leukomalacia: What we learned from animal models. Vol. 12, Neural Regeneration Research. Wolters Kluwer Medknow Publications; 2017. p. 1795–6.
18. Hartnett ME. Pathophysiology and mechanisms of severe retinopathy of prematurity. Vol. 122, Ophthalmology. Elsevier Inc.; 2015. p. 200–10.
19. Hellström A, Smith LEH, Dammann O. Retinopathy of prematurity. In: The Lancet. Elsevier B.V.; 2013. p. 1445–57.
20. McCrea HJ, Ment LR. The Diagnosis, Management, and Postnatal Prevention of Intraventricular Hemorrhage in the Preterm Neonate. Vol. 35, Clinics in Perinatology. 2008. p. 777–92.
21. Owens R. Intraventricular Hemorrhage in the Premature Neonate.
22. Hossain M, Begum M, Ahmed S, Absar MN. Causes, Management and Immediate Complications of Management of Neonatal Jaundice? A Hospital-Based Study. Journal of Enam Medical College. 2015 Jun 29;5(2):104–9.
23. Cibulskis CC, Maheshwari A, Rao R, Mathur AM. Anemia of prematurity: how low is too low? Vol. 41, Journal of Perinatology. Springer Nature; 2021. p. 1244–57.
24. Ballabh P. Intraventricular Hemorrhage in Premature Infants: Mechanism of Disease. 2009.
25. Collins A, Weitkamp JH, Wynn JL. Why are preterm newborns at increased risk of infection? Vol. 103, Archives of Disease in Childhood: Fetal and Neonatal Edition. BMJ Publishing Group; 2018. p. F391–4.
26. Iacovidou N, Varsami M, Syggellou A. Neonatal outcome of preterm delivery. Ann N Y Acad Sci. 2010; 1205:130–4.
27. Koltsida G, Konstantinopoulou S. Long term outcomes in chronic lung disease requiring tracheostomy and chronic mechanical ventilation. Semin Fetal Neonatal Med. 2019 Oct 1;24(5).
28. Roberts G, Howard K, Spittle AJ, Brown NC, Anderson PJ, Doyle LW. Rates of early intervention services in very preterm children with developmental disabilities at age 2 years. J Paediatr Child Health. 2008 May;44(5):276–80.
29. Patel RM. Short- and Long-Term Outcomes for Extremely Preterm Infants. Vol. 33, American Journal of Perinatology. Thieme Medical Publishers, Inc.; 2016. p. 318–28.
30. WHO, March of dimes, UNICEF S the children. Born Too Soon. 2014;1–23.
31. Vinturache AE, Gyamfi-Bannerman C, Hwang J, Mysorekar IU, Jacobsson B. Maternal microbiome - A pathway to preterm birth. Vol. 21, Seminars in Fetal and Neonatal Medicine. W.B. Saunders Ltd; 2016. p. 94–9.
32. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health. 2019 Jan 1;7(1):e37–46.
33. Urząd Statystyczny G. Warszawa Rocznik Demograaczny Demographic Yearbook of Poland.
34. Flint Porter T, Fraser A, Hunter CY, Ward RH, Varner MW. The Risk of Preterm Birth Across Generations.
35. Winkvist A, Mogren I, H6gberg U. Familial patterns in birth characteristics: impact on individual and population risks [Internet]. Vol. 27, Great Britain International Journal of Epidemiology. 1998. Available from: http://ije.oxfordjournals.org/
36. Goldenberg RL, Culhane JF, Iams JD, Romero R. Preterm Birth 1 Epidemiology and causes of preterm birth [Internet]. Vol. 371, www.thelancet.com. 2008. Available from: www.thelancet.com
37. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med (2000). 342(20), 1500-7. doi: 10.1056/NEJM200005183422007. PMID: 10816189.

38. Krohn MA, Hillier SL, Nugent RP, Cotch MF, Carey JC, Gibbs RS, et al. Atlanta (abstract 682) [Internet]. Vol. 171, The Journal of Infectious Diseases. 1990. Available from: http://jid.oxfordjournals.org/
39. Hillier SL, Martius J, Krohn M, Kiviat N, Holmes KK, Eschenbach DA. A case-control study of chorioamnionic infection and histologic chorioamnionitis in prematurity. N Engl J Med. 1988 Oct 13;319(15):972-8. doi: 10.1056/NEJM198810133191503. PMID: 3262199.
40. Gibbs RS, Romero R, Hillier SL, Eschenbach DA, Sweet RL. A review of premature birth and subclinical infection. Am J Obstet Gynecol. 1992;166(5):1515–28.
41. Ernest JM, Wasilauskas B. Capnocytophaga in the amniotic fluid of a woman in preterm labor with intact membranes. Am J Obstet Gynecol. 1985;153(6):648–9.
42. Amsel R, Patricia Totten MA, Carol Spiegel MA, S Chen KC, Eschenbach D, Holmes KK. Nonspecific Vaginitis Diagnostic Criteria and Microbial and Epidemiologic Associations. 1983.
43. Amsel R, Patricia Totten MA, Carol Spiegel MA, S Chen KC, Eschenbach D, Holmes KK. Nonspecific Vaginitis Diagnostic Criteria and Microbial and Epidemiologic Associations. 1983.
44. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol. 1986 Feb;67(2):229-37. doi: 10.1097/00006250-198602000-00013. PMID: 3003634.
45. Meis PJ, Goldenberg RL, Mercer B, Moawad A, Das A, McNellis D, Johnson F, Iams JD, Thom E, Andrews WW. The preterm prediction study: significance of vaginal infections. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Am J Obstet Gynecol. 1995 Oct;173(4):1231-5. doi: 10.1016/0002-9378(95)91360-2. PMID: 7485327.
46. Krohn MA, Hillier SL, Nugent RP, Cotch MF, Carey JC, Gibbs RS, et al. Atlanta (abstract 682) [Internet]. Vol. 171, The Journal of Infectious Diseases. 1990. Available from: http://jid.oxfordjournals.org/
47. Hillier SL, Krohn MA, Cassen E, Easterling TR, Rabe LK, Eschenbach DA. The Role of Bacterial Vaginosis and Vaginal Bacteria in Amniotic Fluid Infection in Women in Preterm Labor with Intact Fetal Membranes. Downloaded from [Internet]. Vol. 20, Clinical Infectious Diseases. 1995. Available from: http://cid.oxfordjournals.org/
48. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. Microbiome. 2015 Dec;3(1).
49. Grice EA, Segre JA. The human microbiome: Our second genome. Vol. 13, Annual Review of Genomics and Human Genetics. 2012. p. 151–70.
50. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The Human Microbiome Project. Vol. 449, Nature. Nature Publishing Group; 2007. p. 804–10.
51. Vartoukian SR, Palmer RM, Wade WG. Strategies for culture of "unculturable" bacteria. Vol. 309, FEMS Microbiology Letters. Blackwell Publishing Ltd; 2010. p. 1–7.
52. Kim M, Morrison M, Yu Z. Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. J Microbiol Methods. 2011 Jan;84(1):81–7.
53. Chakravorty S, Helb D, Burday M, Connell N, Alland D. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. J Microbiol Methods. 2007 May;69(2):330–9.
54. Nuriel-Ohayon M, Neuman H, Koren O. Microbial changes during pregnancy, birth, and infancy. Vol. 7, Frontiers in Microbiology. Frontiers Media S.A.; 2016.

55. Jiao Y, Hasegawa M, Inohara N. The role of oral pathobionts in dysbiosis during periodontitis development. Vol. 93, Journal of Dental Research. SAGE Publications Inc.; 2014. p. 539–46.
56. Hajishengallis G, Lamont RJ. Beyond the red complex and into more complexity: The polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. Mol Oral Microbiol. 2012 Dec;27(6):409–19.
57. Hong BY, Araujo MVF, Strausbaugh LD, Terzi E, Ioannidou E, Diaz PI. Microbiome profiles in periodontitis in relation to host and disease characteristics. PLoS One. 2015 May 18;10(5).
58. Darveau RP. Periodontitis: A polymicrobial disruption of host homeostasis. Vol. 8, Nature Reviews Microbiology. 2010. p. 481–90.
59. Belstrøm D, Fiehn NE, Nielsen CH, Kirkby N, Twetman S, Klepac-Ceraj V, et al. Differences in bacterial saliva profile between periodontitis patients and a control cohort. J Clin Periodontol. 2014 Feb;41(2):104–12.
60. Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis.
61. Hajishengallis G. Aging and its impact on innate immunity and inflammation Implications for periodontitis. In: Journal of Oral Biosciences. Japanese Association for Oral Biology; 2014. p. 30–7.
62. Eskan MA, Jotwani R, Abe T, Chmelar J, Lim JH, Liang S, et al. The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. Nat Immunol. 2012 May;13(5):465–73.
63. Lindroth AM, Park YJ. Epigenetic biomarkers: A step forward for understanding periodontitis. Vol. 43, Journal of Periodontal and Implant Science. 2013. p. 111–20.
64. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. Vol. 10, Nature Reviews Microbiology. 2012. p. 717–25.
65. Divaris K, Monda KL, North KE, Olshan AF, Reynolds LM, Hsueh WC, et al. Exploring the genetic basis of chronic periodontitis: A genome-wide association study. Hum Mol Genet. 2013 Jun;22(11):2312–24.
66. Paropkari AD, Leblebicioglu B, Christian LM, Kumar PS. Smoking, pregnancy and the subgingival microbiome. Sci Rep. 2016 Jul 27;6.
67. Hill GB. Preterm Birth: Associations With Genital and Possibly Oral Microflora.
68. Cassini MA, Pilloni A, Condò SG, Vitali LA, Pasquantonio G, Cerroni L. Periodontal bacteria in the genital tract: are they related to adverse pregnancy outcome? Int J Immunopathol Pharmacol. 2013 Oct-Dec;26(4):931-9. doi: 10.1177/039463201302600411. PMID: 24355228.
69. Mobeen N, Jehan I, Banday N, Moore J, McClure EM, Pasha O, et al. Periodontal disease and adverse birth outcomes: a study from Pakistan. Am J Obstet Gynecol. 2008;198(5):514.e1-514.e8.
70. Takeuchi N, Ekuni D, Irie K, Furuta M, Tomofuji T, Morita M, et al. Relationship between periodontal inflammation and fetal growth in pregnant women: A cross-sectional study. Arch Gynecol Obstet. 2013 May;287(5):951–7.
71. Offenbacher S, Boggess KA, Murtha AP, Jared HL, Lieff S, Mckaig RG, et al. Progressive Periodontal Disease and Risk of Very Preterm Delivery Level of Evidence: II-2. Vol. 107, Obstet Gynecol. 2006.
72. Wardwell LH, Huttenhower C, Garrett WS. Current concepts of the intestinal microbiota and the pathogenesis of infection. Curr Infect Dis Rep. 2011 Feb;13(1):28–34.
73. King JC. Physiology of pregnancy and nutrient metabolism. Am J Clin Nutr. 2000 May;71(5 Suppl):1218S-25S. doi: 10.1093/ajcn/71.5.1218s. PMID: 10799394.

74. Dahl C, Stanislawski M, Iszatt N, Mandal S, Lozupone C, Clemente JC, et al. Gut microbiome of mothers delivering prematurely shows reduced diversity and lower relative abundance of Bifidobacterium and Streptococcus. PLoS One. 2017 Oct 1;12(10).
75. Shiozaki A, Yoneda S, Yoneda N, Yonezawa R, Matsubayashi T, Seo G, et al. Intestinal microbiota is different in women with preterm birth: Results from terminal restriction fragment length polymorphism analysis. PLoS One. 2014 Nov 5;9(11).
76. Cribby S, Taylor M, Reid G. Vaginal Microbiota and the Use of Probiotics. Interdiscip Perspect Infect Dis. 2008; 2008:1–9.
77. Romero R, Chaiworapongsa T, Espinoza J. Micronutrients and Intrauterine Infection, Preterm Birth and the Fetal Inflammatory Response Syndrome. J Nutr. 2003 May;133(5):1668S–1673S. doi: 10.1093/jn/133.5.1668S.
78. Abdulamir AS, Hafidh RR, Bakar FA. The association of Streptococcus bovis/gallolyticus with colorectal tumors: The nature and the underlying mechanisms of its etiological role. Vol. 30, Journal of Experimental and Clinical Cancer Research. 2011.
79. DiGiulio DB, Romero R, Kusanovic JP, Gómez R, Kim CJ, Seok KS, et al. Prevalence and Diversity of Microbes in the Amniotic Fluid, the Fetal Inflammatory Response, and Pregnancy Outcome in Women with Preterm Pre-Labor Rupture of Membranes. American Journal of Reproductive Immunology. 2010 Jul;64(1):38–57.
80. Dunlop AL, Mulle JG, Ferranti EP, Edwards S, Dunn AB, Corwin EJ. Maternal Microbiome and Pregnancy Outcomes That Impact Infant Health: A Review. Vol. 15, Advances in Neonatal Care. Lippincott Williams and Wilkins; 2015. p. 377–85.
81. Dominguez-Bello MG, Godoy-Vitorino F, Knight R, Blaser MJ. Role of the microbiome in human development. Gut. 2019 Jun 1;68(6):1108–14.
82. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome. 2014 Feb 3;2(1).
83. Miller EA, Beasley DAE, Dunn RR, Archie EA. Lactobacilli dominance and vaginal pH: Why is the human vaginal microbiome unique? Front Microbiol. 2016;7(DEC).
84. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011 Mar 15;108(SUPPL. 1):4680–7.
85. Kamada N, Chen GY, Inohara N, Núñez G. Control of pathogens and pathobionts by the gut microbiota. Vol. 14, Nature Immunology. 2013. p. 685–90.
86. Miller EA, Beasley DAE, Dunn RR, Archie EA. Lactobacilli dominance and vaginal pH: Why is the human vaginal microbiome unique? Front Microbiol. 2016;7(DEC).
87. Fox C, Eichelberger K. Maternal microbiome and pregnancy outcomes. Vol. 104, Fertility and Sterility. Elsevier Inc.; 2015. p. 1358–63.
88. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SSK, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011 Mar 15;108(SUPPL. 1):4680–7.
89. Fettweis JM, Paul Brooks J, Serrano MG, Sheth NU, Girerd PH, Edwards DJ, et al. Differences in vaginal microbiome in African American women versus women of European ancestry. Microbiology (United Kingdom). 2014 Oct 1;160:2272–82.
90. Hickey RJ, Zhou X, Settles ML, Erb J, Malone K, Hansmann MA, et al. Vaginal microbiota of adolescent girls prior to the onset of menarche resemble those of reproductive-age women. mBio. 2015 Mar 24;6(2).
91. Stout MJ, Zhou Y, Wylie KM, Tarr PI, Macones GA, Tuuli MG. Early pregnancy vaginal microbiome trends and preterm birth. Am J Obstet Gynecol. 2017 Sep 1;217(3):356.e1-356.e18.

92. Cervantes JL, Hong BY. Role of next-generation sequencing in understanding the interactions between human papillomavirus and the cervicovaginal microbiome. Vol. 76, Gynecologic and Obstetric Investigation. S. Karger AG; 2013. p. 195–202.
93. Smith BC, McAndrew T, Chen Z, Harari A, Barris DM, Viswanathan S, et al. The cervical microbiome over 7 years and a comparison of methodologies for its characterization. PLoS One. 2012 Jul 9;7(7).
94. Hein M, Valore E v., Helmig RB, Uldbjerg N, Ganz T. Antimicrobial factors in the cervical mucus plug. Am J Obstet Gynecol. 2002;187(1):137–44.
95. Kindinger LM, Bennett PR, Lee YS, Marchesi JR, Smith A, Cacciatore S, et al. The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk. Microbiome. 2017;5(1).
96. Kiefer DG, Keeler SM, Rust OA, Wayock CP, Vintzileos AM, Hanna N. Is midtrimester short cervix a sign of intraamniotic inflammation? Am J Obstet Gynecol. 2009;200(4):374.e1-374.e5.
97. Hee L. Likelihood ratios for the prediction of preterm delivery with biomarkers. Acta Obstet Gynecol Scand. 2011 Nov;90(11):1189–99.
98. Vogel I, Thorsen P, Curry A, Sandager P, Uldbjerg N. ACTA REVIEW Biomarkers for the prediction of preterm delivery.
99. Banaem LM, Mohamadi B, Jaafarabadi MA, Moghadam NA. Maternal serum C-reactive protein in early pregnancy and occurrence of preterm premature rupture of membranes and preterm birth. Journal of Obstetrics and Gynaecology Research. 2012 May;38(5):780–6.
100. 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. International Journal of Gynecology and Obstetrics. 2019 Oct 1;147(1):83–8.
101. Lohsoonthorn V, Qiu C, Williams MA. Maternal serum C-reactive protein concentrations in early pregnancy and subsequent risk of preterm delivery. Clin Biochem. 2007 Mar;40(5–6):330–5.
102. Zhang Q, Ananth C v., Li Z, Smulian JC. Maternal anaemia and preterm birth: A prospective cohort study. Int J Epidemiol. 2009;38(5):1380–9.
103. Yi SW, Han YJ, Ohrr H. Anemia before pregnancy and risk of preterm birth, low birth weight and small-for-gestational-age birth in Korean women. Eur J Clin Nutr. 2013 Apr;67(4):337–42.
104. Pawelczyk E, Nowicki BJ, Izban MG, Pratap S, Sashti NA, Sanderson M, et al. Spontaneous preterm labor is associated with an increase in the proinflammatory signal transducer TLR4 receptor on maternal blood monocytes. BMC Pregnancy Childbirth. 2010 Oct 21;10.
105. Pawelczyk E, Nowicki BJ, Izban MG, Pratap S, Sashti NA, Sanderson M, et al. Spontaneous preterm labor is associated with an increase in the proinflammatory signal transducer TLR4 receptor on maternal blood monocytes. BMC Pregnancy Childbirth. 2010 Oct 21;10.
106. Shannon CE. A Mathematical Theory of Communication. The Bell System Technical Journal. 1948.
107. Dahl C, Stanislawski M, Iszatt N, Mandal S, Lozupone C, Clemente JC, et al. Gut microbiome of mothers delivering prematurely shows reduced diversity and lower relative abundance of Bifidobacterium and Streptococcus. PLoS One. 2017 Oct 1;12(10).
108. Consortium HMP. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207-14.

109. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep. 2016;6:23129.
110. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. Genome Med. 2016;8(1):51.
111. Koren O, Knights D, Gonzalez A, Waldron L, Segata N, Knight R, et al. A guide to enterotypes across the human body: meta-analysis of microbial community structures in human microbiome datasets. PLoS Comput Biol. 2013;9(1):e1002863.
112. Hu J, Nomura Y, Bashir A, Fernandez-Hernandez H, Itzkowitz S, Pei Z, Stone J, Loudon H, Peter I. Diversified microbiota of meconium is affected by maternal diabetes status. PLoS One. 2013;8(11):e78257. doi: 10.1371/journal.pone.0078257.
113. DiGiulio DB, Callahan BJ, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci U S A. 2015;112(35):11060-11065. doi: 10.1073/pnas.1502875112.
114. Koren O, Goodrich JK, Cullender TC, Spor A, Laitinen K, Bäckhed HK, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell. 2012 Jun 22;150(3):470-80. doi: 10.1016/j.cell.2012.07.008. PMID: 22863481.
115. Romero R, Hassan SS, Gajer P, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome. 2014;2:4. doi: 10.1186/2049-2618-2-4.
116. Stout MJ, Conlon B, Landeau M, et al. Identification of intrapartum vaginal microbiota-associated bacteria in term and preterm infants. Pediatr Res. 2017;82(4):564-573. doi: 10.1038/pr.2017.125.
117. Walther-António MRS, Jeraldo P, Berg Miller ME, Yeoman CJ, Nelson KE, Wilson BA, et al. Pregnancy's stronghold on the vaginal microbiome. PLoS One. 2014;9(6):1–10.
118. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Bieda J, et al. The vaginal microbiota of pregnant women who subsequently have spontaneous preterm labor and delivery and those with a normal delivery at term. Microbiome. 2014;2(1):1–15.
119. Koren O, Goodrich JK, Cullender TC, Spor AA, Laitinen K, Backhed HK, et al. During Pregnancy. Cell. 2013;150(3):470–80.
120. Corwin EJ, Hogue CJ, Pearce B, Hill CC, Read TD, Mulle J, et al. Erratum: Protocol for the Emory University African American Vaginal, oral, and gut microbiome in pregnancy cohort study. [BMC Pregnancy and Childbirth 17, (2017), (161)] DOI: 10.1186/s12884-017-1357-x. BMC Pregnancy Childbirth. 2017;17(1):1–8.
121. Nelson DB, Shin H, Wu J, Dominguez-Bello MG. The Gestational Vaginal Microbiome and Spontaneous Preterm Birth among Nulliparous African American Women. Am J Perinatol. 2016;33(9):887–93.
122. Park S, Moon J, Kang N, Kim YH, You YA, Kwon E, et al. Predicting preterm birth through vaginal microbiota, cervical length, and WBC using a machine learning model. Front Microbiol. 2022 Aug 2;13.
123. Solgi E, Tavakoli-Far B, Badehnoosh B, Khavandegar A, Bakhtiyari M. Vaginal and oral probiotics effect in the prevention of preterm delivery in patients visiting Kamali Hospital, Karaj, Iran in 2020. Eur J Obstet Gynecol Reprod Biol X. 2022 Dec 1;16.
124. Cristofori F, Dargenio VN, Dargenio C, Miniello VL, Barone M, Francavilla R. Anti-Inflammatory and Immunomodulatory Effects of Probiotics in Gut Inflammation: A Door to the Body. Vol. 12, Frontiers in Immunology. Frontiers Media S.A.; 2021.
125. Baldassarre ME, Palladino V, Amoruso A, Pindinelli S, Mastromarino P, Fanelli M, et al. Rationale of probiotic supplementation during pregnancy and neonatal period. Vol. 10, Nutrients. MDPI AG; 2018.

126. Onubi OJ, Poobalan AS, Dineen B, Marais D, McNeill G. Effects of probiotics on child growth: A systematic review. J Health Popul Nutr. 2015;34(1).

14. Attachments

14.1 Consent of the Bioethics Committee

Uniwersytet Mikołaja Kopernika w Toruniu

Collegium Medicum im L. Rydygiera w Bydgoszczy

KOMISJA BIOETYCZNA

Ul. M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, tel.(052) 585-35-63, fax.(052) 585-38-11

KB 443/2019 Bydgoszcz, 14.05.2019 r.

Działając na podstawie art.29 Ustawy z dnia 5 grudnia 1996 roku o zawodzie lekarza (Dz.U. z 1997 r. Nr 28 poz. 152 (wraz z późniejszymi zmianami), zarządzenia Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych (Dz.U.Nr 47 poz.480) oraz Zarządzeniem Nr 21 Rektora UMK z dnia 4 marca 2009 r. z późn. zm. w sprawie powołania oraz zasad działania Komisji Bioetycznej Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im Ludwika Rydygiera w Bydgoszczy oraz zgodnie z zasadami zawartymi w ICH – GCP

Komisja Bioetyczna przy UMK w Toruniu, Collegium Medicum w Bydgoszczy

(skład podano w załączeniu), na posiedzeniu w dniu **14.05.2019 r.** przeanalizowała wniosek, który złożyła kierownik badania:

lek. med. Alicja Harmoza
Katedra Położnictwa Chorób Kobiecych i Ginekologii Onkologicznej
Collegium Medicum w Bydgoszczy

z zespołem w składzie

- dr hab. n. med. Iwona Sadowska-Krawczenko, dr hab. n. med. Marcin Woźniak prof. UMK, lek. med. Alicja Harmoza.

w sprawie badania:

„Zmienność mikrobioty organizmu kobiet ciężarnych i jej wpływ na częstość występowania porodów przedwczesnych."

Po zapoznaniu się ze złożonym wnioskiem i w wyniku przeprowadzonej dyskusji oraz głosowania Komisja podjęła

Uchwałę o pozytywnym zaopiniowaniu wniosku

w sprawie przeprowadzenia badań, w zakresie określonym we wniosku pod warunkiem:

- poinformowania uczestników badania w tym również uczestników stanowiących grupę kontrolną o celu oraz zakresie badań i uzyskania od każdego z nich osobnej, pisemnej, świadomej zgody na udział w badaniu, zgodnie z obowiązującymi przepisami, datowanej najpóźniej na moment rozpoczęcia badania, a nie wcześniej niż data uzyskania z Komisji Bioetycznej zgody na takie badanie;
- zapewnienia, że osoby uczestniczące w eksperymencie badawczym nie są ubezwłasnowolnione, nie są żołnierzami służby zasadniczej, nie są osobami pozbawionymi wolności, nie pozostają w zależności służbowej, dydaktycznej lub innej z prowadzącym badanie;
- UWAGA! Uczestnicy badania stanowiący grupę kontrolną nie mogą być rekrutowani spośród studentów lub pracowników podlegających zależności służbowej lub dydaktycznej z badaczami.
- zachowania tajemnicy wszystkich danych, w tym danych osobowych pacjentów, umożliwiających ich identyfikację w ewentualnych publikacjach;
- sugerujemy uzyskanie podpisu uczestnika badania pod informacją o badaniu, lub sporządzenie formularza informacji i świadomej zgody na udział w badaniu na jednej kartce.

Jednocześnie informujemy, iż „Zgoda na udział w badaniu" winna zawierać m.in.: imię i nazwisko badanej osoby; Nr historii choroby pacjenta (L.ks.gł. Oddziału/Poradni) oraz datę i podpis badanej osoby, a także

klauzule, że uczestnik badania wyraża zgodę na przetwarzanie danych osobowych dotyczących realizacji tematu badawczego, z wyjątkiem publikacji danych osobowych.

Kierownik badania zobowiązany jest do przechowywania wszystkich dokumentów dotyczących badania przez okres dwudziestu lat.

Zgoda obowiązuje od daty posiedzenia (14.05.2019 r.) do końca 2022 r.

Wydana opinia dotyczy tylko rozpatrywanego wniosku z uwzględnieniem przedstawionego projektu; każda zmiana i modyfikacja wymaga uzyskania odrębnej opinii. Wnioskodawca zobowiązany jest do informowania o wszelkich poprawkach, które mogłyby mieć wpływ na opinię Komisji oraz poinformowania o zakończeniu badania.

Od niniejszej uchwały podmiot zamierzający przeprowadzić eksperyment medyczny, kierownik zakładu opieki zdrowotnej, w której eksperyment medyczny ma być przeprowadzony, mogą wnieść odwołanie do Odwoławczej Komisji Bioetycznej przy Ministrze Zdrowia, za pośrednictwem Komisji Bioetycznej przy Collegium Medicum im. L. Rydygiera w Bydgoszczy, w terminie 14 dni od daty otrzymania niniejszej Uchwały.

Prof. dr hab. med. Karol Śliwka

Przewodniczący Komisji Bioetycznej

Otrzymuje:
lek. med. Alicja Harmoza
Katedra Położnictwa Chorób Kobiecych i Ginekologii Onkologicznej
Collegium Medicum w Bydgoszczy

Lista obecności

na posiedzeniu Komisji Bioetycznej

w dniu 14.05.2019 r.

Lp.	Imię i nazwisko	Funkcja	Podpis,
1.	Prof. dr hab. med. Karol Śliwka	Przewodniczący	
2.	Mgr prawa Joanna Połetek-Żygas	Z – ca przewodniczącego	
3.	Prof. dr hab. med. Mieczysława Czerwionka-Szaflarska		
4.	Prof. dr hab. med. Anna Balcar-Boroń		
5.	Prof. dr hab. med. Marek Grabiec		
6.	Prof. dr hab. med. Zbigniew Włodarczyk		
7.	Dr hab. n. med. Katarzyna Pawlak-Osińska, prof. UMK		
8.	Dr hab. n med. Maria Kłopocka		
9.	Ks. dr hab. Wojciech Szukalski, prof. UAM		
10.	Dr n. med. Radosława Staszak-Kowalska		
11.	Mgr prawa Patrycja Brzezicka		
12.	Mgr farm. Aleksandra Adamczyk		
13.	Mgr Lidia Iwińska-Tarczykowska		