"VAGINAL DYSBIOSIS IN EARLY SECOND TRIMESTER OF PREGNANCY AND ITS ASSOCIATION WITH PPROM: A LONGITUDINAL OBSERVATIONAL STUDY AT KAHER'S DR.PRABHAKAR KORE CHARITABLE HOSPITAL, BELAGAVI."

By

REG. NO. BJ0119017

Dissertation

Submitted to the

KAHER, Belagavi, Karnataka

In partial fulfillment

of the requirements for the degree of

MASTER OF SURGERY (M.S.)

In

OBSTETRICS AND GYNAECOLOGY

J. N. MEDICAL COLLEGE, NEHRU NAGAR

BELAGAVI-590010

APRIL 2022

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA

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Ref No: MDC/PG/ Date: 22-12-2021.

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ABBREVIATIONS

BV	:	Bacterial vaginosis
PPROM	:	Preterm prelabour rupture of membranes
PTB	:	Preterm birth
PROM	:	Premature rupture of membranes
IL	:	Interleukin
IVF	:	In vitro fertilization
LBW	:	Low Birth Weight
MMPs	:	Matrix metalloproteases
PCR	:	Polymerase Chain Reaction
RR	:	Relative Risk
TNF	:	Tumor Necrosis Factor
VMB	:	Vaginal microbiota
C-Section	:	Caesarean section
PCR	:	Polymerase Chain Reaction
LBW	:	Low Birth Weight

ABSTRACT

Background:

Preterm birth is an enormous challenge faced by obstetricians. PPROM is appraised to complicate 3% of pregnancies and is the biggest contributing cause to spontaneous PTB. The presence of an abnormal vaginal microflora in early pregnancy is a risk factor for PPROM, preterm delivery and PROM.

Objectives:

This study was conducted to determine the correlation between vaginal dysbiosis in $14^{+0/7}$ weeks and $20^{+6/7}$ weeks of pregnancy and subsequent development of PPROM, PROM and Spontaneous preterm birth.

Methodology:

This is an Observational longitudinal study conducted in KAHER's Dr. Prabhakar Kore Charitable Hospital and the women attending antenatal clinic in the department of obstetrics and gynecology who met inclusion criteria were recruited and 2 high vaginal swabs were taken from the posterior fornix of vagina. 1st Swab was used for Gram staining for Nugent scoring followed by incubation for aerobic culture. 2nd swab was used for fungal culture. Pregnancy outcome of all these patients was noted. Outcome was correlated to microbiological profiling done in early pregnancy.

Results:

110 participants were analysed. 20 (18.18%) participants had vaginal dysbiosis on nugent scoring (>3) and out of which 1 participant had PPROM ($p=0.3448$), no participants had PROM($p=0.2199$) and 11 participants had spontaneous preterm birth($p<0.001$). No vaginal dysbiosis noted on aerobic and fungal culture was associated with PPROM,PROM and Spontaneous preterm birth.

Conclusion:

There is no significant association of PPROM or PROM with Nugent score, Aerobic culture and Fungal culture. But there is a significant correlation of Abnormal Nugent scoring with Spontaneous Preterm labour.

Keywords: PPROM, PROM, Vaginal dysbiosis, PTB (Preterm birth)

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1. INTRODUCTION

Preterm birth (PTB) is an enormous challenge faced by obstetricians in the current era. It is the world's preeminent cause of childhood mortality and is responsible for almost 80% of all neonatal morbidity(1) leading to major financial and emotional burden to families and society. Preterm prelabour rupture of membranes (PPROM) describes spontaneous rupture of the fetal membranes prior to the completion of 37 weeks of gestation and before the onset of labor (2). PPROM is appraised to complicate 3% of pregnancies and is the biggest contributing cause to spontaneous PTB(3), with 80% of them delivering within nine days and the staggering majority before 37 weeks. PROM/PPROM is associated with neonatal mortality and morbidity. It includes prematurity, neonatal infections including necrotizing enterocolitis, sepsis, respiratory distress syndrome, intraventricular hemorrhage, periventricular leukomalacia, cerebral palsy, retinopathy of prematurity and endocrinological problems including hypoglycemia, transient low hypothyroid levels (4). Inspite of various research efforts, delineating the causes of PPROM are inadequate and the incidence continues to be on the rise on a global scale (5,6).

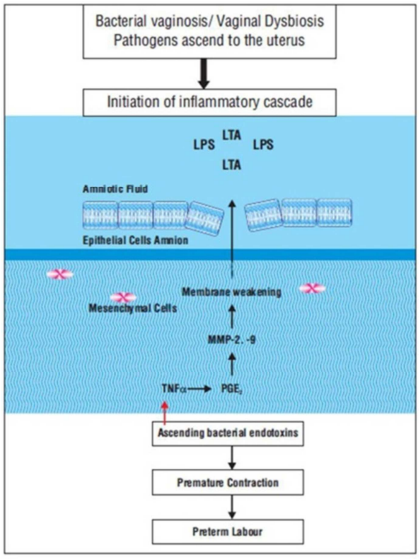


Figure 1: Pathogenesis of preterm birth. (Adapted from (7))

It has been long appreciated that the vaginal microbial composition is critical in maintaining a normal physiological status for the host and its involvement is regarded as indispensable for successful reproduction(8). Since women of diverse races have a singular vaginal microflora with regional variations which makes a global concept of normal vs. dysbiosis of vaginal microbiome debatable(9). Vaginal microflora is affected by age, menstrual cycle, sexual activity, gestational status, and contraceptive use(10).Vaginal flora is normally dominated by lactobacilli chiefly in women of European lineage vs. African American women(11). These microbes provide the host vagina protection from conceivably pathogenic microbes that may lead to urinary tract infections and sexually transmitted diseases(12). Modifications in the vaginal microbiota, counting in deficient lactobacilli abundance and elevated facultative and anaerobic organism populations as well, resulting in bacterial vaginosis, predisposes the host to numerous conditions like low birth weight and heightened risk of incurring bacterial infections (13).

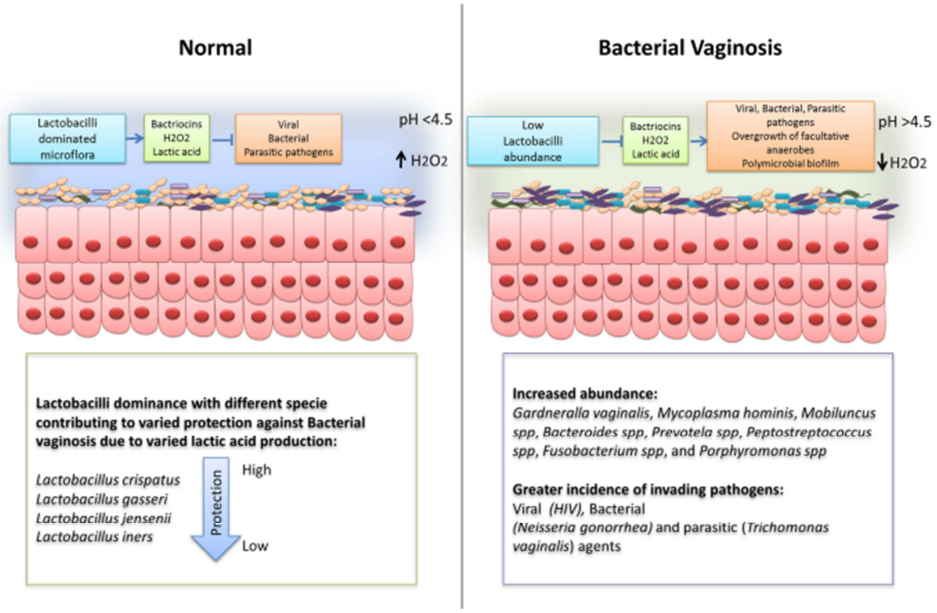


Figure 2: Normal vaginal flora vs. Bacterial vaginosis (Adapted from (14))

Nonetheless, the vaginal microbiome is also modified during pregnancy, with limited microbial diversity with a predominance of Lactobacillus species (15). However, modified vaginal microbiota with lactobacilli inadequacy especially during pregnancy may culminate in the induction of redundant inflammation and preterm labor (16). Since the vaginal microbiome plays a critical role during embryo implantation, it is not unanticipated that bacterial vaginosis is more prevalent in infertile women and associated with decreased rates of conception(17, 18).

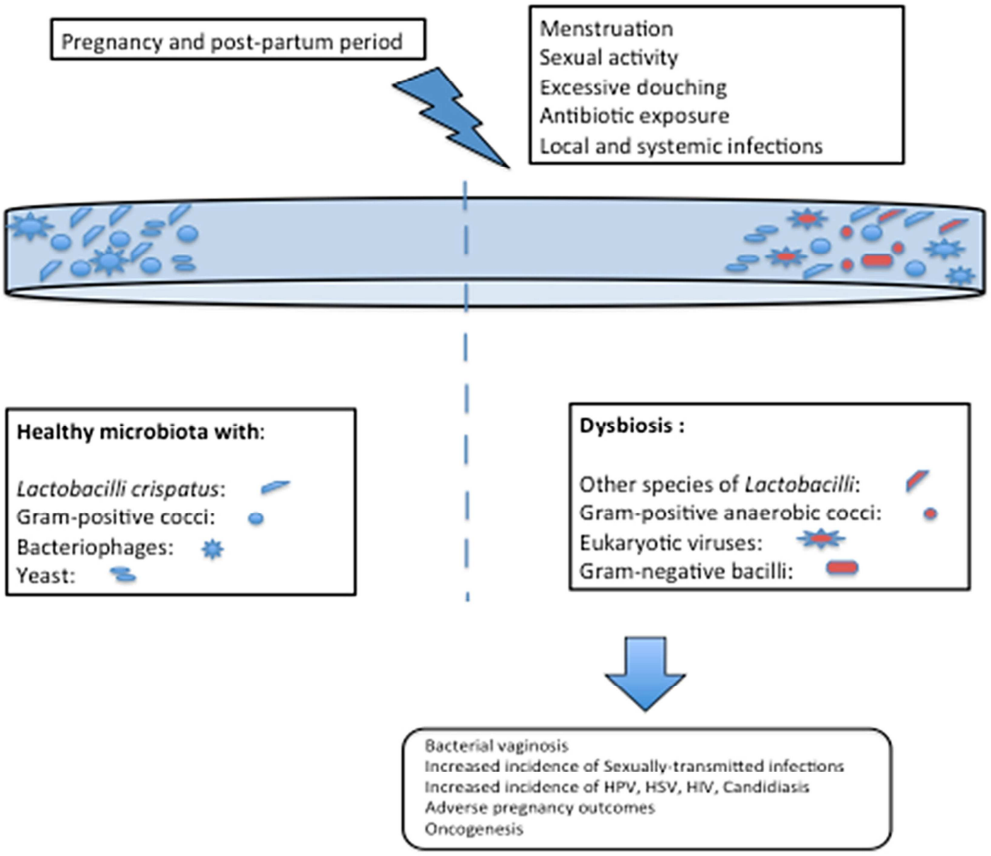


Figure 3: Risk factors for alteration in vaginal microbiome and consequences of dysbiosis. (Adapted from (19))

A widely held rationale is that a percentage of PPROM cases are caused by colonization of the vagina by pathogenic bacteria that stimulate the innate immune system(3,20) triggering an inflammatory response (21–24) that leads to anachronistic

remodelling and disruption of fetal membrane composition and eventually, untimely rupture(25–27). In agreement with this hypothesis, recent investigative studies using techniques that do not rely on cultures have shown that decreased *Lactobacillus* spp. abundance and heightened bacterial diversity are associated with PPROM and PTB. In comparison, healthy pregnancy is characterized by stable, low (28–30) abundance and low diversity community structures monopolised by *Lactobacillus* spp.(31–33). The aforementioned findings are in concurrence with previous culture-based studies that reported deficiency of *Lactobacillus* spp. and polymicrobial establishment in the vagina as risk factors for PPROM and PTB (34–36). In a recent study of 250 pregnant women, Brown et al. have shown that vaginal bacterial configuration defined by *Lactobacillus* spp. depletion and rich diversity were observable prior to the rupture of fetal membranes in approximately one-third of cases(30). *Lactobacillus* spp. depletion and high diversity were absent in women who later delivered at term without complications. However, the juncture during the pregnancy when vaginal bacterial architecture shifts toward a high-diversity state in women who subsequently PPROM remains elusive.

Given the high prevalence and sociological impact of preterm birth and the widely documented correlation of vaginal dysbiosis with preterm delivery, this study has been attempted to determine the correlation between vaginal dysbiosis in the early second trimester of pregnancy and its association with the development of PPROM.

OBJECTIVE

Primary objective-

To determine the correlation between vaginal dysbiosis in 14+0/7 weeks to 20+6/7 weeks of pregnancy and subsequent development of PPROM.

Secondary objective-

To assess the correlation between vaginal dysbiosis in 14+0/7 weeks to 20+6/7 weeks of pregnancy and subsequent development of PROM and Spontaneous preterm birth.

2. REVIEW OF LITERATURE

Childbirth that occurs at less than 37 weeks of gestation is known as preterm delivery; however, the cut-off to determine the low gestational age varies based on the location(37). The occurrence rate of preterm deliveries has risen despite advances in the knowledge of mechanisms of preterm labor and the risk factors associated with it. The introduction of numerous public health and medical interventions designed to reduce preterm birth have been of little avail so far (37,38). Preterm births can be regarded as a cause for 75% of perinatal mortality and also for greater than half the long-term morbidity (39). Even though most preterm babies survive, they have an increased risk of neurodevelopmental impairments and other systemic complications (40). Important obstetric harbingers to preterm birth are: (1) delivery due to maternal or fetal indications, in which the delivery is either induced or the baby is delivered by prelabour cesarean section; (2) spontaneous preterm labor with membranes still intact; and (3) preterm premature rupture of the membranes (PPROM), regardless of vaginal delivery or C-section (Figure-4)(41)

The extent of the effect of the contributory causes of preterm births differs based on the ethnicity. Spontaneous preterm birth is most frequently caused by preterm labor in white women but by PPROM in black women (42). Multiple gestations inherent in assisted reproductive technologies too are an important contributor to the overall increase in the incidence of preterm births. Singleton pregnancies after in-vitro fertilization too are at increased risk of preterm birth(43).

Recently, preterm labor has been understood to be a syndrome established by multiple mechanisms, like infection, uterine overdistension, uteroplacental ischemia stress, and other immunologically mediated processes (44). Accumulating evidence suggests that a number of risk factors interact to cause a transition from uterine repose toward

preterm labor or PPROM. Many of the risk factors implicated in preterm birth result in increased systemic inflammation and stimulation of the infection or inflammatory pathway which might be able to partially explain some of the preterm births (45). Many maternal or fetal characteristics are associated with preterm birth, including maternal demographic details, nutritional status, pregnancy history, current pregnancy statistics, psychological characteristics, infection, uterine contractions and cervical length, biological and genetic markers (46).

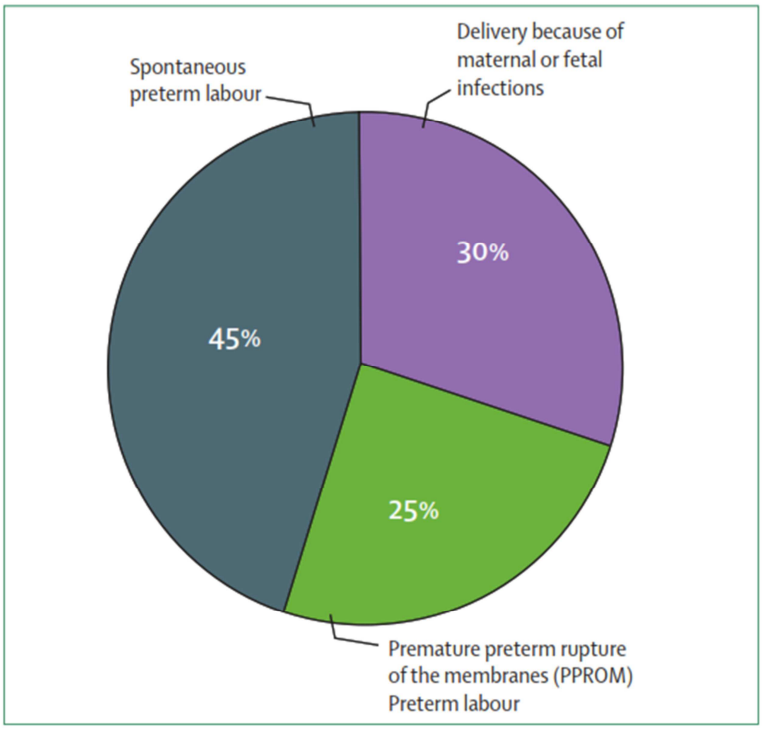


Figure-4: Obstetric precursors of preterm birth (Adapted from (37))

It has been reported by Mercer and colleagues that women with a history of preterm deliveries have a 2·5–fold increased risk for the same in their next pregnancy (47). The risk of successive preterm birth is inversely related to the gestational age of the previous preterm birth. The etiology of the recurrence is not always clear, but women with a history of spontaneous preterm births are far more likely to have

ensuing spontaneous preterm births; women with marked preterm births tend to repeat such births(48,49). Perpetual intrauterine infections can possibly explain many repetitive spontaneous preterm births (49). Disorders indicated as having a contributing effect on preterm births, like obesity, hypertension or diabetes, frequently prevail between pregnancies. Almost all multiple gestations with a higher number of fetuses will result in preterm delivery. Uterine overdistension, leading to contractions and PPROM, is believed to be the cause for spontaneous preterm births in such scenarios (44). Severe fluctuations in the volume of amniotic fluid—oligohydramnios or polyhydramnios —are associated with preterm labor and PPROM.

Intrauterine infection is an incessant and critical mechanism leading to preterm birth (50,51) Intrauterine infections lead to preterm labour via activation of the innate immune system (44). It is evident from microbiological studies that intrauterine infections account for 25–40% of preterm births (51).

Pattern-recognition receptors which elicit the release of inflammatory chemokines and cytokines help the recognition of microorganisms. The production of prostaglandins, matrix-degrading enzymes, and other inflammatory mediators is brought about by the proinflamatory cytokines and the microbial endotoxins. Uterine contractions are stimulated by prostaglandins whereas degeneration of extracellular matrix in the fetal membranes results in PPROM(44,51). Accruing evidence indicates that intra-amniotic infection is a protracted process (51). Women who are positive for *U Urealyticum* in amniotic fluid cultures or PCR analysis at the mid-trimester genetic amniocentesis, frequently are at risk of having spontaneous preterm labor or PPROM after the procedure (52–54). Genital Mycoplasma species and *U urealyticum* are the most commonly reported microorganisms in the amniotic cavity apart from other organisms that have been identified (55–58). Lower genital tract microorganisms,

such as *Streptococcus agalactiae,* which are otherwise common, are rarely seen in the amniotic cavity before membrane rupture (51,59). The frequent absence of overt clinical signs of infection and chronic nature of intrauterine infections can be explained by the fact that the genital mycoplasmas and other organisms observed in the uterus before membrane rupture are typical of low virulence (51).

Intrauterine infection can be limited to the decidua, spread to the chorio-amniotic space, and enter the amniotic cavity and the fetus (44,51). The amniotic cavity is generally devoid of bacteria, however, the implication of microorganisms in the membranes requires more clarity. In approximately15% of non-laboring women undergoing indicated C-section delivery with intact membranes, bacteria have been cultured from the chorioamnion (51).Fluorescence in-situ hybridization analysis with a bacterial DNA (the 16S ribosomal RNA) specific probe has detected bacteria in the membranes of almost 70% of women undergoing elective C-section at term (60).The aforementioned observations suggest that the presence of bacteria in the chorioamnion membranes alone is not sufficient to cause any inflammatory cascade, preterm labor, and preterm delivery (60).Nonetheless, bacteria in the membranes and a related inflammatory response in the amniotic fluid have been detected in more than 80% of women in early preterm labor with intact membranes who underwent c-section delivery (44). Thus, bacterial infection probably is a predisposing factor for preterm birth.

Microorganisms can achieve entry into the amniotic cavity by: (1) moving up from the vagina and the cervix;(2) haematogenous dissemination across the placenta;(3) accidental insertion at the time of invasive diagnostic procedures; and (4) by retrograde advancement through the fallopian tubes (Figure 5)(51,61).

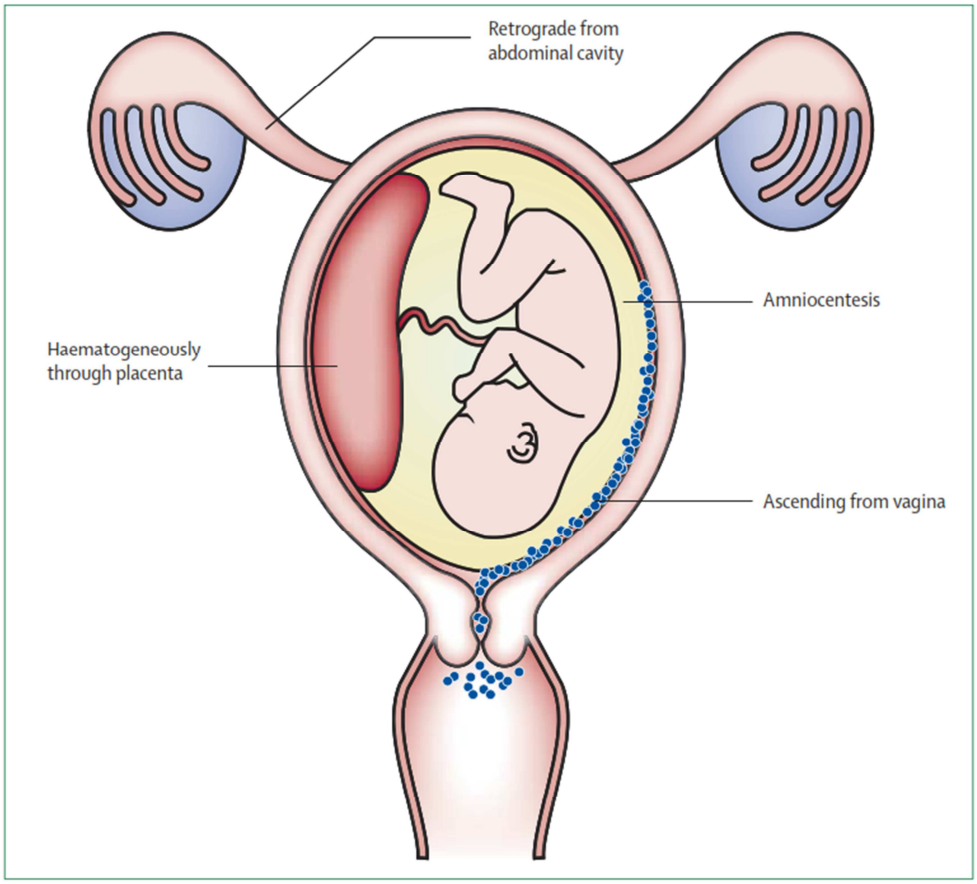


Figure 5: Possible pathways of intrauterine infections. (Adapted from (37))

The most common access way is the ascending route via the vagina and cervix. Even though it is widely believed that ascent occurs during the second trimester, the definite timing is unidentified; some women have endometrial colonization without any related manifestations before pregnancy(62). Irrespective of the timing colonization occurrence, it is hypothesized that at 20 weeks gestation when the membranes become firmly applied to the decidua, typically creating an abscess, only then do the colonized women manifest symptoms and progress to early preterm birth (51).

Fetal infection is the most extreme and severe stage of ascending intrauterine infection. It has been reported by Carroll and colleagues that fetal bacteraemia is

observed in 33% of fetuses with positive amniotic fluid cultures against 4% with negative cultures(63). Another investigative study has evidenced that genital mycoplasma species were observed in 23% of umbilical cord cultures from children born prior to 32 weeks gestation(64). Both the aforementioned studies suggest that subclinical fetal infection is considerably more common than customarily recognized. The microbial incursion of the amniotic cavity is time and again associated with intra-amniotic inflammation and a fetal inflammatory feedback (65,66). The fetal inflammatory cascade has been associated with the onset of preterm labor, fetal injury, and long-term handicaps like cerebral palsy, periventricular leucomalacia, and chronic lung disease(67–69).

Bacterial vaginosis is a disorder defined by the altered microbial ecosystem of the vagina. It is diagnosticated clinically by the presence of a vaginal pH greater than 4·5, clue cells, excessive white discharge which gives a fishy whiff on exposure to potassium hydroxide (70). Laboratorial definition of bacterial vaginosis is based on the Nugent criteria in which Gram-stained smears are scored based on the count of lactobacilli, which generally favor to be low, and the existence of organisms resembling bacteroides and mobiluncus, the numbers of which trend towards higher number (71). A nugent score greater than 3 leads to the diagnosis of bacterial vaginosis and is evidenced to be correlated with a 1·5-fold to 3-fold escalation in the incidence of preterm birth (72–74). It has been observed that black women in the USA and the UK are thrice more likely to have bacterial vaginosis than their white counterparts, and this disparity might explain the excess incidence of preterm births in black women (46,75,76). The mechanistic correlation between bacterial vaginosis and preterm birth is unknown, however, it is believed that infection-causing microorganisms probably move up into the uterus prior to or initial days of pregnancy

(77,78).The clarity of the causal association of genital infections with preterm birth is lacking (79,80). Varying from none to strong a range of associations has been reported for many infections considered to be having a causative effect towards preterm birth. The effect of confounding variables has not been considered in many investigations looking at the role of genital infections as a risk factor. Nonetheless, there seems to be an association of trichomoniasis with preterm birth with a relative risk (RR) of about 1·3(81). In the presence of a maternal immune, chlamydia has shown to be associated with preterm birth with a probable RR of about 2 (82). A probable association with a RR of about 2 has been noted for syphilis and gonorrhea with preterm birth (83).Few studies have reported no association between *U.urealyticum*, vaginal group B streptococcus, and *M. hominus* colonisations and increased risk of preterm birth (79,80). Copious evidence suggests that conditions like pyelonephritis and non-symptomatic bacteriuria, appendicitis, and pneumonia, that are non-genital tract infections, are associated with, and possibly predispose to, preterm birth (80,84). Periodontal disease has been extensively investigated for association with risk for preterm birth independent of other factors and some case-control studies suggest an association of the disorder with an increased risk for preterm delivery (85,86). One likely explanation for the aforementioned association is that gingival crevice organisms, by way of transplacental passage and maternal bacteraemia, result in an intrauterine infection (87); however, after adjusting for other confounding factors, periodontal disease associated with preterm birth was not analogous to increased histological chorioamnionitis or intrauterine bacterial colonization (88). The biological underpinnings of the relation between periodontal disease and preterm births remain ambiguous. Compared to bacterial infections, there is scant indication that viral infections influence the predisposition to preterm birth.

However, when the mother is severely ill with a viral infection, like varicella pneumonia or severe acute respiratory syndrome, the chance of a preterm delivery is high (89,90).Various PCR analysis studies have shown that the viral DNAs identified in the amniotic fluid of non-symptomatic women subjected to genetic amniocentesis were usually unrelated to ensuing preterm births(91); therefore, it seems implausible that maternal viral infection plays a critical role in preterm birth, but the debate persists, and limited information currently available warrants further study (92).

Numerous studies have evidenced an association between uterine contraction frequency and preterm birth (93–95); withal, uterine contractions do not predict preterm birth effectively in singletons due to the wide variation in frequency in normal pregnancy and the huge overlap in number of women who do and do not deliver preterm(95). Similar results were reported in a study in twins by Newman and colleagues(96); however, women who do not deliver in spite of being admitted with a diagnosis of preterm labor, remain at increased risk of subsequent preterm labor and PPROM. Genetic association studies have been utilized to establish single-nucleotide polymorphisms in several genes associated with preterm labor and PPROM(97–99). The maternal and fetal genotypes influence the risk of preterm delivery(97). A gene-environment interaction has been indicated with a mother carrying a particular allele of the TNFα gene and bacterial vaginosis(100). Even though neither attribute alone was associated with spontaneous preterm birth, their interaction increased the risk of preterm birth. In a similar manner, maternal carriage of an allele variant in the IL6 gene did not result in an increased risk of spontaneous preterm birth for white or black women (99); nonetheless, black women who carried the IL6 allele and were affected by bacterial vaginosis had a two-fold greater risk of preterm birth than those who had the variant but did not have such infection. The interactive effect of maternal smoking

and gene polymorphism on birth weight has also been outlined (101). The whole set of proteins encoded by the genome is called proteome, and proteomics studies the entire set of proteins (102). Amniotic fluid and serum collected from women with PPROM and preterm labor have been examined to determine biomarkers for the same.In a study in which bacteria were introduced into the amniotic fluid of rhesus monkeys and the proteomic reactions were monitored over a period (103); several new infection markers were identified. Identical proteins were established in amniotic fluid of pregnant women who had chorioamnionitis-associated preterm labor. Hence, proteomics can be used to determine biomarkers in women with premature delivery and PPROM.

2.1. PPROM

The term premature rupture of membranes (PROM) is used to describe the rupture of fetal membranes prior to the onset of labor or typical uterine contractions. This can occur at term (after 37 weeks of gestation) or preterm (before 37 weeks of gestation)(104). The term PPROM is used to define the spontaneous rupture of the gestational membranes at less than 37 weeks gestation at least 1 hour before the onset of contractions. It is one of the main causes of prematurity and accounts for 30–40% of all preterm births. PROM is the reason for complications in 8–10% of all pregnancies. It arise in about 1–3% of all pregnancies(105). Prematurity is a global health issue accounting for 80% of all neonatal deaths and 60% of all juvenile neurologic handicaps(106). PPROM leads to substantial perinatal morbidity related to prematurity such as neonatal sepsis, respiratory distress syndrome, umbilical cord prolapse, placental abruption and fetal death(107). It leads to about 20% of all perinatal mortality and is also identified to play a role in maternal morbidity.

2.1.1. Pathogenesis

The pathophysiology of PPROM is complex. It is a multifactorial condition. The ulitmate pathway which is common in the occurrence of PROM is disruption of fetal membranes. Fetal membranes are intricate structures with two constituents: relatively thick and cellular choriodecidua, and thinner and stronger the amnion. The chorion and amnion are closely held together and consist of multiple cell types, inclusive of epithelial cells, trophoblast cells, and mesenchymal cells inset in a collagenous matrix (3). The amnion is responsible for almost 20% of the thickness of the fetal membranes but determines the mechanical response of the fetal membranes (108). The stability and cohesiveness of fetal membranes are owed to extracellular membrane proteins, like collagens, fibronectin, and laminin. Matrix metalloproteases (MMPs) diminish membrane strength by escalating collagen degradation. Tissue inhibitors of MMPs attach to MMPs and aid in maintaining membrane integrity by suppressing MMP-related proteolysis. The physical stability of these membranes helps maintain the pregnancy until term, and in most of the women, rupture of these membranes occurs after the natural onset of contractions or after artificial interference during labor. Multiple pathological events (such as, subclinical or apparent infection, inflammation, bleeding, mechanical stress) can disrupt this and other homeostatic mechanisms and trigger a series of biochemical processes that result in PROM. Rupture of the fetal membranes is accelerated by stretch forces manipulating biochemically mediated, pre-weakened fetal membranes (109). The strength of the fetal membranes deteriorates in late gestation as a result of biochemical changes implicated by extracellular matrix remodeling and apoptosis(110). The pre-weakening could develop before term as a result of defective collagen, infections, or other inflammatory mechanisms. Acute inflammation is linked with increased proteolytic enzymes and activation of

cytokines, which precipitate the cascade of matrix breakdown(111). Hemorrhage in early pregnancy can result in membrane weakness as it also provokes the inflammatory cascade. The decidual production of thrombin to commence hemostasis is brought about by decidual hemorrhage or abruptio placenta. Similar to cytokines, thrombin has been indicated to induce dose-dependent deterioration of the fetal membranes *in vitro* with associated remodeling and apoptosis as seen in term fetal membranes(112). Heightened mechanical stretch of fetal membranes can also result in loss of stability and resultant rupture of membranes distant from term (113). Some of the conditions that could result in increased stretching of the membranes are twin gestation and polyhydramnios.

2.1.2. Risk Factors

Even though there seems to be no specific etiology for PPROM, there are a number of risk factors that have been identified(114). Research indicates that the presence of specific risk factors increases the probability of PPROM occurrence compared to others. The risk components that contribute to PPROM can be broadly classified based on their origin as maternal, uteroplacental, or fetal (Table 1). The most prevalent risk factors at play include decidual hemorrhage (placental abruption), excessive uterine stretch, intrauterine infection, and maternal or fetal stress. These factors stimulate mechanisms that cause triggering of factors and subsequent pathways that inhibit uterine quiescence leading to PPROM(115,116).

Intrauterine infection is commonly identified as a precursor. Preterm spontaneous delivery with intact membranes and PPROM, in general, have common etiological factors, however, infections and tobacco exposure are also pivotal in PPROM (117). Most women with PPROM begin labor spontaneously but delivery in a small proportion of women is halted for weeks or months. Under normal

circumstances gestational membranes form a barrier to ascending infection, a prevalent complication of PPROM is the development of intrauterine infection and preterm labor (118)

History of PPROM in a previous pregnancy has been a constant risk factor for PPROM (114,116). The recurrence risk is 16–32 percent when compared to the 4 percent in women with previous uncomplicated term delivery(119). It has also been shown that black women have an increased risk of PPROM due to the greater likelihood of placenta abruption in comparison to other ethnicities(120,121). A decrease in collagen content is linked to PROM, as well as vascular disorders like Ehlers-Danlos and sytemic lupus erythematosus with collagen involvement(116). Risk determinants that have been shown to be associated with PPROM resulting in uterine distension include situations like multiple pregnancies and polyhydramnios. Additional risk factors comprise anemia, lower body mass index, chronic steroid therapy, abdominal trauma, history of sexually transmitted infections, smokers, illicit drug use, medical procedures (cerclage and amniocentesis), and vaginal hemorrhage. Invasive diagnostic procedures executed during pregnancy (such as chorionic villus sampling, amniocentesis, fetoscopy, and cervical cerclage) can impair the membranes, causing them to leak, however, these rarely cause PPROM. Nearly all cases of PPROM develop in otherwise healthy women without detectable risk factors(116). Epidemiological and historical factors like sexual intercourse, maternal exercise, speculum examinations, and parity have shown to be not associated with PPROM (122).

A) ***Maternal factors***
➢ PPROM in a prior pregnancy (recurrence risk is 16–32% as compared with 4% in women with a prior uncomplicated term delivery) ➢ Antepartum vaginal bleeding ➢ Chronic steroid therapy ➢ Collagen vascular disorders (such as Ehlers-Danlos syndrome, systemic lupus erythematosus) ➢ Direct abdominal trauma ➢ Preterm labor ➢ Cigarette smoking ➢ Illicit drugs (cocaine) ➢ Anemia ➢ Low body mass index (BMI <19.8 kg/m2) ➢ Nutritional deficiencies of copper and ascorbic acid ➢ Low socioeconomic status
➢ Unmarried status
B) ***Uteroplacental factors***
➢ Uterine anomalies (such as uterine septum) ➢ Placental abruption (may account for 10–15% of preterm PROM) ➢ Advanced cervical dilatation (cervical insufficiency) ➢ Prior cervical conization ➢ Cervical shortening in the second trimester (<2.5 cm) ➢ Uterine overdistention (polyhydramnios, multiple pregnancy) ➢ Intra-amniotic infection (chorioamnionitis) ➢ Multiple bimanual vaginal examinations (but not sterile speculum or transvaginal ultrasound examinations)
C) ***Fetal factors***
➢ Multiple pregnancy (preterm PROM complicates 7–10% of twin pregnancies)

Table 1: Contributory risk factors towards spontaneous (PPROM) (Adapted from (123))

2.1.3. Clinical features

Generally, a woman shows up with a history of a gush of fluid down the legs, trailed by a steady trickle or a sensation of “wetness”. Consistency, color, and smell of the discharge are critical factors to be considered during history taking and examination. Other typical features which present in cases with intraamniotic infection include abdominal pain, fever, and a foul-smelling vaginal discharge.

An evaluation of vital signs is essential to rule out infection. Tachycardia and increased temperature in the mother along with abdominal tenderness are signs of infection. Reduced amniotic fluid could lead to palpable fetal parts. A sterile speculum examination is necessary for patients who are suspected of PPROM. The examiner is required to keep a lookout for pooling of liquor in the posterior fornix. The patient may be asked to cough or carry out Valsalva maneuver while the speculum exam is being done if liquor is not obviously seen to leak out of the cervical orifice. In case of no pooling of liquor, the vaginal fluid should be obtained for subsequent confirmatory tests. The pooled fluid may also be collected and sent to check for fetal lung maturity, in cases where the gestational age is greater than 32 weeks. At the time of the speculum examination, assessment for cervical dilatation and the presence of cord prolapse is recommended. It is recommended to send the cervical secretions for cultural analysis.

2.1.4. Diagnosis

PPROM is mainly diagnosed clinically. The subject generally presents with a history of vaginal fluid leakage. Subsequently, a sterile speculum examination is performed to check for the rupture of membranes by inspecting for pooling of amniotic fluid in the posterior fornix or clear fluid trickling from the cervical canal.

The confirmation of the clinical diagnosis can later be done using laboratory tests. Sonographic investigations are also initiated to evaluate the amniotic fluid volume, determine the presenting part, estimate gestational age and fetal weight.

A detailed history collection should be performed for all patients presenting with leakage of fluid. Including a history of current illness, obstetric history, gynecological history, medical and surgical history, and other facts related to the socioeconomic background of the subject is recommended. While gathering the details of current illness, it is critical to inquire regarding contractions, fetal movement, the instance of possible rupture, volume, color and odor of fluid, presence of vaginal bleeding, pain, recent physical activity, recent trauma, and recent sexual encounters.

Physical evaluation should be carried out such that it minimizes the risk of infection. Concurrently with the speculum exam, patient should be checked for signs of umbilical cord prolapse, cervicitis, vaginal bleeding, or fetal prolapse. Unless the subject appears to be in active labor or delivery anticipated, the digital examination should be avoided (124).Typical confirmatory diagnosis of membrane rupture is the visualization of amniotic fluid trickling down the cervical canal to pool in the vagina.

Arborization, or ferning, can be identified when amniotic fluid is observed under a microscope. A pH test of vaginal fluid can be carried out. The amniotic fluid generally has a pH of 7.1-7.3, while normal vaginal secretions have a pH of 4.5-6.0. The presence of blood or semen, alkaline antiseptics, or bacterial vaginosis can cause false-positive pH test results. However, prolonged rupture of membranes can give rise to false-negative results. Definitive diagnosis of PROM solely based on the above evaluation can sometimes be difficult for which there are additional tests that may aid

in the diagnosis. A sensitive but nonspecific test for rupture of membranes is fetal fibronectin.

Furthermore, there are numerous commercially available investigation kits for amniotic proteins which are reported to have high sensitivity for the diagnosis of rupture of membranes(125). If the diagnosis remains ambiguous even after a complete evaluation, the ultrasound-guided introduction of indigo carmine dye can be utilized to detect membrane rupture by assessing if the dyed fluid has transferred through the vagina (using a tampon or pad). The staining blue of the tampon or pad from the dye confirms the rupture of membranes.

2.1.5. Treatment and Management

Multiple factors are taken into consideration when it comes to PPROM management. The pivotal factors are gestational age and the presence of infection. Secondary factors include maternal comorbidities, fetal well-being, fetal weight, presence of contractions or cervical dilation. Prematurity is an enormous concern in women with PPROM. Ancillary complications include oligohydramnios, chorioamnionitis, necrotizing enterocolitis, cord compression, abruption placenta, respiratory distress syndrome, neurologic impairment and antepartum fetal death(114). Lack of complications centers the management strategy to expectant management until 34 weeks, while in the cases with gestational age beyond 34 weeks, delivery is deliberated after the fetal lung maturity is ascertained.

The management strategy of patients with premature rupture of membranes is determined by gestational age.

- Gestational age of 37 0/7 weeks or more(Early term and term patients): progresses into delivery and administration of Group B Streptococcus prophylaxis is performed as indicated
- Gestational age of 34 0/7- 36 6/7 weeks (Late Preterm): similar treatment strategy as followed in early term and term subjects
- Gestational age of 24 0/7 – 33 6/7 weeks (Preterm): watchful waiting, administration of latency antibiotics, one course of corticosteroids, if GBS status is unknown antibiotic prophylaxis as indicated is recommended
- Gestational age less than 24 weeks: subject counselling, induction of labor or expectant management, as early as 20 0/7 weeks of gestation antibiotics administration can be considered, before viability tocolysis/magnesium sulfate/GBS prophylaxis/corticosteroids are not advocated

Chorioamnionitis and nonreassuring fetal status (NRFS) are definite indicators for delivery. Vaginal bleeding may be an indicator of placental abruption and delivery considered in such cases. Conditions like fetal status, amount of bleeding, the stability of the mother, and gestational age are to be considered while making the decision for delivery. In case spontaneous labor does not develop near the time of presentation in term subjects, labor should be induced.

Typically, patients with preterm PROM are admitted to hospital with periodic evaluation for infection, umbilical cord compression, placental abruption, fetal well-being, and labor. Periodic sonographic evaluations are performed to monitor fetal statistics.Vitals are monitored and a rise in maternal temperature raise the suspicion of intrauterine infection. Continual monitoring of inflammatory markers and leukocytes have not been proven to be useful in the diagnosis of infection as they are identified to

be nonspecific if there is no clinical evidence of infection. A transient leukocytosis can also be caused by administration of corticosteroids.

The administration of tocolytics to patients with preterm PROM is debatable. Though prophylactic tocolytics are found to be associated with a longer duration of latency and a curtailed risk of delivery within 48 hours, it is also correlated to a greater risk of chorioamnionitis in pregnancies prior to 34 weeks of gestation. Significant maternal or neonatal benefit has not been evidenced for the use of tocolytics(126).

Antenatal corticosteroids administration after preterm PROM has been demonstrated to decrease respiratory distress syndrome, neonatal mortality, necrotizing enterocolitis, and intraventricular hemorrhage.One course of corticosteroids is suggested for all pregnant women between 24 0/7 weeks and 34 0/7 weeks of gestation in case of a predetermined risk of delivery in the next 7 days. Magnesium sulfate is administered in cases in which delivery is anticipated before 32 0/7 weeks of gestation in order to reduce the risk of cerebral palsy(127).

Prolonged pregnancy, reduction in maternal and neonatal infections, and reduced fetal morbidity have been observed by the administration of antibiotics. A seven-day medication course of therapy is suggested for women who are at less than 34 weeks of gestation and have presented with preterm PROM. The recommended administration plan is the intravenous application of ampicillin (2g each 6 hours) and erythromycin (250 mg each 6 hours) for two days, subsequent oral administration of amoxicillin (250 mg each 8 hours) and erythromycin base (333 mg each 8 hours).The aforementioned seven-day regimen is to be completed. Due to increased rates of necrotizing enterocolitis amoxicillin-clavulanic acid is not

recommended.When there is a patient is a candidate for GBS prophylaxis due to predetermined factors,the subject is administerd antibiotics to prevent vertical transmission of GBS (128). A previous history of preterm PROM puts the woman at an increased risk of recurrent PROM and preterm birth. Progesterone supplementation have been shown to reduce the risk of spontaneous preterm birth.

Gestational age	Management
34 weeks or more	Proceed to delivery, by induction of labor or a by cesarean section if indicated Group B prophylaxis is recommended
32–33 completed weeks	Expectant management unless fetal lung maturity is documented Group B streptococcus prophylaxis is recommended Corticosteroids should be administered Antibiotics to prolong latency
21–31 completed weeks	Group B streptococcus prophylaxis is recommended Corticosteroids should be administered Antibiotics to prolong latency Magnesium sulfate should be administered incase delivery is imminent for neuroprotection
Before 24 weeks	Patient counseling Induction of labor or expectant management Group B streptococcus prophylaxis is not recommended Corticosteroids are not recommended Antibiotics administration

Table 2: Summary of treatment and management of PPROM (Adapted from (123))

2.1.6. Complications

Of the many complications associated with PPROM, the most common and crucial one is early or premature delivery. An inverse relationship exists between the latency period, the time duration between membrane rupture until delivery, and the gestational age at which PPROM occurs (129). An international investigative study conducted in Canada, United Kingdom and Australia established that marginally more than half of the women who presented with PPROM at 16–26 weeks' gestation had a latency period of 1 week with almost one fourth of such subjects delivering after 4 weeks. Comparatively, almost 95% of women presented with PROM near term had a

latency interval of nearly 24 hours(130). There is a 3 to 4-fold increase in risk of neonatal or perinatal mortality(116,131).

Neonates surviving PPROM are mostly affected by multiple complications, both before and after delivery. Some of the likely complications are oligohydramnios and intrauterine infection which develops in 15–30% of women who presents with preterm PROM and is responsible for 3–20% of neonatal deaths, umbilical cord prolapse, umbilical cord compression, and abruption placenta. Soon after delivery a neonate is at risk of neurological impairment, necrotizing enterocolitis, intraventricular hemorrhage, respiratory distress syndrome (occurring in 10–40% of neonates), patent ductus arteriosus, retinopathy of prematurity, limb restriction deformities which presents in and complicate nearly12% of preterm PROM, pulmonary hypoplasia which happens in 26% of preterm PROM before 22 weeks of gestation and fetal asphyxia(132–137). Cord accident, infection, and other risk determinants contribute to the 1–2% risk of intrauterine fetal fatality (stillbirth) after preterm PROM(116,131).

Probable maternal complications include, coagulopathy, chorioamnionitis and funisitis, postpartum endometritis, septicemia (in almost 33% of the mothers) and delivery by cesarean section(136,138,139).

2.2. Vaginal dysbiosis as a risk factor of preterm premature rupture of membrane (PPROM)

The vagina is a crucial and composite ecosystem, with predominantly *Lactobacilli* species, but also comprising of a limited number of parasites and fungi. A balance in the aforementioned microbial communities is vital for female health(140,141). Nevertheless, this microbial balance can be disturbed due to various

reasons and result in multiple infectious diseases (140) and non-infectious diseases, like induced abortions, miscarriage, preterm birth, infertility, and menstrual disorders (142–147) presenting a grievous threat to the reproductive health of women.

There has been an increase in discussions in the past decade regarding the influence of vaginal dysbiosis (explained as not dominated by *Lactobacilli* species), the lack of balance of the vaginal commensal bacterial communities (microbiota), on the pregnancy outcomes and whether it should be tracked antenatally(148,149). There is an especially huge burden of vaginal-dysbiosis-related health issues sub-Saharan Africa(150).

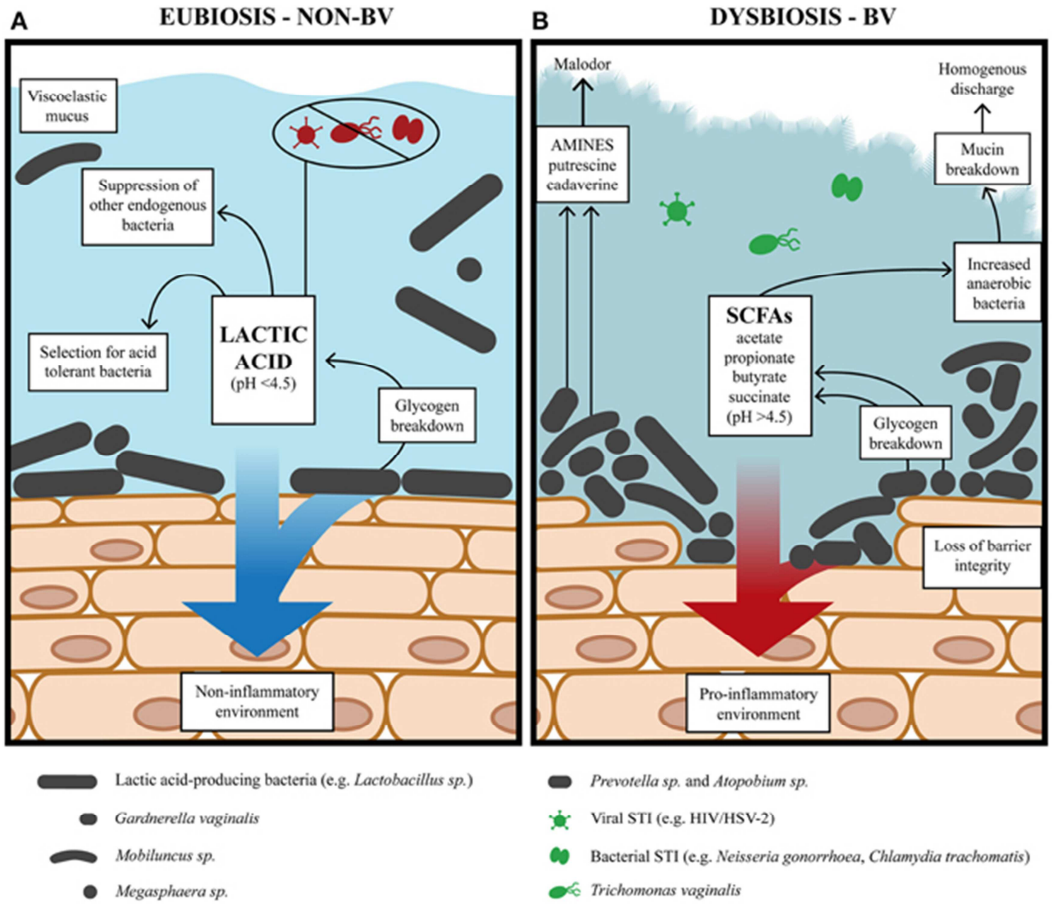


Figure 6: Representation of the vaginal environment during alternative states of eubiosis and vaginal dysbiosis (Adapted from (151)).

In general, the vaginal microbiota (VMB) comprises microorganisms that exist in a composite dynamic, and mutually beneficial association with the host (152). Healthy VMB are critical to the female genital tract. Normal VMB comprise predominantly of hydrogen-peroxide-producing *Lactobacillus* species that bring about an immunological equilibrium and thus support a healthy reproductive tract(153–155). Nevertheless, multiple factors modulate the VMB architecture, like sexual and hygienic practices, ethnicity, nutrition, and hormonal fluctuations during menstruation or pregnancy (156).

Bacterial vaginosis (BV) the most prevalent vaginal dysbiotic condition worldwide. At any given time, approximately 10–30% of women presents with this condition,with a higher predominance in sub-Saharan African women in comparison to rest of the world (157–159). The aforementioned vaginal condition arises as a result of a shift in the *Lactobacillus*-dominant microflora to a more diverse microfloral community, rich with anaerobic bacteria like species of *Gardnerella, Atopobiumgenera* and *Prevotella* (160). The prevalence of bacterial vaginosis diversify with socioeconomic conditions, ethnicity, and gestational age.

Previous investigations have shown that pregnant women with bacterial vaginosis(BV) had around two-fold increased risk of preterm birth than women without BV, which was hypothesized to be caused by moving up of the BV-related bacteria from the vagina to the uterus, leading to chorioamnionitis, deciduitis, endometritis, and infection of the amniotic fluid infection(161,162). Research exploring the mechanisms of BV facilitating miscarriage showed that BV-related bacteria produce lytic enzymes (like phospholipases, proteases, etc.) which cause lysis of the fetal membranes, and thereby induce the production of prostaglandin, which in turn promote uterine muscle contraction, reduce the cervical resistance, and trigger

the release of metalloproteinases (MMPs) to degrade the chorioamniotic membranes (163). In addition to this, elevated levels of inflammatory cytokines IL-6 and IL-8 were also observed in the amniotic fluid of pregnant women presented with BV (164).A randomized controlled trial demonstrated that pregnant women who were administered clindamycin for BV had little chance for miscarriage than their counterparts who were given placebo treatment (165).

These findings suggest that early screening and treatment of BV have a preventive effect on infection-induced miscarriage. Furthermore, a clinical study showed that hydrogen peroxide producing *Lactobacillus* in the vagina could enhance vaginal health by modifying the microbial and inflammatory status, as well as creating a supportive uterine environment for implantation and placentation(166). Mounting of evidence show that BV could also escalate the risk of preterm birth and infertility, primarily facilitating sexually transmitted infection and evoking intrauterine inflammation(143,167). Recent evidence reveal that *Lactobacillus*-dominated vaginal microbiota is negatively correlated with preterm birth and could help prevent adverse fertility outcome by regulating vaginal microbiota and inflammatory cytokines like IL-4 and IL-10 (143,168). Hence it can be hypothesized that antibiotics and probiotics can prevent the preterm delivery and infertility by remodeling the vaginal and uterine microbiota and eubiosis.

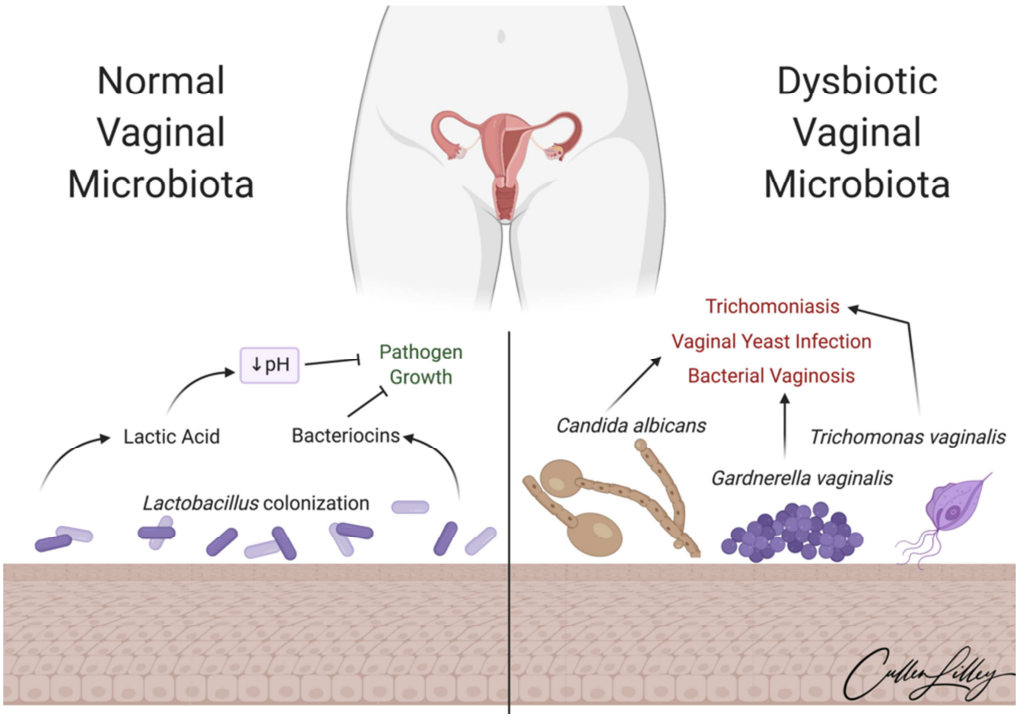


Figure 7: Vaginal Microbiota and Dysbiosis. (Adapted from https://www.pathelective.com/micromeded)

Various independent studies have demonstrated an association between BV and preterm birth (PTB), along with maternal infection, miscarriage, and low birth weight (LBW) (158,160,169). It has been confirmed by two meta-analyses that BV at the time of pregnancy raised the risk of preterm birth by greater than 2-fold (35,170). A distinct vaginal dysbiotic condition, aerobic vaginitis (AV), is indicated by anomalous composition of VMB predominantly of commensal aerobic microorganisms of intestinal origin, mostly *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative staphylococci essentially *Staphylococcus epidermidis*, *Enterococcus faecalis*, and group B *Streptococcus* (*Streptococcus agalactiae*)(171–176). AV has also been linked to an increased risk of preterm prelabour rupture of membranes, spontaneous miscarriage, chorioamnionitis, and preterm delivery (PTD)(177–179). Accumulating evidence is suggest that vaginal dysbiosis is associated with multiple adverse pregnancy outcomes, essentially an increased risk of

early and late miscarriage, post-abortion infection, postpartum endometritis, histological chorioamnionitis, premature ruptureof membranes (PROM), and preterm birth (PTB)(3,169,180–188). A considerable decrease in vaginal microfloral richness (number of species), heterogeneity (the overall richness and evenness of species), and relative abundance of each species at the time of pregnancy is associated with PTB(189).

Moreover, when bacterial pathobionts colonise in the vagina individually,(especially symbionts with potential pathogenic characteristics, like *streptococci*, *staphylococci*, or *Enterobacteriaceae* species) during pregnancy can result in adverse pregnancy outcomes(190,191). Son et al. has demonstrated that a *Klebsiella pneumoniae*-dominant vaginal microflora may perhaps be linked to PTB prior to 28 weeks of gestational age (GA), *Lactobacillus iners* with PTB prior 34 weeks of GA, and *S. agalactiae* with late miscarriage(192). On the contrary, VMB predominated by *L. crispatus* has not been correlated with PTB (192–194).

It has been hypothesized that the heightened risk of negative reproductive and pregnancy outcomes could be the result of the BV-related bacterial species.Considering the cases of in-vitro fertilization (IVF) studies, patients with rich incidence of *Gardnerella*, *Atopobium*, and *Prevotella* failed to have a successful pregnancy after embryo transfer(195,196). Most certainly, in initial stages of pregnancy, a decline in lactobacilli and the overgrowth of *Gardnerella*, *Atopobium*, and *Prevotellagenera* disturb the microbial homeostasis, impeding embryo implantation (196). It has been observed that anaerobe bacteria impact the later stages of pregnancy. For example, DiGiulio et al. has documented a strong association between PTB and the presence of *Gardnerella* and *Ureaplasma*, both of which are BV-related bacteria (197). In contrast to the aforementioned findings no such

correlation was reported in a larger study conducted by Romero et al. (198). The modest sample sizes and diversity in the study populations may be able to explain the discrepancies between the two studies. The former study was conducted in Caucasian women (60%), while the latter was mostly African American women (86%)(197,198).

The basic differences in the VMB amid women of disparate ethnic backgrounds have been reported in a comparative study by Ravel et al. (199). It has been detailed in the study that healthy nonsymptomatic women of African ancestry living in North America had higher diversity and less lactobacillus-rich vaginal microflora than women from other ethnic backgrounds living in the same lifestyle setting(199). Accumulating evidence regarding vaginal dysbiosis suggests that non-Lactobacillus-dominant vaginal microflora may not be comparable to clinically significant dysbiosis in nonsymptomatic healthy non-Caucasian women(200). Till date, most vaginal dysbiosis profiling studies, specifically those with respect to pregnancy, have been done among Caucasians in Europe or North America. There is accumulating research interest in comprehending how vaginal microflora composition and the associated conditions might confer to, and extend the diagnostic potential for, antagonistic pregnancy outcomes. There is a lacuna in our understanding of vaginal dysbiosis and the associated consequences of pregnancy under the circumstances with increased rates of pregnancy complication.

3. MATERIALS AND METHODS

3.1. Study design

An observational longitudinal study design was adopted. The clinical assessments and sample collection was done on the same day of subject recruitment after obtaining written informed consent from the subjects on the consent forms approved by the institute's ethics committee.

3.2. Selection of patients

The subjects were recruited from the outpatient services of the Department of Obstetrics and Gynaecology of KAHER's Dr. Prabhakar Kore Charitable Hospital, Belgaum, Karnataka. The demographic and clinical information (history of presenting illness as well as any other medical illness, family & personal history, nutritional & life style related history) regarding the patients were collected with the help of structured scales and proforma.

Inclusion criteria:

Antenatal women attending the outpatient department of obstetrics and gynecology at KAHER's Dr. Prabhakar Kore Charitable Hospital, Belagavi during $14^{+0/7}$ to $20^{+6/7}$ weeks of gestation.

Exclusion criteria

- History of preterm birth in previous pregnancies
- Uterine Malformations
- Cervical Incompetence/Cervical Encirclage

- Multiple pregnancies
- History of threatened abortion in 1st trimester

3.3. Study period

The study extended for a period of 15 months.(January 2020 to March 2021)

3.4. Sample size

The sample size for the study was determined using the formula

$$n = \frac{Z^2_{1-\alpha} * SD^2}{d^2}$$

where $Z_{1-\alpha} = 1.96$ (for 95% CI)

z_α is linked with the level of significance.

d is variate. Here, d=45% of standard deviation

Based on the above formula, the sample size of the study came upto n=107 (with 10% attrition)

3.5. Sample collection and processing

Two high vaginal swabs from posterior vaginal fornix were taken. One swab was used for gram staining (primary smear) . The same swab was used for primary plating which was done on media consisting of blood agar and Macconkey agar. All cultures were incubated at 37°C overnight (18-24hours) and next day colony morphology and gram staining was done from the colony. The organism identification was carried out subsequently.

Another swab was saved for subsequent slide preparation to look for any fungal growth. Sabauraud's dextrose agar (SDA) was used for that and incubated at 35°C for 18-24 hours.

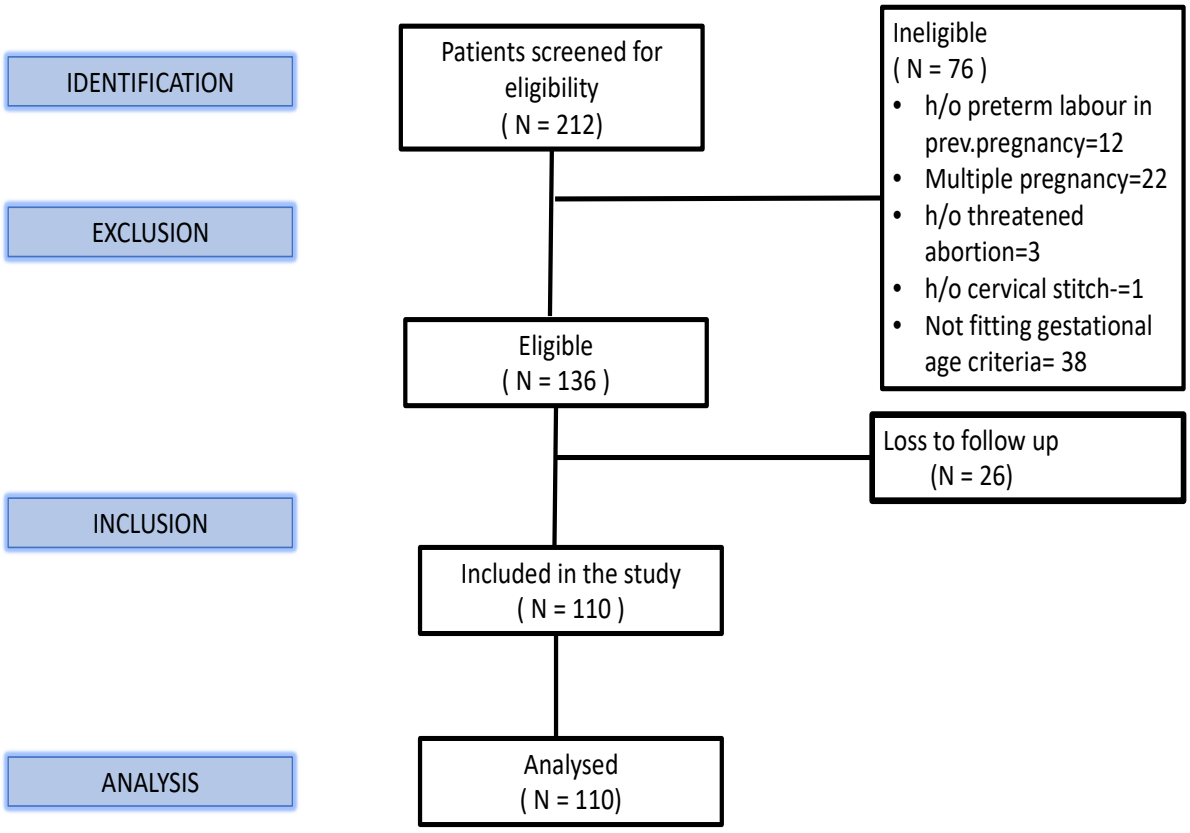
All the patients were followed up until their delivery and the pregnancy outcome was recorded. Four outcome categories were recorded namely PPROM, PROM, Preterm birth or Term delivery. The outcomes were then correlated with the microbiological profiling done in early second trimester.

3.6. Statistical analysis

The plan of analysis for the data generated were as follows:

The quantitative variables mean and standard deviation were calculated. The continuous variables were compared using suitable tools of statistics like unpaired student's t test. Suitable graphs were used to depict the comparison. The categorical data were expressed in terms of rates, ratios and percentages. For intergroup comparison of quantitative data, paired and unpaired t-tests were used. Chi square test was used for intergroup comparison of qualitative data. Discrete variables over time were analyzed using repeated measures ANOVA. For all the tests the value of p less than 5% (0.05) was considered as significant.

4. RESULTS


IDENTIFICATION
EXCLUSION
INCLUSION
ANALYSIS
Patients screened for eligibility (N = 212)
Ineligible (N = 76)
• h/o preterm labour in prev.pregnancy=12
• Multiple pregnancy=22
• h/o threatened abortion=3
• h/o cervical stitch-=1
• Not fitting gestational age criteria= 38
Eligible (N = 136)
Loss to follow up (N = 26)
Included in the study (N = 110)
Analysed (N = 110)

4.1. Sample attributes

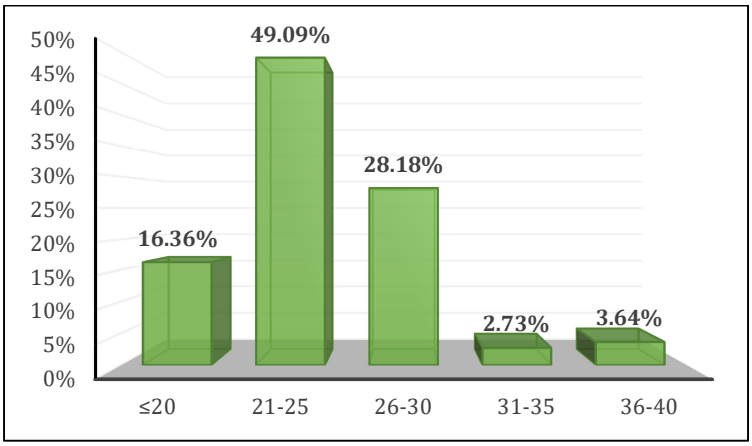
The study was a longitudinal observational study and comprised of 110 antenatal women between $14^{+0/7}$ to $20^{+6/7}$ weeks of gestation. The demographic and clinical profile of subjects has been described in **Table 3.** Out of 110 subjects, 54.55% were multigravida and 45.45% were primigravida. 72.73% had gestation age >15 weeks. There was no history of preterm delivery/PROM/PPROM, short cervix/ cervical cerclage threatened abortion or uterine anomalies in any of the subjects. 81.82% of the patients had normal Nugent scores (Nugent <=3). The distribution of the study subjects based on the different study parameters (like age, parity, gestational age, Nugent score, aerobic culture, fungal culture, PPROM, PROM, spontaneous preterm, uncompleted term vaginal delivery, term C-section delivery, and preterm C-Section delivery) is graphically represented in **Figures 8,9,10 and 11.**

Table 3: Demographic and clinical profile of the study participants

Variables	Sub Category	Number of subjects (%)
Age (years)	≤20	18 (16.36%)
	21-25	54 (49.09%)
	26-30	31 (28.18%)
	31-35	3 (2.73%)
	36-40	4 (3.64%)
	Mean ± SD Median (Min, Max)	24.55 ± 4.12 24 (18, 40)
Parity	Multi	60 (54.55%)
	Primi	50 (45.45%)
Gestational Age (weeks)	>15	80 (72.73%)
	≤15	30 (27.27%)
H/O Preterm delivery/PROM/PPROM	No	110 (100%)
H/O Short cervix/ Cervical cerclage	No	110 (100%)

H/O Threatened abortion	No	110 (100%)
H/O Uterine anomalies	No	110 (100%)
Nugent Scoring	Abnormal	20 (18.18%)
	Normal	90 (81.82%)
Aerobic culture	Klebsiella pneumoniae	1 (0.91%)
	Methicillin sensitive staph aureus	1 (0.91%)
	Vaginal commensals	28 (25.45%)
	Nil	80 (72.73%)
Fungal culture	Budding yeast cells	5 (4.55%)
	Candida	5 (4.55%)
	Nil	100 (90.91%)
PPROM	No	108 (98.18%)
	Yes	2 (1.82%)
PROM	No	101 (91.82%)
	Yes	9 (8.18%)
Spontaneous Preterm	No	95 (86.36%)
	Yes	15 (13.64%)
Uncomplicated Term Vaginal Delivery	No	58 (52.73%)
	Yes	52 (47.27%)
Term C-section Delivery	No	76 (69.09%)
	Yes	34 (30.91%)
Preterm C-Section Delivery	No	108 (98.18%)
	Yes	2 (1.82%)
Indication for LSCS	ANAMNIOS	2 (1.82%)
	BOH with gestational hypertension at 34W2D	1 (0.91%)
	Breech	1 (0.91%)
	CDMR	3 (2.73%)
	CPD	2 (1.82%)
	Fetal Distress	2 (1.82%)
	K/C/O Epilepsy	1 (0.91%)
	MSL with fetal distress	1 (0.91%)

	MSL with an unfavorable cervix	1 (0.91%)
	NPL	2 (1.82%)
	Placenta previa	1 (0.91%)
	Previous pregnancy	1 (0.91%)
	Previous 2 LSCS	1 (0.91%)
	Previous 2 LSCS in labour	1 (0.91%)
	Previous LSCS in labour	5 (4.55%)
	Previous LSCS NOT W/F VBAC	4 (3.64%)
	Previous LSCS with PPROM with severe OLIGO	1 (0.91%)
	Severe FGR with pathological trace	1 (0.91%)
	No indication	79 (71.82%)



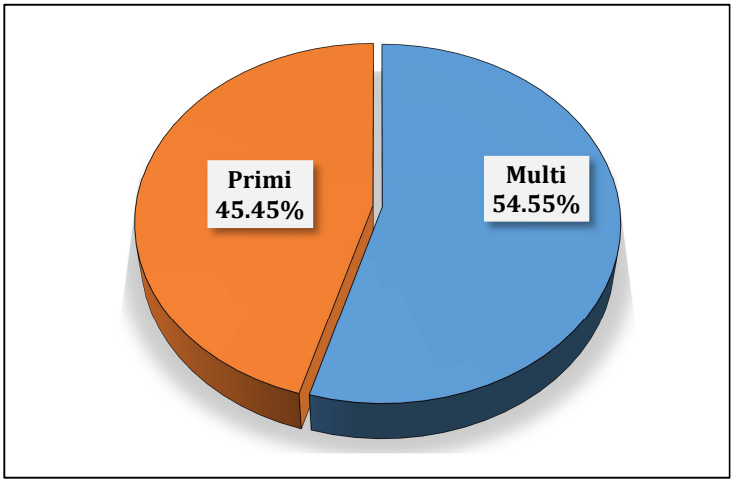
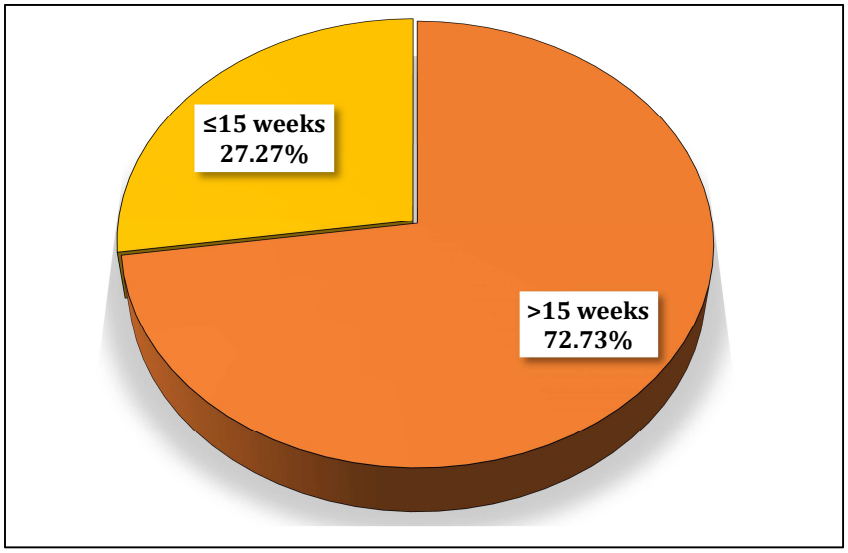


Figure 8: Distribution of subjects according to age and parity.



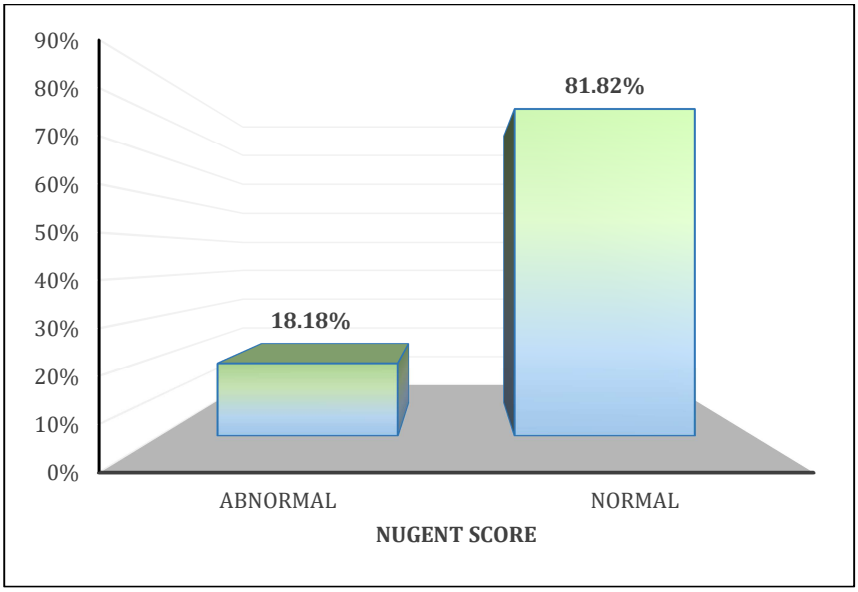
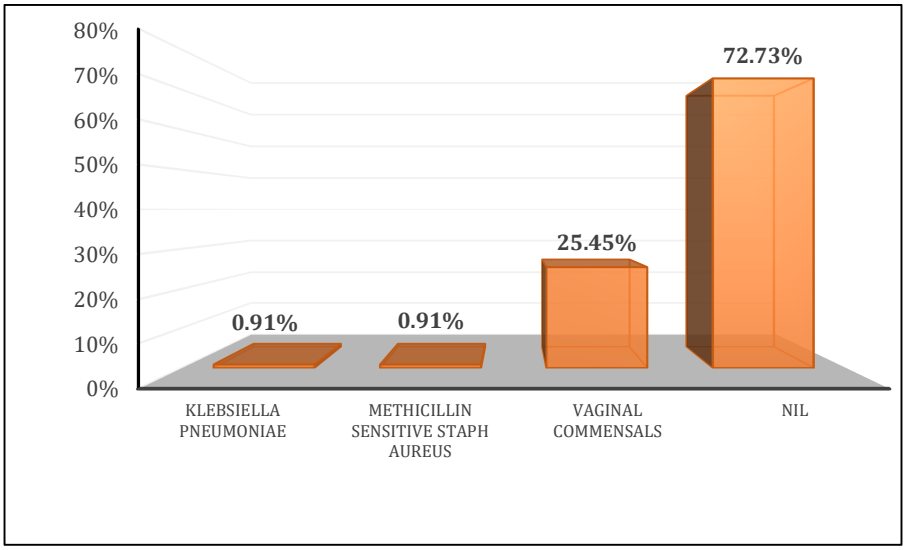


Figure 9: Distribution of subjects according to gestational age and Nugent score.



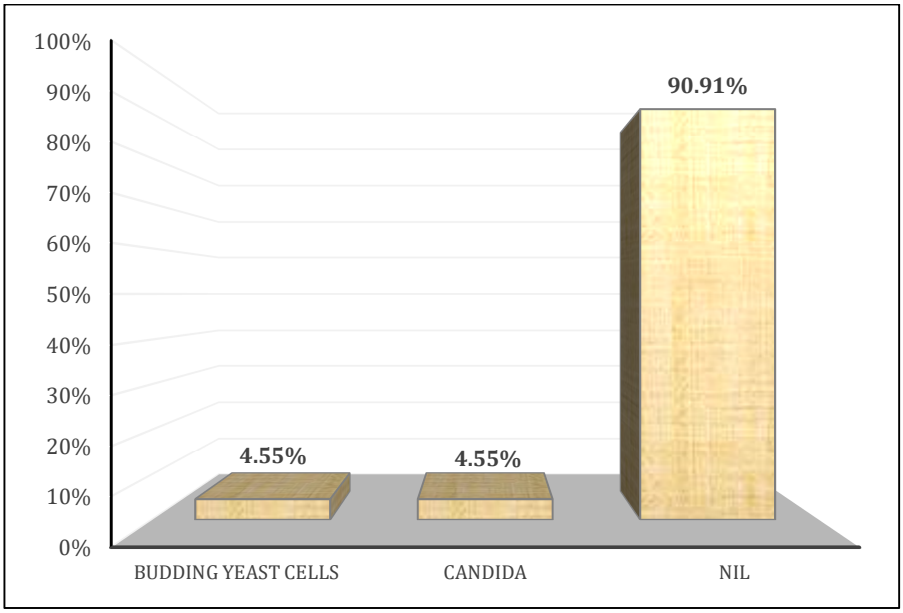


Figure 10: Distribution of subjects according to aerobic culture and fungal culture.

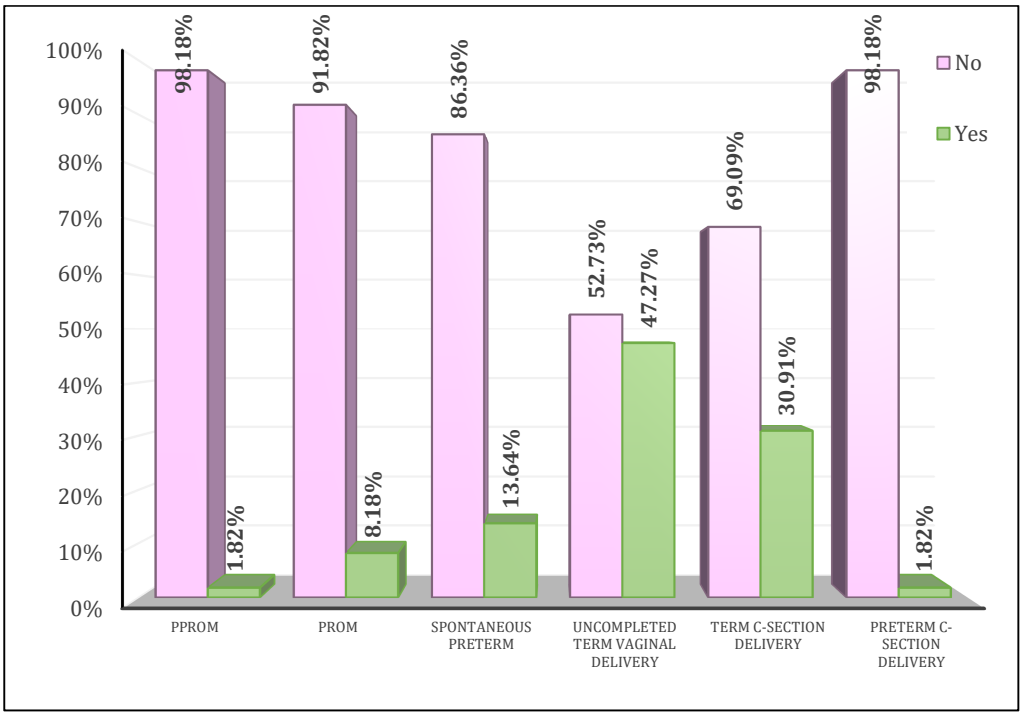


Figure 11: Distribution of subjects according to PPROM, PROM, spontaneous preterm, uncomplicated term vaginal delivery, term C-section delivery, and preterm C-Section delivery.

4.2. Association of Nugent score with other study parameters

Chi-square analysis test revealed that there is a significant association of gestational age, spontaneous preterm, and uncomplicated term delivery with Nugent score **(Table 4) (Figures 12 and 13)**. Abnormal nugent score of >3 signify vaginal dysbiosis and are associated with spontaneous preterm delivery whereas normal nugent score of <=3 is associated with term delivery. However, there is no significant association of age, parity, aerobic culture, fungal culture, PPROM, PROM with Nugent score. A two sample t-test showed no significant difference in the mean age when compared alongside the Nugent score.

Table 4: Comparison of different variables with Nugent score.

Variables	Sub Category	Nugent score		p-value
		Abnormal	**Normal**	
Age (years)	≤20	5 (25%)	13 (14.44%)	0.7361^{MC}
	21-25	9 (45%)	45 (50%)	
	26-30	5 (25%)	26 (28.89%)	
	31-35	0	3 (3.33%)	
	36-40	1 (5%)	3 (3.33%)	
	Mean ± SD Median (Min, Max)	24.35 ± 4.64 24 (18, 38)	24.59 ± 4.02 24 (18, 40)	0.8157^{t}
Parity	Multi	12 (60%)	48 (53.33%)	0.5881^{C}
	Primi	8 (40%)	42 (46.67%)	
Gestational Age (weeks)	>15	11 (55%)	69 (76.67%)	**0.0491^{C}***
	≤15	9 (45%)	21 (23.33%)	
Aerobic culture	No	15 (75%)	65 (72.22%)	0.0808^{C}
	Yes	5 (25%)	25 (27.78%)	
Fungal culture	No	18 (90%)	82 (91.11%)	1^{MC}
	Yes	2 (10%)	8 (8.89%)	
PPROM	No	19 (95%)	89 (98.89%)	0.3448^{MC}
	Yes	1 (5%)	1 (1.11%)	
PROM	No	20 (100%)	81 (90%)	0.2199^{MC}
	Yes	0	9 (10%)	
Spontaneous Preterm	No	9 (45%)	86 (95.56%)	**< 0.001^{MC}***
	Yes	11 (55%)	4 (4.44%)	
Uncomplicated Term Vaginal Delivery	No	16 (80%)	42 (46.67%)	**0.0069^{C}***
	Yes	4 (20%)	48 (53.33%)	
Term C-section Delivery	No	16 (80%)	60 (66.67%)	0.2432^{C}
	Yes	4 (20%)	30 (33.33%)	
Preterm C-Section Delivery	No	19 (95%)	89 (98.89%)	0.3488^{MC}
	Yes	1 (5%)	1 (1.11%)	

*Abbreviation: C – Chi-square test, MC – Chi-square test with Monte Carlo simulation, t – Two-sample t-test, * indicates statistical significance.*

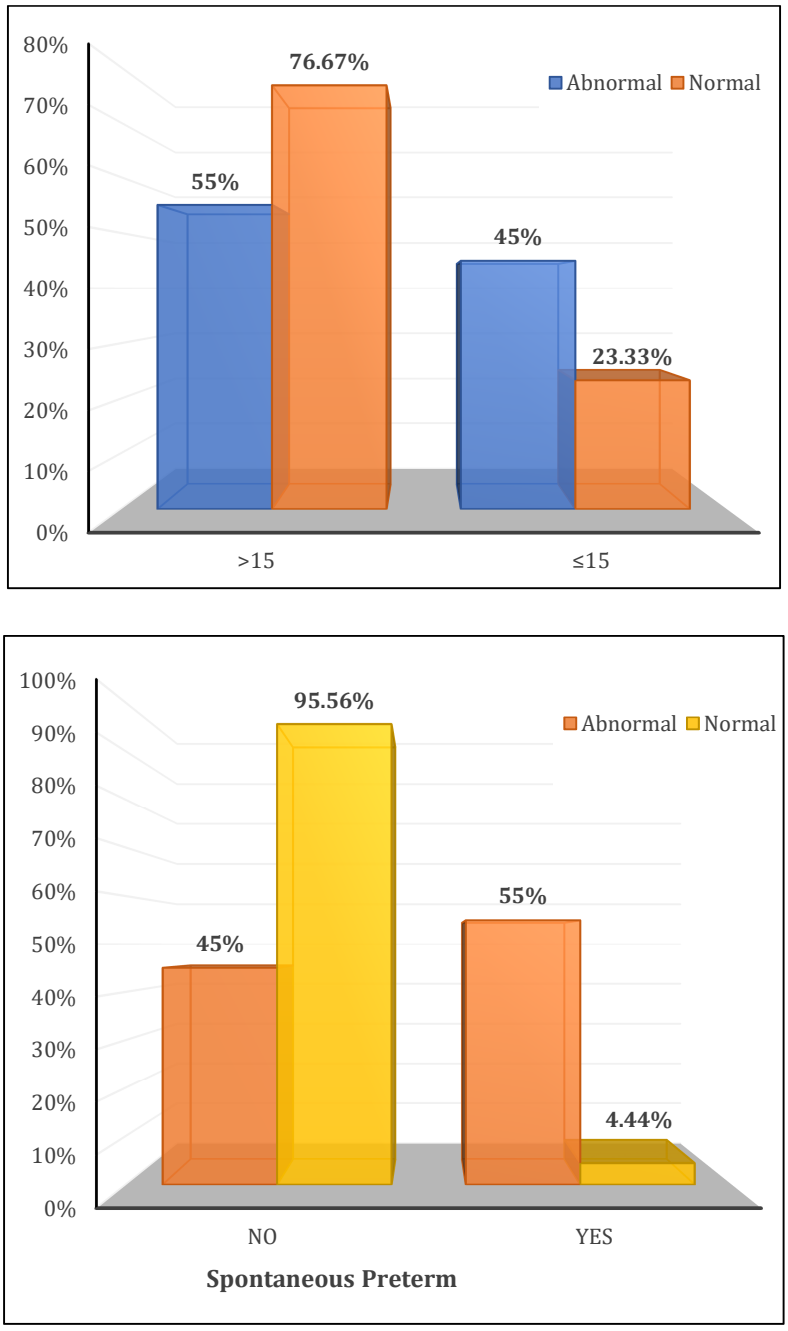


Figure 12: Distribution of gestational age and spontaneous preterm with Nugent Score.

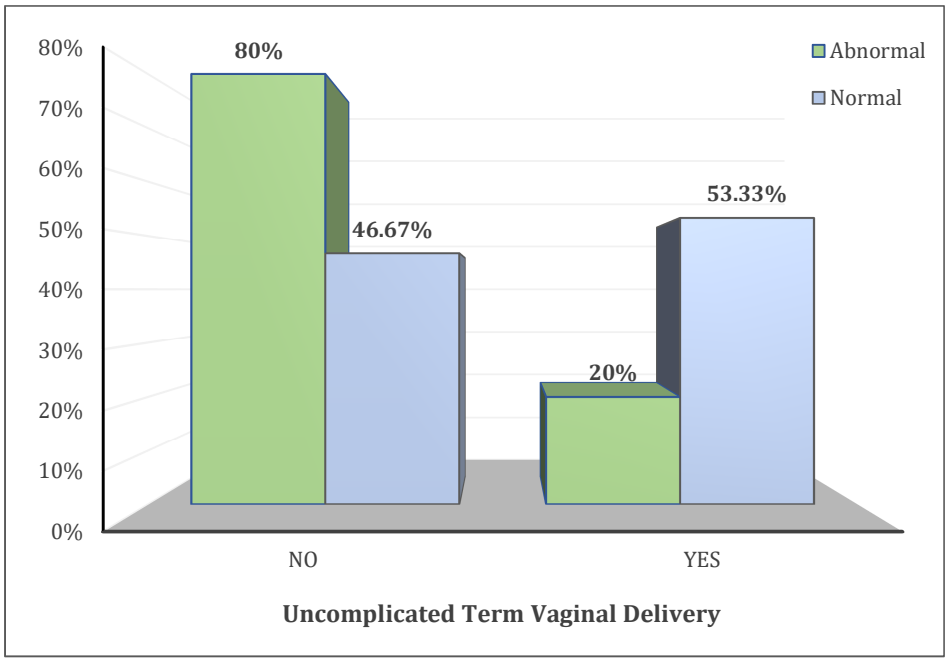


Figure 13: Distribution of uncomplicated term vaginal delivery with Nugent Score.

4.3. Association of aerobic culture with other study parameters

A Chi-square test revealed no significant association of age, parity, gestational age, PPROM, PROM, spontaneous preterm, uncomplicated term vaginal delivery, term C- section delivery, and Preterm C-section delivery with aerobic culture. From the two-sample t-test, we observed that there is no significant difference in the mean age over aerobic culture **(Table 5) (Figures 14, 15, 16, 17, and 18)**.

Table 5: Comparison of different variables with aerobic culture.

Variables	Sub Category	Aerobic culture		p-value
		No	Yes	
Age (years)	≤20	10 (12.5%)	8 (26.67%)	0.0985[MC]
	21-25	43 (53.75%)	11 (36.67%)	
	26-30	24 (30%)	7 (23.33%)	
	31-35	1 (1.25%)	2 (6.67%)	
	36-40	2 (2.5%)	2 (6.67%)	
	Mean ± SD Median (Min, Max)	24.46 ± 3.93 24 (18, 40)	24.77 ± 4.66 24 (19, 37)	0.7319[t]
Parity	Multi	46 (57.5%)	14 (46.67%)	0.3095[C]
	Primi	34 (42.5%)	16 (53.33%)	
Gestational Age (weeks)	>15	57 (71.25%)	23 (76.67%)	0.57[C]
	≤15	23 (28.75%)	7 (23.33%)	
PPROM	No	80 (100%)	28 (93.33%)	0.0615[MC]
	Yes	0	2 (6.67%)	
PROM	No	74 (92.5%)	27 (90%)	0.7151[MC]
	Yes	6 (7.5%)	3 (10%)	
Spontaneous Preterm	No	69 (86.25%)	26 (86.67%)	1[MC]
	Yes	11 (13.75%)	4 (13.33%)	
Uncomplicated Term Vaginal Delivery	No	42 (52.5%)	16 (53.33%)	0.9379[C]
	Yes	38 (47.5%)	14 (46.67%)	
Term C-section Delivery	No	53 (66.25%)	23 (76.67%)	0.2924[C]
	Yes	27 (33.75%)	7 (23.33%)	
Preterm C-Section Delivery	No	80 (100%)	28 (93.33%)	0.0614[MC]
	Yes	0	2 (6.67%)	

*Abbreviation: C – Chi-square test, MC – Chi-square test with Monte Carlo simulation, t – Two-sample t-test, * indicates statistical significance.*

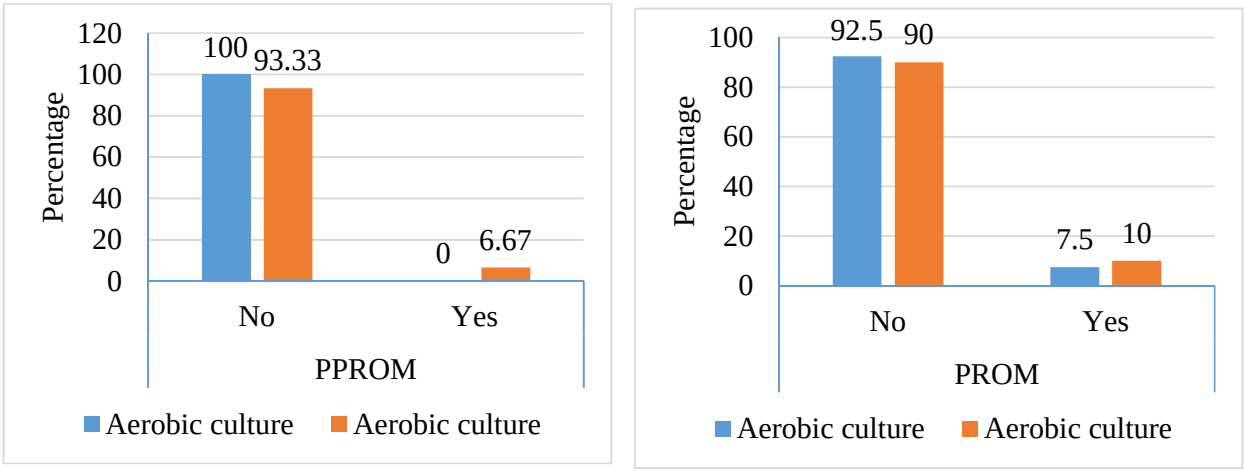


Figure 14: Plot of distribution of PPROM and PROM against aerobic culture

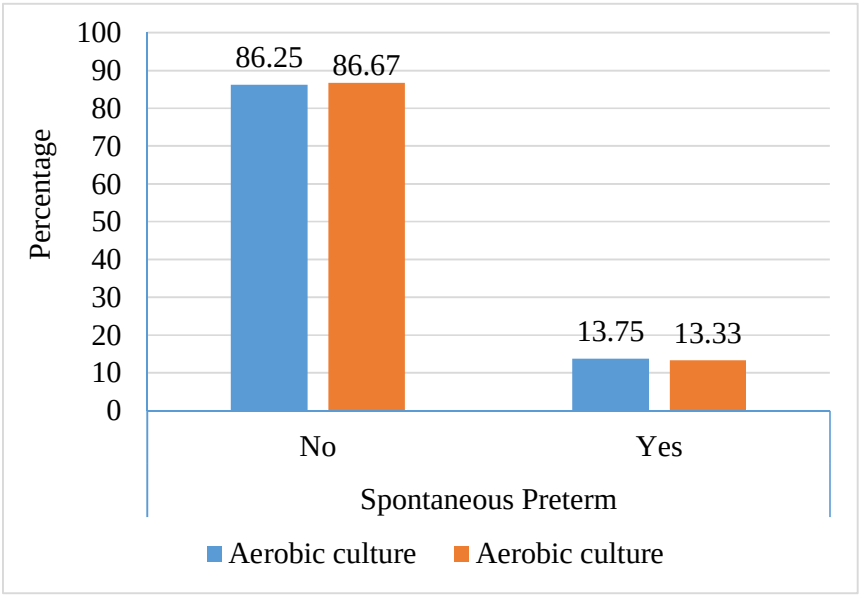


Figure 15: Plot of distribution of spontaneous Preterm against aerobic culture

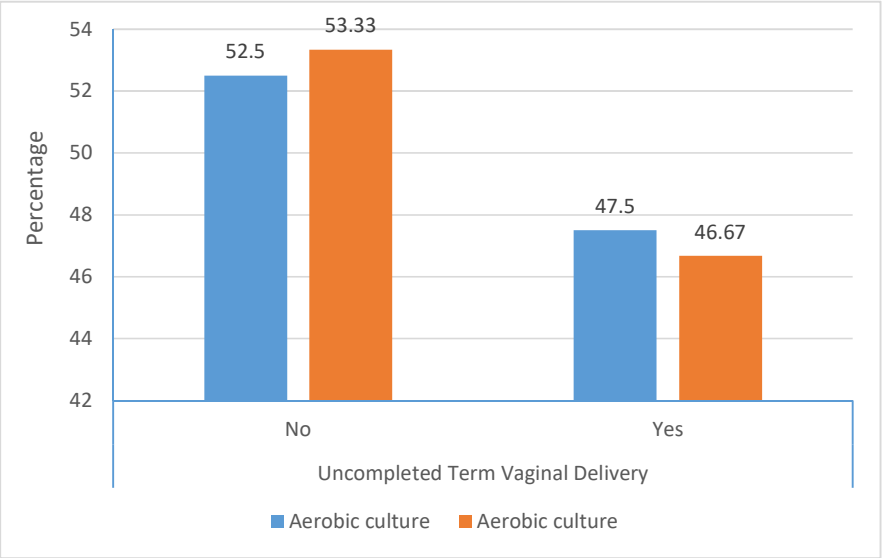


Figure 16: Plot of distribution of uncomplicated term vaginal delivery against aerobic culture

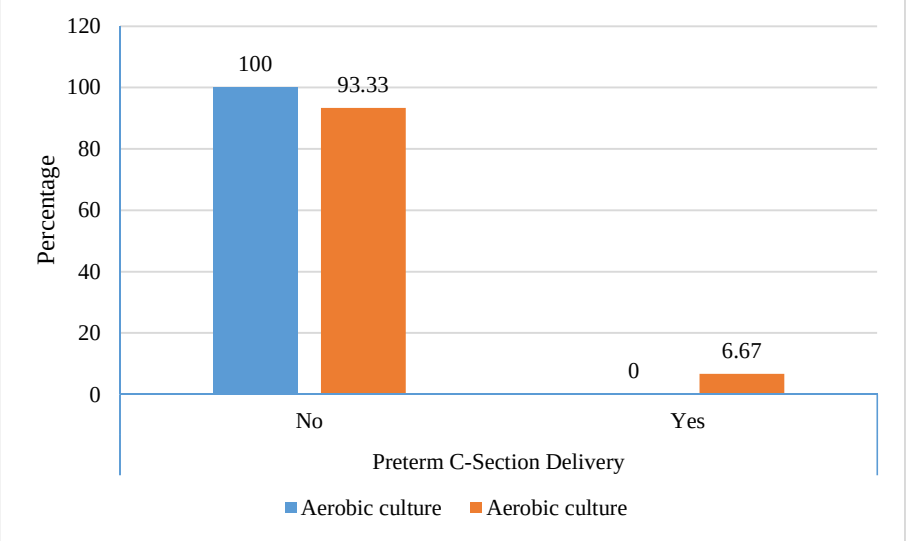


Figure 17: Plot of distribution of Preterm C-section delivery against aerobic culture

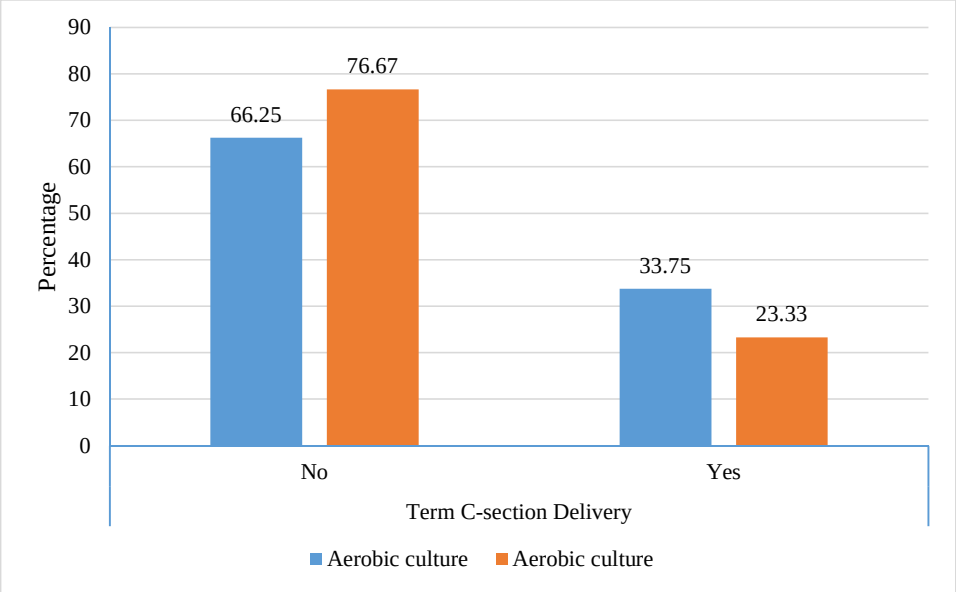


Figure 18: Plot of distribution of Term C-section delivery against aerobic culture

4.4. Association of fungal culture with other study parameters

Chi-square analysis revealed no significant association of age, parity, gestational age, PPROM, PROM, spontaneous preterm, uncomplicated term vaginal delivery, term C- section delivery, and Preterm C-section delivery with a fungal culture. The two-sample t-test, showed no significant difference in the mean age when compared alongside the fungal culture **(Table 6) (Figures 19,20,21,22,23 and 24)**.

Table 6: Comparison of different variables with a fungal culture.

Variables	Sub Category	Fungal culture		p-value
		No	Yes	
Age (years)	≤20	18 (18%)	0	0.5222[MC]
	21-25	48 (48%)	6 (60%)	
	26-30	27 (27%)	4 (40%)	
	31-35	3 (3%)	0	
	36-40	4 (4%)	0	
	Mean ± SD Median (Min, Max)	24.51 ± 4.22 24 (18, 40)	24.9 ± 3.07 25 (21, 30)	0.7767[t]
Parity	Multi	56 (56%)	4 (40%)	0.5432[MC]
	Primi	44 (44%)	6 (60%)	
Gestational Age (weeks)	>15	74 (74%)	6 (60%)	0.4723[MC]
	≤15	26 (26%)	4 (40%)	
PPROM	No	98 (98%)	10 (100%)	1[MC]
	Yes	2 (2%)	0	
PROM	No	91 (91%)	10 (100%)	0.5982[MC]
	Yes	9 (9%)	0	
Spontaneous Preterm	No	86 (86%)	9 (90%)	1[MC]
	Yes	14 (14%)	1 (10%)	
Uncomplicated Term Vaginal Delivery	No	53 (53%)	5 (50%)	1[MC]
	Yes	47 (47%)	5 (50%)	
Term C-section Delivery	No	70 (70%)	6 (60%)	0.7356[MC]
	Yes	30 (30%)	4 (40%)	
Preterm C-Section Delivery	No	98 (98%)	10 (100%)	1[MC]
	Yes	2 (2%)	0	

*Abbreviation: C – Chi-square test, MC – Chi-square test with Monte Carlo simulation, t – Two-sample t-test, * indicates statistical significance.*

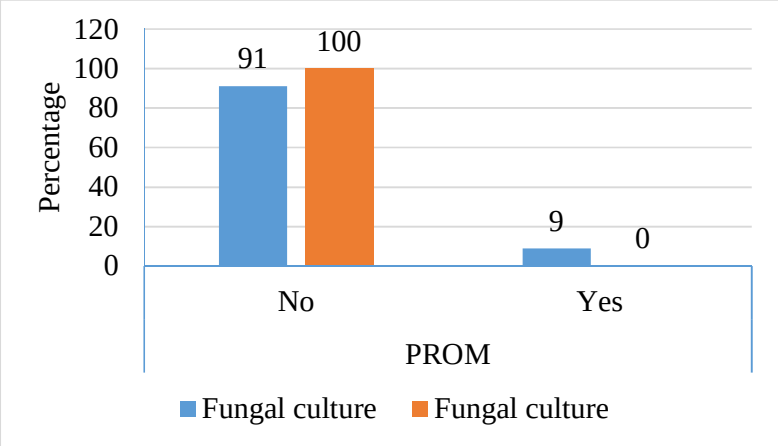


Figure 19: Plot of distribution of PROM against fungal culture

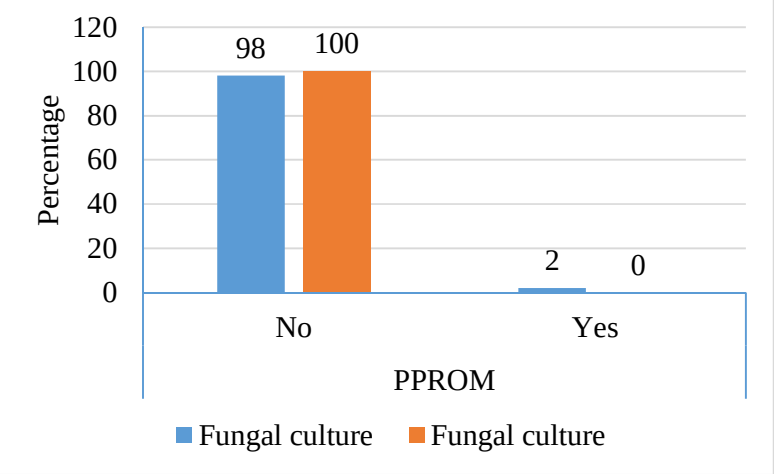


Figure 20: Plot of distribution of PPROM against fungal culture

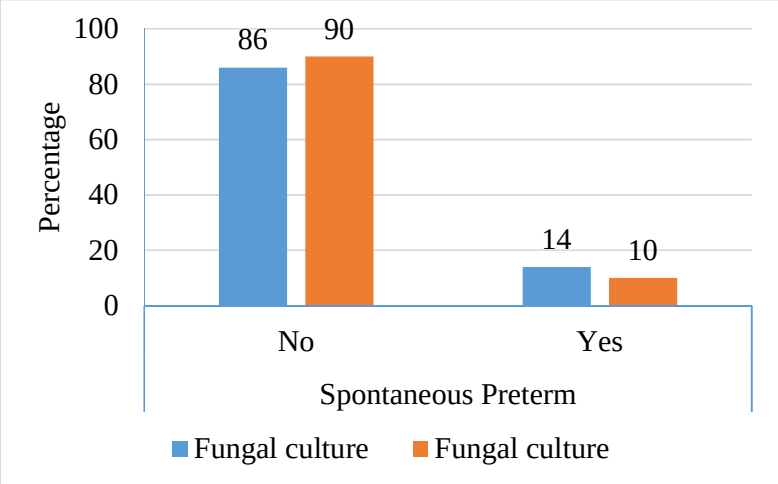


Figure 21: Plot of distribution of spontaneous preterm against fungal culture

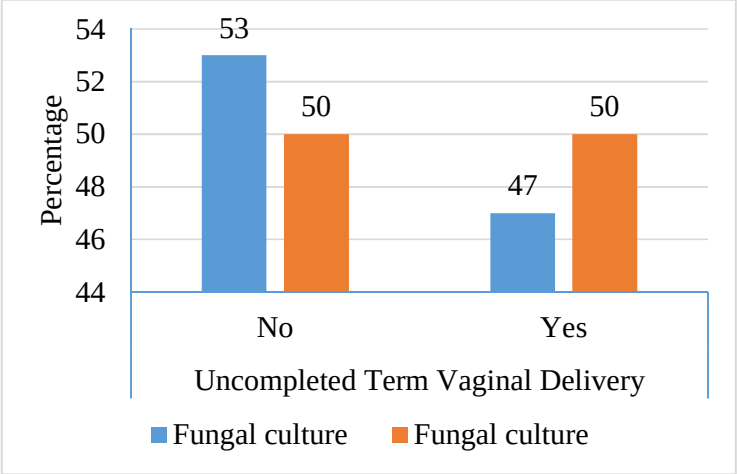


Figure 22: Plot of distribution of uncomplicated term vaginal delivery against fungal culture

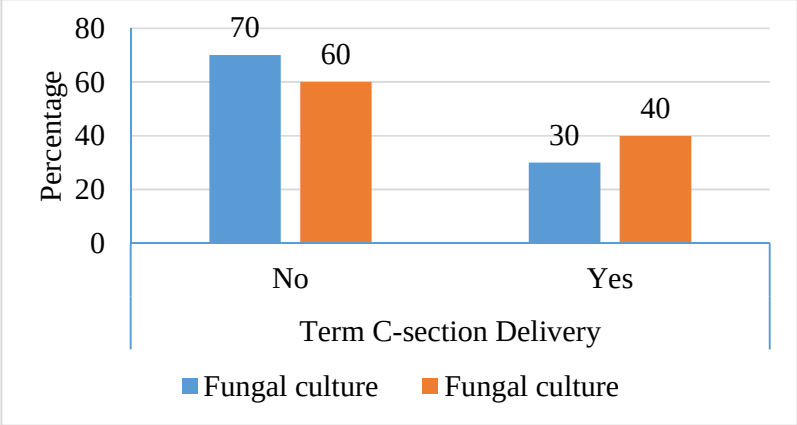


Figure 23: Plot of distribution of Term C-section against fungal culture

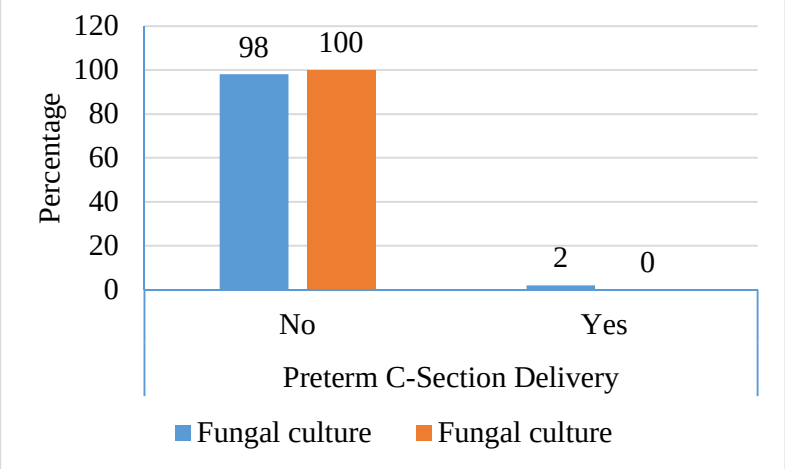


Figure 24: Plot of distribution of Preterm C-section against fungal culture

5. DISCUSSION

As per the global statistics, nearly fifteen million preterm births (birth before 37 weeks of gestation) occur annually (201). Preterm birth (PTB) continues to be the second prevailing cause of neonatal death worldwide, and the predominant reason for infant mortality in high-income and middle-class economies(202). Nearly 1 million children die annually as a result of complications of preterm birth (WHO 2013 data). Over 184 countries, the incidence of preterm birth range from 5% to 18% of children born. In India, out of 27 million babies born every year (2010 data), 3.5 million babies born are premature(203,204). The repercussions of PTB prevail from early childhood through to adolescence and even adulthood (205,206). In spite of these statistics, there is a continuous paucity of effective approaches for the prediction and prevention of PTB. Even though maternal and fetal genetic make-up, and gene-environment interactions, quintessentially play roles in deciding the duration of gestation, environmental factors, inclusive of the microbiome, are the principal contributors to PTB, notably among women of African descent (207). Inflammation induced by the microbial activity caused as a result of trichomoniasis, sexually transmitted infections, urinary tract infection, or bacterial vaginosis is speculated to be a causative agent of PTB (26,177). The ascent of microorganisms(26,208) from the lower genital tract to the placental tissues, fetal membrane layers, and uterine chamber have also been cited as an explanation for approximately 40–50% of preterm births that are correlated with microbial etiologies(209,210).

The present study is an attempt to determine the correlation between vaginal dysbiosis in the early second trimester of pregnancy and the subsequent development of PPROM. Analysis of the data revealed a significant association of gestational age, spontaneous preterm, and uncompleted term vaginal delivery with Nugent score. A

Nugent score of more than 3 signifies vaginal dysbiosis and resulted in an increased frequency of spontaneous preterm delivery whereas a Nugent score of 3 or less is assumed to be normal and was associated with uncompleted term vaginal delivery. These observations are in line with the well-evidenced fact that the presence of an abnormal vaginal microflora in early pregnancy is a risk factor for PPROM, preterm delivery, and PROM(211).

A uniform *Lactobacillus*-dominated microbiome has always been regarded as an indicator of a healthy female reproductive tract. Generally, *L. iners, L. jensenii, L. crispatus,* and *L. gasseri* have been shown to dominate the vaginal microbiome in normal women of reproductive age in various proportions (212–214). Around 120 species of *Lactobacillus* have been described and about 20 are established to be inhabiting the vagina. The vaginal microbiome of healthy women includes one or two lactobacilli species (215,216). Nonetheless, recent advancements in culture independent techniques reveal the variability and diversity in the composition of a healthy vaginal microbiome. The healthy vaginal tract consists of greater than 50 non-pathogenic microbial species (217–219). Specific bacterial arrays associated with different ethnic groups have been observed (199,212). Remarkably, various anaerobic bacteria were predominant, indicating that Lactobacillus dominance is not a decisive characteristic of healthy vaginal flora (199). Remarkably, various anaerobic bacteria were predominant, indicating that Lactobacillus dominance is not a decisive characteristic of healthy vaginal flora(199). Further, bacterial genera comprising *Prevotella, Atopobium, Gardnerella, Megasphaera*, and *Mobiluncus* have been associated with abnormal vaginal microbiota (199,212) in non-symptomatic reproductive-age women.

Vaginal microbiome rich in species diversity, as seen in bacterial vaginosis, has been linked with increased risk for acquiring and transmitting sexually transmitted infections, inflammatory pelvic diseases, and PTB (220–224). It is implied that ethnicity plays a critical role in ascertaining the risk of bacterial vaginosis and is often associated with increased risk of PTB(199,225). Additionally, a reduction in microbial diversity was also recognized in the vaginal microbiome of pregnant women as gestation advanced, and an increased vaginal microflora diversity was detected in mothers delivering on term compared with preterm births (31,33,226). Furthermore, the affluence of *Lactobacillus* species in healthy pregnant women was observed (33).

Bacterial vaginosis during pregnancy causes a nine-fold increase in the risk of preterm labor (227–230). The inflammatory cascade triggered by bacterial dysbiosis results in the release of proinflammatory cytokines such as IL-6, IL-1 beta, IL-8, and TNF-α (231). These proinflammatory cytokines further stimulate MMP release, specifically MMP-8 which are produced by neutrophils. MMP8 causes the breakdown of the membranes. MMP-8 is capable of degradation of a large number of proteins found in the extracellular matrices like collagen I and III. The infection results in elevated MMP8 levels, and an increased proteoglycan degradation process that compromises the integrity of the membranes. This kind of disruption of the membrane integrity causes premature rupture of the membranes(231,232).

Bacterial vaginosis is abetted by alteration in vaginal microflora with ensuing loss of protective *Lactobacillus* species and an accompanying rise in the abundance of anaerobic and facultative microorganisms in the vaginal environment. Disturbance in the normal environmental balance leads to the inundate growth of many anaerobes including *Gardnerella vaginalis, Peptostreptococcus spp., Mycoplasma hominis,*

Mobiluncus spp., *Fusobacterium spp.*, *Bacteroides spp.*, *Prevotela spp.*, and *Porphyromonas spp.* A deficit of lactobacilli hinders the natural protective mechanism resulting in a higher-than-normal pH (>4.5) due to lactic acid depletion (233,234). It is held in belief that atypical vaginal flora is a potential risk component for pre-term birth. Women with *Lactobacillus* rich vaginal microenvironment in the first trimester of gestation have a 75% decreased risk of giving birth pre-term compared to those with aberrant flora represented by the establishment of bacteria associated with bacterial vaginosis. Moreover, the species diversity of *Lactobacilli* is also a crucial factor influencing the outcome of pregnancy. Nonetheless, many non-symptomatic healthy women have disparate vaginal microbiota. More precise strategies are required for the evaluation of the risk, prevention, and treatment of the condition and thereby promote health (15,199,235–238).

A recent case-control study conducted in Indonesian population with a sample size of 76 has shown that bacterial vaginosis presents a higher risk for PPROM (Wiraguna et al., 2019). These findings are in line with observations made by studies conducted in the India subcontinent(240–243). In a prospective cohort study conducted amongst pregnant women by Brown et. al, it was observed that a vaginal microbiome with reduced *Lactobacillus* spp. abundance and increasing vaginal bacterial diversity were associated with an increased relative risk of PPROM(211). Another study was conducted in Italy in which 600 pregnant women at 28 weeks of gestation were subjected to a vaginal swab to search for streptococcus agalactiae and also to assess lactobacillary grading and/or presence of any pathogenic bacteria and/or candida. It showed that women with a vaginal infection and/or abnormal vaginal microbiota had a higher incidence of PPROM (244). A longitudinal study comprising of 111 pregnant women, of which 98 gave birth at term and 13 gave birth at pre-term,

hypothesized that women retaining only one species of *Lactobacillus* during pregnancy were at an increased risk of delivering before the term in comparison to those who have vaginal microbiota comprising of more than one species. It also established that *L. iners* was the predominant species linked with pre-term birth(245). A contrary observation to the hypothesis of a causal association of atypical vaginal microflora and pre-term birth was made by Romero et al. As per their evaluation, no significant difference in the microbiota was found between group A (comprising of 18 pre-term delivering) and group B (70 term delivering mothers) concerning bacterial taxa and relative abundance when compared alongside gestational age(198).

Recent literature on the microbiome in pregnant women (30,180,189,192,194,197,198,226,246–255) have evidenced that the architecture of the vaginal microbiota has a consequential population-specific influence on PTB risk. Numerous studies focusing on populations mainly of European ancestry (30,194,197,246) have associated *Lactobacillus crispatus* with a reduced risk for PTB. The aforementioned findings were replicated in a cohort of principally of African ancestry(246). As originally described by Ravel et al.(199), and later corroborated by other research groups (156,235), the vaginal microbiome composition of women of European and African descent vary substantially. While the fact that specific taxa have been linked to PTB in women of African descent in a few studies(246,247), no significant associations were observed in others (189,198). It is less likely that women of African descent to exhibit vaginal lactobacilli, usually have vaginal *L. crispatus* prevalence, and are more inclined to present with high vaginal microbial diversity (199,235). A case-control study in the Vietnamese population has provided further evidence that vaginal dysbiosis increased the risk for PTB and

PPROM. They have also observed that aerobic bacteria were closely associated with PPROM while fungal infection did not have the same associative relation(256).

Rapidly advancing research has been directed to the prenatal vaginal microflora in influencing the risk for PTB. However, till date, these studies have yielded moderately conflicting findings across as well as within racial/ethnic groups. The contradiction in findings across studies may be a reflection of the differences in the study sample. Across studies, there is appreciable variation in participant racial/ethnic and socioeconomic diversity, attributes themselves that are linked with PTB (26,257,258), and many studies have small sample size. In addition, across studies there is substantial disparateness the classification of PTB, with many studies not differentiating spontaneous PTB from any PTB, including the medically-indicated cases due to fetal or maternal complications. The clinical factors leading to the different PTB scenarios vary; spontaneous preterm labour is more often linked with intrauterine infection or inflammation, whereas the indicated delivery is often related to medical complications. Lastly, there is also reasonable variability in the gestational age of vaginal sample collection across studies, and it is determined that the composition of the vaginal microbiome changes with advancing gestational age(31,198). A direct comparison of the findings of different studies of the vaginal microflora and PTB is ambitious as there is considerable heterogeneity in methods across all the published studies.

Thence, future analyses should be population-specific investigations focusing on exploring particular environmental, social, and behavioral exposures that may contribute to the microfloral diversity. These kinds of investigations are required to gauge the broad influence of the vaginal microbiome as a risk factor of PTB and to also to classify contributing taxa. In contrast to the contributory effect of factors like

anatomical abnormalities, genetic factors, and antepartum hemorrhage, antagonistic vaginal microbiome is a resilient risk factor for PPROM. Identification the subset of patients with vaginal dysbiosis followed by management of the microflora via a combination of antibiotic, prebiotic and probiotic therapies calls for further investigation and serve as a promising approach for the prevention and/or reduction of PPROM and PTB.

6. CONCLUSION

Vaginal microfloral dysbiosis-associated conditions are the most recurrent causes of preterm birth. This study made an attempt to establish any correlation between vaginal dysbiosis and subsequent development of PPROM, PROM and spontaneous preterm birth. We found that vaginal dysbiosis in early second trimester of pregnancy did not have any correlation to PPROM and PROM but there was a significant association with spontaneous preterm labor. However, a larger sample size and using novel microbiological profiling techniques of the vaginal microbiome would have given us a more accurate and conclusive interpretation of data.

7. SUMMARY

This was a longitudinal observational study conducted on antenatal women between $14^{+0/7}$ to $20^{+6/7}$ weeks of gestation attending the outpatient services of the Department of Obstetrics and Gynaecology of KAHER's Dr. Prabhakar Kore Charitable Hospital, Belgaum, Karnataka. Employing a convenience sampling technique, a sample of 110 pregnant women who satisfied inclusion criteria were recruited for the study. All the recruited subjects were followed up until their delivery and the pregnancy outcome was recorded namely PROM, PROM, Preterm birth or Term delivery. The outcomes were then correlated with the microbiological profiling done in early second trimester.

Chi-square analysis test revealed that there is a significant association of gestational age, spontaneous preterm, and uncompleted term vaginal delivery with Nugent score. However, there is no significant association of age, parity, aerobic culture, fungal culture, PPROM, PROM, term C- section delivery, and Preterm C-section delivery with Nugent score. Moreover, no association was established for aerobic or fungal cultures with other study parameters. The observation that a positive Nugent score led to an increased frequency of spontaneous preterm delivery and uncomplicated term vaginal delivery are in agreement with the well-supported fact that the presence of an abnormal vaginal microflora in early pregnancy is a risk factor for PPROM, preterm delivery, and PROM.

Identification the subset of patients with vaginal dysbiosis using modern microbiological profiling techniques and subsequent management of the microbiota through a combination of antibiotic, prebiotic and probiotic therapies holds promise for the reducing the incidence of PPROM and PTB.

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ANNEXURE - I - ETHICAL CLEARANCE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
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Ref: MDC/DOME/ 165 **Date: 24/12/2019**

To,

REG. NO. BJ0119017
PG student in Obstetrics & Gynecology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"VAGINAL DYSBIOSIS IN EARLY SECOND TRIMESTER OF PREGNANCY AND ITS ASSOCIATION WITH PPROM: A LONGITUDINAL OBSERVATIONAL STUDY AT KAHER'S DR. PRABHAKAR KORE CHARITABLE HOSPITAL, BELAGAVI ",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

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ANEXXURE II- CONSENT FORM

Purpose of the study

I have been informed by **REG. NO. BJ0119017,** Post Graduate in M.S. Obstetrics and Gynaecology under the guidance of Dr._____, Department of Obstetrics and Gynaecology, J.N. Medical College, KAHER, Belagavi is conducting a study to determine To determine the correlation between vaginal dysbiosis in $13^{+0/7}$ weeks and $20^{+6/7}$ weeks of pregnancy and subsequent development of PPROM in women receiving antenatal care at KAHER's Dr.Prabhakar Kore Charitable Hospital, Belagavi.

PROM/PPROM is associated with neonatal mortality and morbidity. It includes prematurity, neonatal infections including necrotizing enterocolitis, sepsis, respiratory distress syndrome, intraventricular haemorrhage, periventricular leukomalacia, cerebral palsy, retinopathy of prematurity and endocrinological problems including hypoglycaemia, transient low hypothyroid levels. All these contribute to economic and emotional burden for the parents and the family members.

The data on prevalence of vaginal dysbiosis in early pregnancy is scarce in this part of the country with a prevalence of 6.4 % in KAHER. This study aims to determine the correlation between vaginal dysbiosis in early second trimester of pregnancy and its association with development of PPROM.

Study procedure:

Once I have signed the informed consent form, the personal details like name, age, place, address, my education, my health, reproductive history and other information will be noted down. Vaginal swab will be taken and processed for culture and gram staining. The reports will be noted and I will be followed up.

Potential Risks

There are no observable risks associated with the study.

Benefits

There is a benefit as I will be followed up in my antenatal period on a regular basis and will be delivering in KAHER's Dr.Prabhakar Kore Charitable Hospital, Belagavi.

Financial incentive for participation

I will not receive any payment for taking part in this research study.

Alternatives

If I decide not to participate in the study, my health care provider will provide the usual standard care during my pregnancy, delivery and up to through 6 weeks after delivery**.**

Privacy

To protect my privacy, all the collected information will be given a number rather than using my name. Any information collected during the study will remain confidential. My medical files will be reviewed only at the hospital (or study doctor's office) to check the information and verify the result without breaking my confidentiality. Only de-identified information on my pregnancy will be shared so as to learn the results of the study.

Authorisation to publish results

The information about me will be analysed together with other study participants.

Results of this study will be published and presented to scientific groups for scientific purposes, but I will never be individually identified in the presentation of the study results.

Institutional Policy

In case I have any questions related to the study, in future or in case of study related injury or illness, I can contact **REG. NO. BJ0119017,** Department of Obstetrics and Gynaecology, KLE KAHER, J.N Medical College, Ph. No. 0831-2551292 or phone number:_____ or Dr._____, Dept. of Obstetrics and Gynaecology, KAHER, J.N Medical College, Belagavi Ph.: 0831-2551292 or phone number: _____.

Voluntary Participation

My participation in the study is voluntary. In case I need any further information regarding my rights as study participant, I may contact Dr. Roopa M Bellad, Professor of Paediatrics, as Chairman of J. N. Medical College Institutional Ethics Committee on Human Subjects Research, Phone No.0831 2473777 ext-1527 at J. N. Medical College, Belagavi. My doctor will take care of me during this pregnancy or in the future. I am free to stop participation in this study at any time and for any reason.

Screening form

Screening number: ☐☐☐

Date of screening (dd-mm-yyyy): ☐☐ - ☐☐ - ☐☐☐☐

Full name: _____

First name : _____ Middle name : _____ Last name: _____

Age (years): ☐☐

OP number: ☐☐☐☐☐☐☐

Husband's name: _____

Address: House number-_____

Street _____

Taluka_____

District_____

Phone number: _____

Landline (optional): _____

RECRUITMENT FORM

1) Is the period of gestation between $13^{+0/7}$ weeks and $20^{+6/7}$ weeks of gestation yes ☐ no ☐

2) Past history (1=yes 2=no)

History of any preterm delivery/PROM/PPROM in the past pregnancy ☐

History of short cervical length/cervical encirclage ☐

History of threatened abortion in 1st trimester ☐

History of uterine anomalies ☐

The woman is eligible to consent only if answer to 1 is yes and 2 is no:

Eligible ☐

Consented ☐

PROFORMA

Enrollment number: ☐☐☐

I. **Socio demographic information**:

1. Age (years) ☐☐

2. What is the level of schooling? ☐

1= no formal schooling, illiterate

2= no formal schooling, literate

3= schooling

a. number of years of schooling ☐☐

4= Don't know

Socio-economic status (according to modified B.J Prasad classification)

1 =upper class ☐

2=upper middle class ☐

3=middle class ☐

4=Lower middle class ☐

5=lower class ☐

BMI ☐

II. **Present pregnancy**

Obstetric score :

Married life:

Consanguinity:

Gravida: ☐

Para: ☐

Living: ☐

Abortion: ☐

Stillbirth: ☐

MENSTRUAL HISTORY

3. What was the last day of your menstrual cycle? -☐☐- ☐☐ ☐☐☐☐

d d m m y y y y

4. Estimated date of delivery (EDD) by LMP

☐☐ ☐☐ ☐☐☐☐

5. Corrected Estimated date of delivery (EDD) if any

☐☐ ☐☐ ☐☐☐☐

6. Gestational age- ☐ weeks ☐ days ☐

Past history:

History of any preterm delivery/PROM/PPROM in the past pregnancy ☐ 1=yes 2=no

History of short cervical length/cervical encirclage ☐ 1=yes 2=no

History of threatened abortion in 1st trimester ☐ 1=yes 2=no

History of uterine anomalies ☐ 1=yes 2=no

III. Vaginal swab culture:

1) Nugent score:
2) Organisms isolated on aerobic culture:

Organism: (1= yes, 2= no)

Lactobacillus	☐
Gardnerella vaginalis	☐
Chlamydia trachomatis	☐
E.coli	☐
Group B streptococii	☐
Enterococci	☐
Lactobacilli	☐
Bacteroides	☐
Propionibacterium	☐
Trichomonas vaginalis	☐
M.hominis	☐
Streptococcus agalctiae	☐
Staph. Aureus	☐
U. urealyticum	☐
Non haemolytic streptococci	☐
Prevotella	☐
Vaginal commensals	☐

If others, specify the organism _____

3) Fungal organisms isolated:

   Yeast:

   Candida albicans:

   Other candida species:

IV. Pregnancy outcome

1) PPROM ☐
2) PROM ☐
3) SPONTANEOUS PRETERM ☐
4) UNCOMPLICATED TERM VAGINAL DELIVERY ☐
5) CESAREAN SECTION ☐

ANNEXURE IV – MASTERCHART

SL NO	SREENING DATE	AGE	OP/IP NUMBER	PARITY	LMP	EDD	C.EDD	GESTATION AGE	H/O PRETERM DELIVERY/PROM/PPROM	H/O SHORT CERVIX/CERVICAL ENCIRCLAGE	H/O THEATENED ABORTION	H/O UTERINE ANOMALIES	NUGENT SCORING	AEROBIC CULTURE	FUNGAL CULTURE	PPROM	PROM	SPONTANEOUS PRETERM	UNCOMPLICATED TERM DELIVERY	INDICATION FOR LSCS
1	17-01-2020	23	5528232	G2A1	28-09-2019	04-07-2020		15WK 6D	N	N	N	N	1	NIL	NIL				Y	
2	17-01-2020	24	3596307	G2P1L1	04-01-2021	11-10-2021		16WK 2D	N	N	N	N	1	NIL	NIL				Y	CPD
3	20-01-2020	25	3326589	G2P1L1	02-09-2019	08-06-2020		20WK	N	N	N	N	2	NIL	NIL				Y	
4	20-01-2020	32	5507743	G2P1L1	30-09-2019	09-07-2020		16WK	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	FETAL DISTRESS
5	20-01-2020	22	1016972	G2P1L1	08-10-2019	15-07-2020		14WK 6D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	
6	20-01-2020	29	4674924	G3P2L2	01-09-2019	06-06-2020		20WK 1D	N	N	N	N	3	NIL	NIL				Y	
7	20-01-2020	28	5578134	PRIMI	15-10-2019	24-07-2020		13WK 6D	N	N	N	N	1	NIL	NIL				Y	PLACENTA PREVIA
8	27-01-2020	25	4599873	G2P1L1	08-10-2019	14-07-2020		15WK 6D	N	N	N	N	2	NIL	NIL			Y(34WK 6D)		
9	28-01-2020	29	5599224	G3P2L2	13-09-2019	19-06-2020		19WK 4D	N	N	N	N	1	NIL	NIL				Y	
10	31-01-2020	22	5604494	G2A1	22-09-2019	28-06-2020		18WK 5D	N	N	N	N	2	NIL	NIL				Y	
11	31-01-2020	26	5417341	PRIMI	16-12-2020	22-09-2021		18WK 6D	N	N	N	N	3	NIL	NIL				Y	PRECIOUS PREGNANCY
12	14-02-2020	26	5622441	PRIMI	01-11-2019	13-08-2020		14WK 1D	N	N	N	N	3	NIL	CANDIDA				Y	NPL
13	14-02-2020	26	3937840	G2P1L1	09-11-2019	21-08-2020		15WK 2D	N	N	N	N	2	NIL	NIL				Y	
14	14-05-2020	22	5696023	G2A1	07-01-2020	13-10-2020		18WK2D	N	N	N	N	1	NIL	NIL				Y	
15	14-05-2020	18	4249936	G3P1L1A1	20-01-2020	29-10-2020		14WK 6D	N	N	N	N	1	NIL	NIL				Y	K/C/O EPILEPSY
16	14-05-2020	37	3698106	G3P1L1A1	27-12-2019	06-10-2020		19WK 6D	N	N	N	N	2	VAGINAL COMMENSALS	NIL				Y	
17	14-05-2020	20	5696648	PRIMI	02-01-2020	09-10-2021		16WK 4D	N	N	N	N	1	NIL	NIL				Y	
18	14-05-2020	23	5696589	G4P2L2A1	24-01-2020	30-10-2020		15WK 6D	N	N	N	N	1	NIL	NIL				Y	PREV LSCS IN LABOUR
19	18-05-2020	24	5679748	G2P1L1	11-02-2019	20-11-2020		13WK 6D	N	N	N	N	1	NIL	BUDDING YEAST CELLS				Y	
20	18-05-2020	24	5699866	PRIMI	10-01-2020	16-10-2020		18WK 6D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	
21	18-05-2020	30	3240359	G3P2L2	25-12-2019	30-09-2020		20WK 5D	N	N	N	N	2	NIL	BUDDING YEAST CELLS				Y	PREV 2 LSCS
22	18-05-2020	21	5699904	PRIMI	24-12-2019	29-09-2020		20WK 6D	N	N	N	N	1	NIL	NIL				Y	NPL
23	18-05-2020	22	5699868	PRIMI	26-12-2019	01-10-2020		20WK 4D	N	N	N	N	1	NIL	NIL		Y(39WK 6D)		Y	CDMR
24	18-05-2020	20	5699696	G2A1	31-12-2019	06-10-2020		19WK 6D	N	N	N	N	2	VAGINAL COMMENSALS	NIL			Y(35WK 4D)		
25	18-05-2020	23	5696791	PRIMI	10-12-2019	16-09-2020		20WK 3D	N	N	N	N	1	NIL	NIL				Y	FETAL DISTRESS
26	26-05-2020	26	5656763	PRIMI	08-02-2020	15-11-2020		15WK 2D	N	N	N	N	2	NIL	NIL				Y	
27	26-05-2020	21	5706588	G2P1L1	21-01-2020	28-01-2020		13WK 6D	N	N	N	N	1	NIL	NIL				Y	
28	26-05-2020	38	5362751	PRIMI	11-02-2020	17-11-2020		14WK 6D	N	N	N	N	6	NIL	NIL				Y	CDMR
29	26-05-2020	20	5411523	PRIMI	24-01-2020	31-10-2020	15-11-2020	15WK 2D	N	N	N	N	2	NIL	NIL				Y	
30	26-05-2020	28	5682047	G3P2L2	14-01-2020	20-10-2020		19WK	N	N	N	N	0	NIL	NIL				Y	
31	26-05-2020	22	5686822	PRIMI	16-01-2020	22-10-2020		18WK 5D	N	N	N	N	4	NIL	NIL				Y	
32	26-05-2020	22	5706684	PRIMI	10-01-2020	19-10-2020		19WK 4D	N	N	N	N	1	NIL	NIL				Y	
33	26-05-2020	25	5707763	G2P1L1	04-01-2020	10-10-2020		20WK 3D	N	N	N	N	0	NIL	NIL				Y	
34	26-05-2020	21	5707811	PRIMI	20-02-2020	26-11-2020		13WK 5D	N	N	N	N	1	NIL	NIL				Y	
35	26-05-2020	25	4798063	G2P1L1	22-02-2020	28-11-2020		13WK 3D	N	N	N	N	5	NIL	NIL				Y	PREV LSCS IN LABOUR
36	26-05-2020	31	2884499	G3P2L1D1	19-01-2020	25-10-2020		18WK 2D	N	N	N	N	2	NIL	NIL				Y	

37	29-05-2020	26	5665243	G3P2L2	07-01-2020	14-10-2020		20WK 3D	N	N	N	N	4	NIL	NIL			Y(36WK)		
38	29-05-2020	23	4115761	G2P1L1	20-02-2020	26-11-2020		14WK 1D	N	N	N	N	2	NIL	NIL				Y	
39	29-05-2020	25	4500577	G2P1L1	13-01-2020	20-10-2020		19WK 4D	N	N	N	N	4	NIL	BUDDING YEAST CELLS				Y	PREV LSCS IN LABOUR
40	29-05-2020	25	5327158	G2A1	04-01-2020	10-10-2020	21-10-2020	19WK 2D	N	N	N	N	2	NIL	NIL				Y	MSL WITH UNFAVOURABLE CERVIX
41	29-05-2020	23	5703999	G4P2L1D1	08-01-2020	15-10-2020		20WK 2D	N	N	N	N	2	NIL	NIL				Y	
42	29-05-2020	29	5709850	G2P1L1	28-12-2019	30-09-2020		21WK 6D	N	N	N	N	1	NIL	NIL				Y	
43	29-05-2020	21	5710308	PRIMI	01-02-2020	08-11-2020		16WK 6D	N	N	N	N	1	NIL	NIL				Y	
44	29-05-2020	20	5638972	PRIMI	12-11-2019	17-09-2020		19WK 6D	N	N	N	N	4	NIL	NIL			Y(32WK 1D)		
45	29-05-2020	21	5652385	PRIMI	11-01-2020	18-10-2020		19WK 6D	N	N	N	N	6	NIL	BUDDING YEAST CELLS				Y	
46	29-05-2020	24	5710535	PRIMI	22-02-2020	28-05-2020		13WK 6D	N	N	N	N	4	VAGINAL COMMENSALS	NIL				Y	
47	29-05-2020	21	5711528	G3P1L1A1	12-01-2020	18-10-2020		19WK 5D	N	N	N	N	1	NIL	NIL				Y	PREV LSCS IN LABOUR
48	29-05-2020	23	5710818	PRIMI	11-01-2020	20-10-2020		19WK 6D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	
49	14-07-2020	24	5750537	G2A1	16-04-2020	21-01-2021		13WK	N	N	N	N	4	VAGINAL COMMENSALS	NIL			Y(36WK 6D)		
50	14-07-2020	19	5723399	PRIMI	12-04-2020	17-01-2021		13WK 2D	N	N	N	N	1	NIL	NIL				Y	
51	14-07-2020	25	5768703	PRIMI	28-02-2020	04-12-2020		13WK 4D	N	N	N	N	1	NIL	NIL				Y	
52	14-07-2020	22	3828549	G2P1L1	10-04-2020	17-01-2021		19WK 4D	N	N	N	N	2	NIL	NIL		Y(38WK)			
53	14-07-2020	26	4177186	G4P2L1A1	23-03-2020	28-12-2020		16WK 1D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	PREV LSCS IN LABOUR
54	14-07-2020	27	5750544	G3P2L2	25-02-2020	01-12-2020		20WK	N	N	N	N	1	NIL	NIL				Y	
55	28-07-2020	28	4883747	G2A1	23-03-2020	28-12-2020		18WK 1D	N	N	N	N	3	VAGINAL COMMENSALS	NIL				Y	
56	28-07-2020	23	5783169	PRIMI	10-03-2020	15-12-2020		20WK	N	N	N	N	1	VAGINAL COMMENSALS	NIL		Y(39WK)			
57	28-07-2020	25	5766449	PRIMI	09-02-2020	15-11-2020	15-12-2020	20WK 1D	N	N	N	N	1	NIL	NIL				Y	
58	28-07-2020	24	5729742	G2P1L1	12-03-2020	17-12-2020		19WK 5D	N	N	N	N	6	NIL	NIL			Y(35WK 2D)		
59	28-07-2020	26	5784495	G3P2L2	14-03-2020	19-12-2020		19WK 3D	N	N	N	N	4	VAGINAL COMMENSALS	NIL	Y(35W K 1D)				ANAMNIOS
60	01-09-2020	27	4253285	G2P1L1	27-04-2020	01-02-2021		18WK 1D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	PREV LSCS NOT WILLING FOR VBAC
61	01-09-2020	20	5750649	PRIMI	16-04-2020	21-01-2021	28-01-2021	18WK 5D	N	N	N	N	3	VAGINAL COMMENSALS	NIL				Y	
62	01-09-2020	20	5701223	G2A1	UNKNOWN		10-03-2021	13WK 6D	N	N	N	N	4	VAGINAL COMMENSALS	NIL			Y(34WK 2D)		
63	01-09-2020	27	4648016	G4P1L1A2	12-05-2020	16-02-2021		16WK	N	N	N	N	2	NIL	NIL				Y	BOH WITH GESTATIONAL HYPERTENSION AT 34W2D
64	08-09-2020	29	5825509	G3P1L1A1	05-06-2020	12-03-2021		13WK 4D	N	N	N	N	1	NIL	NIL				Y	PREV LSCS NOT WILLING FOR VBAC
65	08-09-2020	24	5749267	G2P1L1	20-04-2020	25-01-2021	02-02-2021	18WK 6D	N	N	N	N	6	NIL	NIL			Y(35W 5D)		
66	08-09-2020	29	3677738	PRIMI	23-05-2020	27-02-2021		15WK 3D	N	N	N	N	2	VAGINAL COMMENSALS	NIL		Y(38WK 6D)			
67	08-09-2020	40	5825853	G2P1L1	25-06-2020	01-04-2021	15-02-2021	17WK	N	N	N	N	1	NIL	NIL				Y	
68	08-09-2020	30	5826238	G3P2L2	22-04-2020	27-01-2021		19WK 6D	N	N	N	N	6	NIL	NIL				Y	
69	08-09-2020	20	5568357	G2P1L1	05-05-2020	09-02-2021		18WK	N	N	N	N	6	NIL	NIL			Y(29WK 6D)		
70	08-09-2020	29	5826428	G4P2L2A1	12-05-2020	16-02-2021		14WK 4D	N	N	N	N	2	NIL	NIL				Y	PREV 2 LSCS IN LABOUR
71	08-09-2020	23	5812728	PRIMI	20-04-2020	25-01-2021		18WK 1D	N	N	N	N	1	NIL	NIL				Y	CDMR
72	08-09-2020	23	5826831	PRIMI	14-04-2020	19-01-2021		20WK 5D	N	N	N	N	2	NIL	NIL		Y(38WK 2D)		Y	ANAMNIOS
73	20-10-2020	36	5866857	G3P2L2	18-06-2020	25-03-2021		17WK 5D	N	N	N	N	1	VAGINAL COMMENSALS	NIL	Y(34W K)				PREV LSCS WITH PPROM WITH SEVERE OLIGO
74	20-10-2020	20	5867530	PRIMI	17-07-2020	23-04-2021		13WK 4D	N	N	N	N	2	NIL	NIL		Y(37WK 5D)			
75	20-10-2020	26	5832639	PRIMI	27-05-2020	03-03-2021		20WK 6D	N	N	N	N	1	METHICILLIN SENSITIVE	NIL				Y	

														STAPH AUREUS						
76	20-10-2020	21	5812196	PRIMI	28-05-2020	04-03-2021		20WK 5D	N	N	N	N	2	NIL	CANDIDA				Y	
77	20-10-2020	22	5854521	PRIMI	20-07-2020	26-04-2021		13WK 1D	N	N	N	N	6	VAGINAL COMMENSALS	NIL				Y	
78	23-10-2020	22	5819793	PRIMI	29-06-2020	05-04-2021		16WK 4D	N	N	N	N	2	NIL	CANDIDA				Y	CPD
79	23-10-2020	19	5862779	PRIMI	20-05-2020	24-02-2021		20WK 6D	N	N	N	N	1	KLEBSIELLA PNEUMONIAE	NIL				Y	
80	04-11-2020	20	5867530	PRIMI	17-06-2020	06-05-2021		20WK	N	N	N	N	2	NIL	NIL				Y	FETAL DISTRESS
81	18-11-2020	25	4782830	G2P1L1	18-06-2020	25-03-2021		20WK 6D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	PREV LSCS NOT WILLING FOR VBAC
82	18-11-2020	27	5850909	PRIMI	10-07-2020	16-04-2020		18WK 5D	N	N	N	N	2	VAGINAL COMMENSALS	NIL				Y	
83	18-11-2020	31	4653761	G5P1L1A3	14-07-2020	20-04-2021		18WK 1D	N	N	N	N	2	VAGINAL COMMENSALS	NIL				Y	PREV LSCS IN LABOUR
84	18-11-2020	20	5843843	PRIMI	16-06-2020	23-03-2021	05-04-2021	20WK 2D	N	N	N	N	1	VAGINAL COMMENSALS	NIL			Y(36WK)		
85	01-12-2020	24	3767488	G3P2L2	25-07-2020	01-05-2021		18WK 3D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	SEVERE FGR WITH PATHOLOGICAL TRACE
86	01-12-2020	26	5869286	G5P2L2A1	09-08-2020	16-05-2021		16WK 1D	N	N	N	N	1	NIL	NIL				Y	
87	02-12-2020	27	5850909	PRIMI	10-07-2020	16-04-2020		18WK 5D	N	N	N	N	1	NIL	NIL				Y	
88	02-12-2020	22	4558756	G2P1L1	08-08-2020	15-05-2021	26-05-2021	15WEEKS	N	N	N	N	1	NIL	NIL				Y	PREV LSCS NOT W/F VBAC
89	02-12-2020	24	5898091	PRIMI	10-07-2020	16-04-2021		20WK 5D	N	N	N	N	2	NIL	NIL		Y(38WK 2D)			
90	02-12-2020	23	5977493	PRIMI	23-07-2021	29-04-2021	20-04-2021	20WK 1D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	MSL WITH FETAL DISTRESS
91	02-12-2020	20	5983654	PRIMI	12-07-2020	18-04-2021		20WK 3D	N	N	N	N	2	VAGINAL COMMENSALS	NIL				Y	
92	02-12-2020	29	5912956	G2P1L0	20-08-2020	27-05-2021		14WK 6D	N	N	N	N	1	NIL	NIL				Y	
93	05-12-2020	23	5916610	PRIMI	23-08-2020	30-05-2021		14WK 6D	N	N	N	N	1	NIL	NIL				Y	
94	05-12-2020	25	5916669	PRIMI	18-07-2020	24-04-2021		20WK	N	N	N	N	2	NIL	CANDIDA				Y	
95	05-12-2020	23	5916733	PRIMI	24-08-2020	31-05-2021		13WK 4D	N	N	N	N	1	NIL	NIL		Y(38WK 5D)			
96	05-12-2020	25	6018991	PRIMI	15-07-2020	21-04-2021	05-03-2021	14WK 3D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	
97	09-12-2020	20	5849177	PRIMI	06-08-2020	13-05-2021		17WK 6D	N	N	N	N	1	VAGINAL COMMENSALS	NIL				Y	
98	09-12-2020	22	6064979	PRIMI	07-08-2020	14-05-2021		17WK 5D	N	N	N	N	2	NIL	NIL				Y	
99	16-12-2020	26	5926711	PRIMI	24-08-2020	31-05-2021		13WK 6D	N	N	N	N	1	NIL	CANDIDA			Y(35WK 6D)		
100	17-12-2020	24	4108187	G3P2L1D1	17-07-2020	23-04-2021		21WK 6D	N	N	N	N	1	NIL	NIL				Y	
101	17-12-2020	20	5916982	PRIMI	13-09-2020	20-06-2021		13WK 4D	N	N	N	N	1	VAGINAL COMMENSALS	NIL		Y(38WK 6D)			
102	17-12-2020	26	5920534	G2A1	24-08-2020	31-05-2021		16WK 3D	N	N	N	N	2	NIL	NIL				Y	FETAL DISTRESS
103	22-12-2020	21	3852338	G2P1L1	03-09-2020	10-06-2021		15WK 5D	N	N	N	N	1	NIL	NIL				Y	
104	23-12-2020	20	6016094	PRIMI	09-09-2020	16-06-2021		15WK	N	N	N	N	5	NIL	NIL			Y(36WK 2D)		
105	23-12-2020	24	4690677	G4P2L2A1	06-08-2020	13-05-2021		19WK 6D	N	N	N	N	2	NIL	NIL				Y	BREECH
106	05-01-2021	29	5419200	G2P1L1	02-09-2020	09-06-2021		15WK	N	N	N	N	2	NIL	BUDDING YEAST CELLS				Y	
107	02-02-2021	30	6103568	G3P1L1A1	29-09-2020	09-07-2021		18WK	N	N	N	N	6	NIL	NIL			Y(35WK)		
108	17-02-2021	28	6124487	G2P1L1	04-11-2020	11-08-2021		15WK	N	N	N	N	6	NIL	NIL			Y(33WK 3D)		
109	03-03-2021	18	6109214	PRIMI	28-11-2020	04-09-2021		13WK 4D	N	N	N	N	5	NIL	NIL			Y(34WK 6D)		
110	03-03-2021	21	5552614	G2P1L1	28-10-2020	05-08-2021		17WK 6D	N	N	N	N	1	NIL	NIL				Y	