

**MICROBIAL PATTERN IN AMNIOTIC FLUID AND HISTOLOGY OF THE
SMOOTH CHORION IN WOMEN WITH PREMATURE RUPTURE OF
MEMBRANES AND PRETERM PREMATURE RUPTURE OF MEMBRANES AT
THE KENYATTA NATIONAL HOSPITAL**

Principal Investigator:

Dr. Angela Anzeze. MBChB

Senior House Officer

H58/80841/2015

Department of Obstetrics and Gynaecology

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University of Nairobi

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DECLARATION

This dissertation is my original work and has not been presented for a degree in any other University.

Signature _____ **Date:** _____

Dr Angela Anzeze, MBChB.

Resident in the department of Obstetrics and Gynaecology (University of Nairobi)

CERTIFICATE OF SUPERVISION

This dissertation has been submitted with our approval as University supervisors:

Professor S.B.O Ojwang’, MBChB, M.Med (Obs/Gyn), Dip.Oncology

Professor, Department of Obstetrics and Gynaecology,

Consultant, Obstetrician and Gynaecologist, University of Nairobi.

Signature: _____ **Date:** _____

Dr George Gwako, MBChB, M.Med(Obs/ Gyn)

Lecturer, Department of Obstetrics and Gynaecology,

Consultant, Obstetrician and Gynaecologist, University of Nairobi

Signature: _____ **Date:** _____

Dr. Moses M. Obimbo, (MBChB, MSc, MMED (Obs/ Gyn), PhD, Postdoc,

Senior Lecturer Department of Human Anatomy,

Consultant, Obstetrician and Gynecologist, University of Nairobi

Signature: _____ **Date:** _____

The dissertation was prepared in consultation with,

Dr. Edwin Walong’, MBChB, MMED Pathology, FCPATH ECSA,

Lecturer, Department of Human Pathology, University of Nairobi

Signature: _____ **Date:** _____

CERTIFICATE OF AUTHENTICITY

This is to certify that **DR. ANGELA ANZEZE**, registration number **H58/80841/2015**, has completed this dissertation in the department of Obstetrics and Gynaecology, University of Nairobi, under the guidance and supervision of Professor S.B. Ojwang', Dr. Obimbo Moses, Dr. George Gwako and Dr. Edwin Walong'

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The study was funded by the Kenyatta National Hospital research and programmes department, upon approval by the KNH/UON ethics research committee.

Signature:..... Date:.....

Professor, Omondi Ogutu, MBChB, M.Med (Obs/Gyn), PGDRM

Associate professor, Department of Obstetrics and Gynaecology,

Consultant, Obstetrician and Gynaecologist,

Chairman, Department of Obstetrics and Gynaecologist,

University of Nairobi.

ABBREVIATIONS

ANC	Antenatal care
ANW	Antenatal Ward
BV	Bacterial Vaginosis
ECM	Extracellular matrix
FIRS	Foetal Inflammatory Response Syndrome
GBS	Group B Streptococci
HVS	High vaginal swab
ICU	Intensive Care Unit
IL's	Interleukins
KEMRI	Kenya Medical Research Institution
KMTC	Kenya Medical Training College
KNH	Kenyatta National Hospital
NBU	New Born Unit
NG	Neisseria Gonorrhoea
NNICU	Neonatal Intensive Care Unit
PROM	Premature Rupture of Membranes
PPROM	Preterm Premature Rupture of Membranes
SS	Sample Size
UoN	University of Nairobi

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OPERATIONAL DEFINITIONS

Premature Rupture of Membranes: Rupture of membranes prior to the onset of labour beyond 37 weeks' gestation.

Preterm Premature Rupture of Membranes: Rupture of membranes prior to the onset of labour, before 37 weeks' gestation.

Prolonged Rupture of Membranes: Prolonged ROM is any ROM that persists for more than 24 hours and prior to the onset of labour.

Chorion: The outermost layer of the foetal membranes

Amnion: The inner most layer of the foetal membranes.

Smooth Chorion: The part of the chorion that is in contact with the decidua capsularis undergoes atrophy, so that by the fourth month, a trace of the villi is left. This part of the chorion becomes smooth, and is named the chorion laeve (from the Latin word *levis*, meaning smooth)

Nitrazine Test: It's a test that uses a pH indicator dye ranging from 4.5 – 7.5. It changes colour from yellow to blue, which confirms membrane rupture.

Culture and Sensitivity: Tests to identify specific microorganisms that cause infection, and their susceptibility to antimicrobial agents.

Preterm Birth: Birth of a child before 37 completed weeks

Preterm Labour: Onset of labour before 37 completed weeks.

Chorioamnionitis: Chorioamnionitis is defined as acute inflammation of the foetal membranes due to ascending infection.

Neonatal sepsis: It is associated with acquisition of microorganisms from the mother. Trans - placental infection or an ascending infection from the cervix may be caused by organisms that colonize the mother's genitourinary (GU) tract. The neonate acquires the microorganisms as it passes through the colonized birth canal at delivery. (Early neonatal sepsis)

ABSTRACT

Background: Premature membrane rupture complicates 2% of pregnancies but is linked with 40% of premature delivery and high perinatal morbidity and mortality. Ascending genital infections have been associated with occurrence of premature membrane rupture. Multiple pathogens are implicated.

Objective: To examine the associations between amniotic fluid microbial growth and the histology of the smooth chorion of the placenta with pregnancy outcomes among women with PROM and PPRM.

Methods: This was a prospective cohort study conducted at the Kenyatta National Hospital labour ward between May and August 2018. A total of 50 women were recruited, 29 with preterm premature rupture of membranes, and 21 with rupture of membranes at term. Eligible mothers were interviewed to obtain medical history, and physical examination performed. High vaginal swab/ amniotic fluid samples were collected for microscopy culture and sensitivity. The placentae were collected at delivery for histology. Placental pathological changes and amniotic fluid bacteriology were the exposure variables. Clinical outcomes (maternal disease, neonatal disease) were the outcome variables.

Results: Women with PPRM had a mean age of 25.7 years; the mean age for PROM was 29.2 years. ($p=0.017$). Among women with PPRM, candida species (17.2%), Group B streptococcus (13.8%) and E.coli (10.3%), were the most common microbes, while in PROM, candida (19.1%), Group B streptococcus (14.3%), E.coli (5%), E. faecalis (5%) were isolated. *Group B streptococcus* was sensitive to other Penicillins (80%), Cephalosporin's (80%), Beta lactamase inhibitors (80%), Azithromycin (20%), Erythromycin (20%), and resistant to Amoxicillin 0%. *E. coli* was sensitive to Cephalosporin (100%), other Penicillins (80%), Beta lactamase inhibitors (100%), resistant to Amoxicillin (0%) and Erythromycin (0%). No sensitivity patterns were performed for *candidiasis*. On placental histological examination, 51.7% had maternal inflammation ($p=0.008$, RR 4.0), while 27.6% had foetal inflammation in patients with PPRM. For those who presented with PROM, 14.3% had maternal inflammation

Conclusion and Recommendations: In our set up, erythromycin is commonly used for prophylaxis in premature rupture of membranes to prevent chorioamnionitis. Sensitivity patterns have demonstrated increased resistance to these organisms. Histological chorioamnionitis was more common in PPRM than PROM. Antibiotic therapy reduces incidence of adverse maternal and neonatal outcomes. Update guidelines should be put in place to initiate antimicrobial cover for these patients, with culture sensitive drugs recommended as 1st line treatment, while awaiting amniotic fluid culture results.

CHAPTER ONE: INTRODUCTION

1.0 Background of the Study

Preterm premature rupture of membranes (PPROM) is rupture of membranes at <37 weeks gestation(1). Premature rupture of membranes (PROM) is rupture of membranes at ≥ 37 weeks. PPRM occurs in 2% of pregnancies and contributes almost 1/5 of perinatal mortality(2). Prematurity, sepsis, and haemorrhage(3) are the main causes of neonatal death following PROM. Women with sexually transmitted and intrauterine infections are prone to earlier delivery(4).

Collagen is the main component responsible for the tensile strength of foetal membranes, which comprise the amnion and the chorion fused by the extracellular matrix (ECM). The foetal membranes form the inner lining of the intrauterine cavity that encases the gestation. Alterations in production or degeneration of collagen may therefore predispose to rupture of membranes(5).

The amnion is a quintet layer derived from the embryonic ectoderm (6).It lacks neurovascular input and derives nutrition from the adjacent liquor(7). The layer in proximity with the amniotic fluid is the amniotic epithelium, which secretes the collagen types 3 and 4 and other glycoproteins that form the basement membrane (7). The chorion is four times thicker than the amnion. During the course of the pregnancy, it forms following avascular degeneration of villi.(8). The amnion and the chorion fuse at 12 weeks gestation, expand to fill the endometrial cavity by 20 weeks gestation and serve a protective role(8,9). The extracellular matrix of the chorioamnion is composed of fibrous proteins in a carbohydrate medium, which collectively facilitate their strength and elasticity(10). Collagens are the main component of the foetal membranes. These collagens undergo constant remodelling throughout the

pregnancy to accommodate for the growing foetus. During the last eight weeks there is decreased collagen in the membranes.

The precise role of pathogens in rupture of membranes remains shrouded in mystery (11). Thinning of the chorion layer in patients with PROM and PPROM tends to be more marked at the site of rupture(12). The presence of vaginal infections in the 3rd trimester is a known risk factor for PROM and PPROM. They stimulate the production of cytokines and a systemic foetal inflammatory response syndrome (FIRS)(11). These organisms produce bacterial collagenases, and matrix degrading enzymes which are known to significantly reduce the tensile strength and elasticity of the membranes, predisposing to rupture (11). Maternal serum Interleukins 1 α , 1 β & 6 levels are higher in PPROM than in PROM (13).

Chorioamnionitis refers to an inflammatory process occurring in the foetal membranes following a scant vaginal infection (14). Histologically, it's defined as presence of polymorphnuclear cells in the amnion and chorion (14). Currently published literature presents a hiatus of data on the pathophysiologic sequelae that culminate in chorioamnionitis, including the most culpable microbes and the gestational age incurring the highest risk. Chorioamnionitis is the most significant maternal consequence of PROM and PPROM.

Various microbes have been implicated in the pathophysiologic basis of PROM and PPROM following assurgent vaginal infection. A study done in 2014 in India by Shikha R *et al* to evaluate vaginal flora in PPROM found that *Escherichia. coli* and *Staphylococcus auerus* were the most common isolates. *Trichomonas vaginalis* and *Bacterial Vaginosis* have also been implicated(15). A case control study in Uganda in 2015 implicated *N. gonorrhoea* and

Group B Streptococcus (GBS)(16). Onyango (2009) in KNH, concluded that *E. Coli* caused >2/3 of cases of PROM(17).

The aim of this study was to identify the microbial pattern in amniotic fluid, the histological profile and their relation to selected pregnancy outcomes among women with PROM and PPROM at the Kenyatta National Hospital in Nairobi, Kenya. The findings from this study will influence policy, clinical practice and provide opportunities for research, to establish these associations further.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

PROM complicates 1 in 10 pregnancies (18), but is associated with 2/5 of preterm deliveries and adverse neonatal outcomes(19). There is evidence demonstrating an association between ascending infection from the lower genital tract and PROM, PPROM (20). In patients with PROM, 1/3 of pregnancies have positive cultures and studies have shown that bacteria have the ability to cross intact membranes(20). Vaginal pooling of liquor confirms the diagnosis.

Following a suggestive history (sudden vaginal gushing of fluid that runs down the legs) and a sterile speculum examination, diagnosis confirmed using the following tests on pooled fluid in the vaginal fornix(21), the Nitrazine test (detects pH change) (20); the ferning crystallization microscopic evaluation of vaginal fluid; and examination for lanugo hair and foetal epithelial cells stained with Nile blue(22). Digital vaginal examination is contraindicated unless delivery is imminent in order to avoid iatrogenic contamination that may trigger preterm labour(23).

2.2 Non Infective Causes of PROM/PPROM

The possible causes include: Increased friability of the membranes, decreased tensile strength of the membranes, polyhydramnios, multiple pregnancy, cervical insufficiency, prior preterm labour, low BMI ($< 19\text{kg/m}^2$) and smoking(24).

2.3 Prevalence and Microbial Causes of Premature Rupture of Membranes

A case control study done in 2009 by Onyango at KNH to identify the current microbial patterns of patients presenting with PROM at KNH labour ward, concluded that *E. Coli* was the most common bacterial isolate accounting for 66–70% in PROM. Other isolates were

Staphylococcus aerus and *Streptococcus viridans*(17). Literature from a 2015 research at Mulago Hospital discovered that *Neisseria gonorrhoea* and *Group B Streptococcus* were associated with PROM(16). *T vaginalis* has been discovered to weaken membranes and prospective studies have discovered a link between *T. vaginalis* and PROM (16).

Women with *bacterial vaginosis* (BV) were more likely than women without BV to have PROM. Maternal syphilis is associated with adverse obstetric outcomes (16). *GBS* and *T. vaginalis* were associated with PROM at the Mulago hospital(16).

A 2014 study in India discovered that the most prevalent organisms were gram-negative bacteria such as *E. coli* and gram-positive bacteria such as *Staphylococcus aerus*(15). A 2017 research on the causes of preterm premature rupture of membranes discovered that four lactobacillus non-lactobacillus subtypes and *Gardnerella vaginalis* were observed in females with PPRM in the cervical microbiota. It was found that non-lactobacillus species presence was associated with greater cervical inflammation rates and the attack of the amniotic cavity by the microbe(25).

A Study by Kimberly in 2014 found that the fetal membrane integrity plays an important role in the maintenance of pregnancy(18). Cell death within the chorion with premature activation of the cellular apoptotic pathways has been demonstrated when infection is present. In term patients, apoptotic cell death was greater with histological chorioamnionitis. The greatest degree of chorion thinning and apoptotic cell death was found among subjects with PPRM (18).

2.4 Outcomes Associated with Premature Rupture of Membranes

Maternal complications include abruption placenta (26), chorioamnionitis (13-60%), endometritis (2%-13%) and cord prolapse. Fetal complications include pulmonary hypoplasia, skeletal deformities related to oligohydramnios, fetal inflammatory response syndrome, respiratory distress, long-term neurological complications and sepsis(27). Without antibiotics >50% mothers will deliver within 48 hours(26).

The acute histological manifestation of chorioamnionitis is increased polymorphonuclear cells. Key clinical manifestations include fever, fundal tenderness, tachycardia (maternal and fetal) and offensive liquor (19).

Fetal inflammatory response syndrome (FIRS) is defined as acute systemic inflammatory response to intra-amniotic infection. The syndrome is characterized by elevated levels of interleukin 6, neutrophils, and sepsis and organ injury. Its histological hallmarks are funisitis and chorionic vasculitis. FIRS is a risk factor for adverse neonatal outcome in preterm infants(28).

2.5 Conceptual Framework

Microbial colonization leads to ascending infection that predisposes to rupture of membranes. Ascending infection causes maternal inflammation (chorioamnionitis). Non-infective causes (multiple pregnancy, cervical incompetence and prior preterm labor) may also culminate in PROM and PPROM. Regardless of the underlying cause, PROM, PPROM, and chorioamnionitis share a final common pathway that enhances the risk of adverse obstetric outcomes.

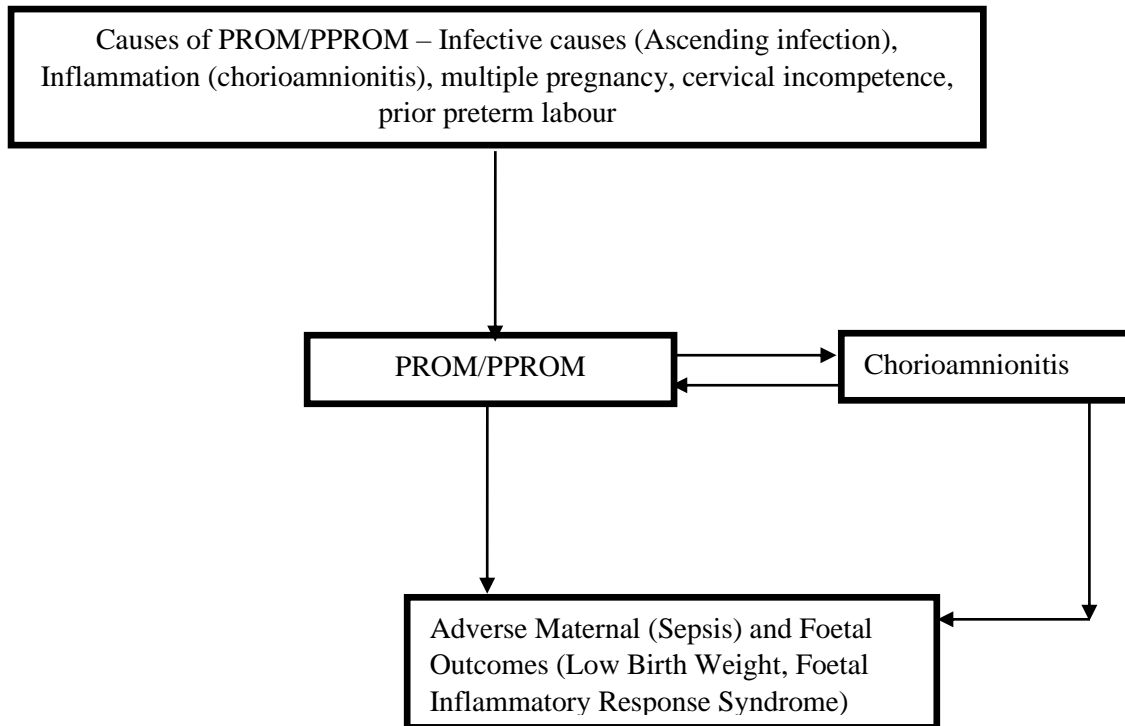


Figure 1: Conceptual Framework

2.6 Study Justification

PROM and PPROM are associated with unacceptably high obstetric morbidity and mortality. Because of PPROM, infants born with sepsis have a fourfold higher mortality rate. Chorioamnionitis associated with PROM and PPROM may result in adverse maternal and fetal outcomes.

Locally, no data exists on the microbial patterns in PROM and PPROM, and the clinical outcomes of histologically confirmed chorioamnionitis. The bacterial etiological agents of chorioamnionitis complicating PROM and PPROM are unknown. Insight into the impact of chorioamnionitis and its bacterial etiology on adverse maternal outcomes has established association and causality. It has identified at risk pregnancies for the purpose of clinical and public health intervention.

2.7 Research Question

What are the microbial patterns and clinical outcomes of histologically confirmed chorioamnionitis associated with PROM and PPRM at KNH?

Null hypothesis: There is no difference in the microbial pattern, histological findings and clinical outcomes among mothers with PROM and PPRM.

2.8 Study Objectives

2.8.1 Broad Objective

To identify the microbial pattern in amniotic fluid, histology of the placenta and maternal and neonatal outcomes in women with PROM and PPRM at the KNH, Nairobi, Kenya.

2.8.2 Specific Objectives

Primary Objectives

Among women with PROM and PPRM at KNH:

1. To identify the microbial pattern in amniotic fluid.
2. To identify the histological characteristics of the chorion, umbilical cord and the placenta.

Secondary Objective

3. To analyze the association between microbial patterns, chorioamnionitis and adverse maternal and neonatal outcomes.

CHAPTER THREE: METHODOLOGY

3.1 Study Design

A prospective descriptive cohort study in which 21 women with PROM and 29 women with PPRM at admission had amniotic fluid obtained for microbial evaluation and followed for histological analysis of the smooth chorion, umbilical cord and placenta for chorioamnionitis, funisitis and placental infarct. Adverse maternal and neonatal outcomes were also determined.

3.2 Study Site and Setting

Research work was done at the KNH, labour ward and antenatal ward. KNH was founded in 1901 as a National Referral and Teaching Hospital to fulfil its function. In 1987 it became a state corporation. KNH serves both as the largest referral hospital in Kenya as well as the training site for the school of Medicine (UoN). The hospital also serves the residents of Nairobi County.

The KNH has 50 wards, 22 clinics, 24 theatres, and a department for accidents and emergencies. The complete bed capacity is 1800. The hospital serves an average of 80,000 inpatients and more than 500,000 out patients each year. It provides a complete variety of health facilities. The department of reproductive health is made up of ANC, maternity theatre, antenatal wards, and gynaecology / oncology wards, NBU, NICU, and adult ICU. KNH's labour ward has 2 maternity theatres, 32 beds for patients (including 7 couches). The unit serves between 60-120 patients per day, with a monthly average of 1400 patients.

The unit handled an average of 14,872 deliveries in 2017, out of which only 149 patients had PROM and PPRM according to data from the records department at KNH. The low numbers were due to the prolonged doctor's strike in 2017. The units are managed by several

specialists and registrars. The labour ward unit is ran by a total of 61 nurses trained in midwifery and emergency obstetric care. At any given 12 hour shift, there are 2 residents (one monitoring the acute patients), 2 residents covering the maternity theatre and 2 specialist consultants on call .KNH has a high patient turn over and a large population to recruit participants for the study.

3.3 Study Population

Participants in this research were pregnant women 14 years of age and above with PPRM (28 - <37 weeks) and PROM (\geq 37 weeks) seen at KNH labour ward and antenatal wards

3.4 Study Recruitment

3.4.1 Inclusion Criteria

Gravidae with PROM and PPRM. Emancipated minors (pregnant women 14 years of age) and above, willing and able to give consent.

3.4.2 Exclusion Criteria

Gravidae who underwent instrumentation or incurred trauma resulting in rupture of membranes were excluded from the study.

3.5 Sample Size Determination

The main outcome of this study is the culture positive bacterial characterization for patients with PROM and PPRM. A study by Hailemariam Segni et al (29) in 2017 concluded that the incidence of PROM was 14.6%. Taralekar Varshali in 2014 found the incidence of PPRM was 56.3% (30). It was hypothesized that patients with PPRM are more likely to have culture-positive bacterial chorioamnionitis than those with PROM.

Therefore taking the above findings and applying in this study, a sample size calculation using Allan Donner's proportions formula to detect a 46.3 % difference, was estimated using

the sample size formula $n = \frac{2(z_{1-\alpha/2}\sqrt{2\bar{p}(1-\bar{p})} + z_{1-\beta}\sqrt{p_c(1-p_c) + p_a(1-p_a)})^2}{(p_c - p_a)^2}$ [Allan Donner; Stat.

Medicine (1984)

Where $\bar{p} = (p_c + p_a)/2$ ($Z_{0.25} = 1.960$, and $Z_{0.8} = 0.842$).

Using statcalc software, that we would need to study a total of 20 women per group to achieve a 80% power at a two-sided alpha=0.05 level of significance.

Working with an assumed response rate of 80%, our recalculated sample size was $100/80 * 40 = 50$ mothers; applying a ratio of 1:1.4, the sample per group was 21 with PROM and 29 with PPROM.

3.6 Sampling Procedure

All women in the KNH maternity unit with PPROM and PROM were approached, screened and consent taken from eligible mothers to participate in the study.

3.7 Recruitment and Consent

Participants were recruited from the antenatal wards and labour ward. Informed consent was administered by the principal investigator and the study clinician. The same participants were followed up to labour and delivery where specimen from the smooth chorion of the placenta was obtained after delivery. History from the patients was taken by the research assistant or principal investigator.

3.8 Variables and Confounders

The primary outcome/dependent variables of interest are the microbial patterns and histological chorioamnionitis in women with PROM and PPROM at KNH. The secondary outcome variables are maternal outcomes (clinical chorioamnionitis, postpartum sepsis) and neonatal outcomes (low birth weight and sepsis,). The exposure/independent variables are PROM and PPROM.

3.9 Data Collection

Data was collected by questionnaires, after obtaining informed consent. Obstetric data relevant for the study was obtained from the patient's medical records and by history taking. The data was collected by the principal investigator and research assistant.

The following information was obtained as per the questionnaire (annex 2); demographic characteristics, socio economic status, last normal menstrual period, parity, duration of drainage of amniotic liquor, amount (number of pads changed and if fully soaked), smell and colour of fluid, any accompanying symptoms like fever, labour like pain, past obstetric history (previous deliveries and complications), antenatal clinic attendance (number of visits and antenatal profile results).

The principal investigator/research assistant performed physical examination immediately at admission which included: General examination and vital signs, abdominal exam to illicit tenderness, fundal height, foetal lie, foetal heart rate, speculum examination to obtain amniotic fluid sample and to confirm the diagnosis. Upon delivery, the placenta was collected for histological profile and data on maternal and neonatal outcomes were obtained.

3.10 Study Procedures

3.10.1 Amniotic Fluid Sampling

Clinical examination was performed immediately at admission, using a sterile speculum exam. Amniotic fluid that had pooled in the posterior vaginal fornix was collected using a sterile syringe and transferred to a sterile container, for gram stain, microbial culture and sensitivity respectively. High vaginal swab was performed for patients that were not actively draining. Details of cytological and microbial processing are in appendix 7.

3.10.2 Amniotic Fluid Examination (Described in Annex 4)

The amniotic fluid was collected from the posterior fornix of the vagina, using a sterile pipette and placed into a sterile bottle. The amniotic fluid was transported to the lab immediately for analysis. Amniotic fluid microscopy then culture was done to isolate the organisms. Specific culture media was used for each organism as follows:

Group B Streptococcus - Blood agar

E. Coli – Blood agar

Neisseria Gonorrhoea – Thayer Martin agar

Candidiasis species - Sabouraud Dextrose agar

Trichomonas Vaginalis – It was identified using wet preparation of the sample

Bacterial Vaginosis – It was identified using gram stain of the sample by calculating the score as per the Nugent's criteria below;

A score of:

0– 3 is considered negative for bacterial vaginosis

4- 6 is considered as altered vaginal flora

≥ 7 is considered positive for bacterial vaginosis

The details of specimen processing and preparation are described in appendix 4.

3.10.3 Examination of the Placenta

After delivery, gross examination was performed by the principle investigator and clinical research assistant, including; in the following order: membranes, cord, foetal surface and maternal surface. Any abnormalities were noted down and the doctor on duty was informed. The membranes were examined for completeness, insertion, decidual necrosis, oedema, retro membranous haemorrhage, meconium staining and transparency.

The cord was examined for completeness, point of insertion, length, presence of knots, torsion, strictures, number of vessels and haematomas. The foetal surface of the placenta was examined for: colour, opacity, cysts (number and size), amnion nodosum, and thrombosis of the foetal vessels. The principle investigator and research assistant examined the maternal surface for: completeness, normal fissures, lacerations (extent), depressed areas, retro placental haemorrhage (size and distance from margin).

Specimen for histopathological examination was obtained using a standard approach. These included a cross section of the umbilical cord, cotyledon, amniotic and chorionic membranes. These were processed using standard histopathological techniques. Sections were stained with haematoxylin and eosin (H/E) and gram stains. These were examined by a pathologist to establish evidence of chorioamnionitis.

The fresh placenta were sectioned for histological examination using a sharp blade. Sections of the placenta were taken to include the smooth chorion. Placenta awaiting examination were initially stored in individual, labelled containers containing 10% formalin. Details of tissue processing and staining are described in Annex 5.

3.10.4 Interpretation of Results

Gram stain – gram positive and negative organisms were identified. If gram positive, a catalase test was done to differentiate between *staphylococcus* and *streptococcus* species. For gram negative organisms, lactose and non-lactose fermenters were identified. Lactose fermenters such as *E.coli* were identified using the indole test. Non lactose fermenters were identified by the urease test. The Nugent's criteria was used to make a diagnosis of bacterial vaginosis. A score of 7-10 qualifies for bacterial vaginosis.

Research assistants trained as lab technicians analysed and interpreted the results in the lab and provided a report. The placenta and cord were examined for histological changes as a result of PROM and PPRM such as: sub chorionic thrombi, funisitis, chorionic vasculitis, villitis, trophoblastic proliferation and the presence of increased polymorph nuclear cells in chorioamnionitis.

3.10.5 Quality Assurance and Control Measures

Pre-analytical: Standard operating procedures were developed for specimen collection. Collection of amniotic fluid specimen was performed using sterile conditions as described in the appendix. Collection of placental tissue was performed while fresh, and appropriately fixed in 10% neutral buffered normal saline.

Analytical: Analysis of the amniotic fluid specimen was performed using cytopathological techniques. Gross pathological examination of the placenta was performed by the pathologist. Digital photography was performed in all the specimen with adequate exposure of the maternal and foetal surfaces of the placenta. Histopathology of the placental tissue was performed using standard operating procedures.

These were performed at the University of Nairobi Core Anatomic pathology laboratory. (ISO 9001:2015). Analysis of stained cytopathological smears and histopathological sections was done using a standardized approach by the pathologist. For external quality assurance, 20% of randomly selected placental photographs and pathological specimen were obtained and examined by a second pathologist. Microbial cultures were examined at the Lancet lab using standard procedures.

Post Analytical: Results were issued using standardized (synoptic) report formats. These had relevant diagnostic formulations and codes. Final reports were in cooperated as cytopathological and microbiological features.

3.10.6 Materials

The following equipment and supplies were used to conduct the study:

- Stationery
- Hospital equipment – sterile speculum, sterile gloves, specimen bottles, Doppler fetoscope, formalin, antiseptics.
- Laboratory equipment – microscope, microscope slides, culture media, reagents.

3.11 Data Management and Statistical Analysis

Pre coded paper and electronic questionnaires were used to collect the data. The questionnaires were pre- tested and analysed before a final draft was administered to the study participants. The research assistants were trained on confidentiality, interviewing, information retrieval and filling the questionnaire.

The principal investigator ensured that regular monitoring and supervision of the research assistants was done during the data collection period. This included checking of each filled questionnaire for completeness. Ten percent of the completed questionnaires were manually checked against the primary data source to ensure data accuracy.

All participant data did not bear the names of participants, but a serial number. Filled questionnaires were kept in a secure lockable cabinet only accessible by the principal investigator and research assistants.

After ethical approval, the principal investigator sought the assistance of two research assistants (one to collect specimen from patients in labour ward and antenatal wards, and the other to assist in specimen preparation for analysis in the lab). They were recruited based on their previous experience in data collection in the medical field. The research assistants were trained on confidentiality, interviewing, information retrieval and filling the questionnaire.

The main researcher ensured that the research assistants were regularly monitored and supervised during the information collection period. This included checking for completeness of each completed questionnaire. Ten percent of the completed questionnaires were checked manually against the primary data source to guarantee precision of the information.

No participant data bore the names of participants but a serial number. The principal investigator and research assistants kept filled questionnaires in a secure lockable cabinet only accessible by them. A data manager cleaned, coded and entered the data into a password protected MS Access database before data analysis.

Research assistants were trained on suitable interview methods and questionnaire filling. After thorough screening, recording of clinical findings was entered. Patient numbers were registered in a serialization register. This register was regularly checked against. If double entries were found, one of the questionnaires was removed, discarded and rectified for serialization. Any mistakes were inspected and fixed for information filled out on the questionnaires. Samples were closely stored for microscopy and culture to be analysed in the laboratory before expiry.

3.12 Statistical Analysis

Data was transferred to STATA software for analysis. Summary statistics was performed on demographic, clinical and laboratory data which was presented in charts and tables. Inferential statistics: To achieve specific objective 1 and 2: diagnosed microbial agents was compared to the outcome (chorioamnionitis) using 2 by 2 tables. Relative risks, chi square and Fischer's exact examination was done.

In addition, when compared to histopathological proof of chorioamnionitis as a gold standard, the sensitivity, specificity, favourable predictive values and adverse predictive values were determined. To achieve specific objective 3, chorioamnionitis was the exposure while outcomes were the maternal and foetal disease. Relative risk in addition to chi square and Fischer's exact test (where required) was performed. All statistical inferential tests was performed at 95% level of significance.

3.2 Ethical Considerations

- **Ethical Approval:** This was sought from the Kenyatta National Hospital and University of Nairobi Ethical Review committee prior to commencement of the study. Institutional approval was sought from the KNH Scientific and Research department, and the Department of Obstetrics and Gynecology.
- **Informed Consent:** Informed consent was administered to all participants. Because we anticipated that some participants will be minors, informed consent was sought from emancipated minors, defined as pregnant women 14 years and above, who are legally married and got pregnant out of will (not through rape or coercion). The informed consent form was administered by the principal investigator and study assistants, both of whom are medical doctors who have undergone training in ethical conduct of research. Persons who declined to provide informed consent were not allowed to participate in the study. Persons who chose to withdraw from the study were not coerced to participate.
- **Benefits of the Study:** This study identified the etiology of PPRM and PROM, a common pregnancy complication, its risk factors and outcomes. The individual study subjects benefited from close clinical follow up and early diagnosis of chorioamnionitis. The benefits to the community include a greater insight into the risk factors and outcomes which can be used to identify individuals at risk. Further, this study examined underserved populations, as pregnant women and particularly pregnant minors who are frequently excluded from studies.
- **Potential Risks:** The potential risks to the patients were injuries sustained during speculum examination, and aggravation of labour following examination. To minimize these risks, the principal investigator trained the research assistants on examination using speculums, and to avoid digital examinations at all times. All procedures were performed by trained and competent persons with strict adherence to

standard operating procedures and relevant quality assurance. Confidentiality was maintained by storing all study related documentation in lockable cabinets and password protected computers. Diagnostic information was offered to the clinician who discussed the findings with the study subjects in addition to providing clinical intervention where necessary.

4.0 STUDY LIMITATIONS

Our study was unable to distinguish vaginal flora from potential isolates from amniotic fluid. Because of this, histopathological evidence of chorioamnionitis provided evidence of infection related to chorioamnionitis. To control for this limitation, statistical analysis of the sensitivity and specificity of amniotic fluid gram stain in relation to histopathology was performed. The sensitivity pattern results were obtained after the participants were discharged from hospital care, hence they were not able to receive antibiotics that were targeted to the specific infectious agents.

CHAPTER FIVE: RESULTS

A total of 50 women with PROM/PPROM who qualified for the study were recruited at the KNH labour ward. Of the 50 participants, 21 had PROM and 29 had PPROM. Informed and written consent was taken, history and physical examination was performed for all the study participants. Posterior fornix of the vagina provided amniotic fluid/HVS samples that were later collected and taken to the KNH laboratory for microscopy/culture and sensitivity. The mothers were followed up to delivery for collection of the placenta for histological examination of the smooth chorion as shown in figure 2 below:

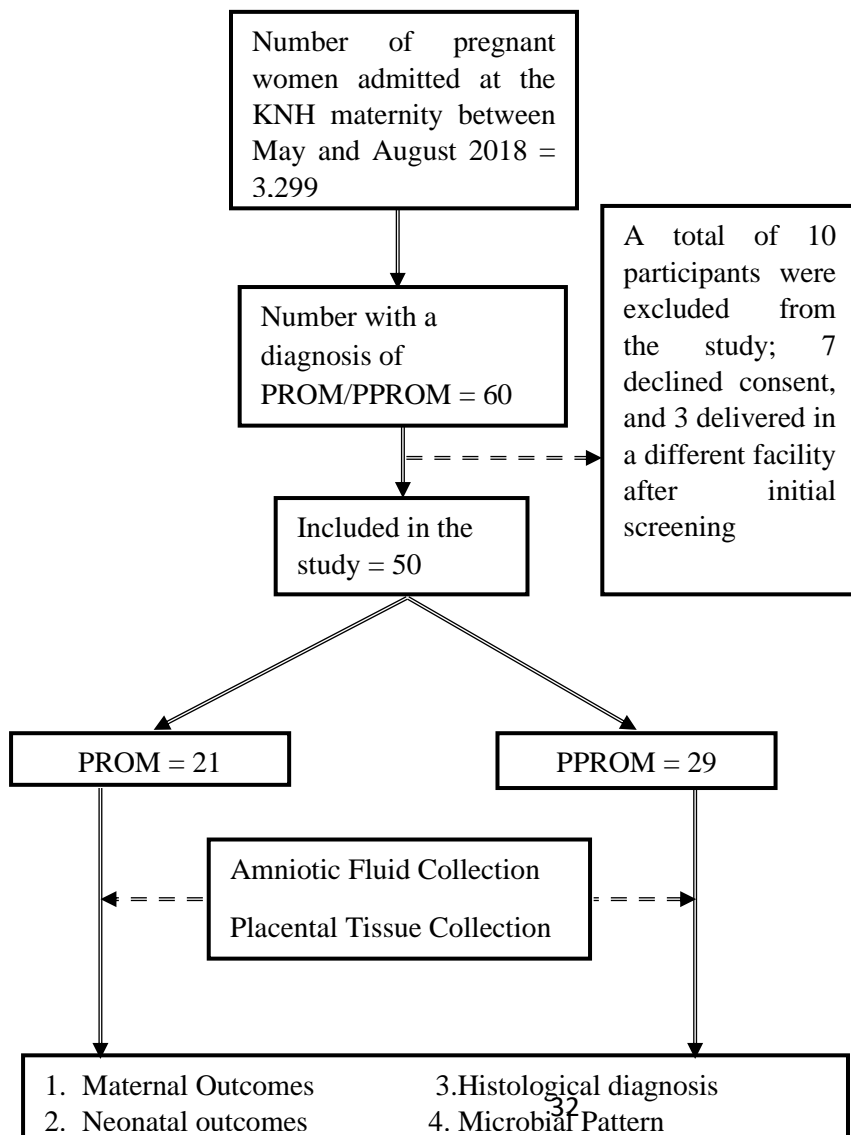


Figure 2:Flow chart showing recruitment of participants in the study.

5.1 Socio-demographic Characteristics

The socio – demographic characteristics of the women are summarised in table 1. Women who presented with PPRM were older than those with PROM (mean age 29 years Vs. 25 years; P val 0.017), had a longer hospital stay (7 days Vs 3 days; p val. 0.018), had higher levels of education, marital status and more in employment than those presenting with PROM, although these differences did not attain statistical significance.

Table 1: Socio Demographic Characteristics of study participants amongst women with PROM and PPRM at KNH between May and August 2018.

		PROM(N=21) n (%)	PPROM(N=29) n (%)	P value
Age (mean and SD)		25(4)	29 (6)	0.017
Mean hospital stay in days (mean and SD)		3(01.4)	7 (9)	0.018
Level of Education (Proportions)	College	10(48)	11(38)	0.920
	Secondary	3(14)	12(41)	
	Primary	8(38)	5(17)	
	None	0(0)	1(4)	
Employment Status (Proportions)	Employed	5(24)	12(41)	0.570
	Self employed	6(29)	5(17)	
	Student	1(15)	1(4)	
	Unemployed	10(43)	11(38)	
Marital Status (Proportions)	Married	15(71)	26 (90)	0.170
	Single	5(24)	3 (10)	
	Divorced	10(5)	0 (0)	

Abbreviations: PROM, Premature rupture of membranes, PPRM, Preterm premature rupture of membranes, SD, Standard deviation, KNH, Kenyatta National Hospital.

5.2 Microbial pattern in both PROM and PPRM

The microbial pattern in figure 3 indicates that *candida* species was the most common organism isolated (18%) followed by *Group B streptococcus* (14%), *E.coli* (8%), and 44% had no growth, in the entire sample.

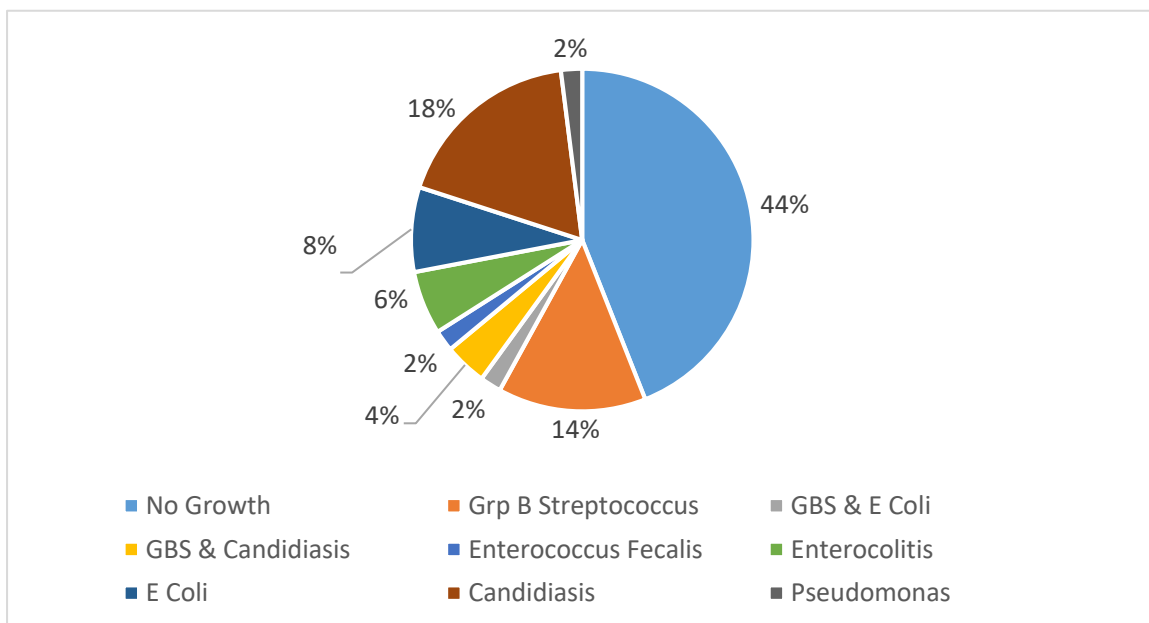


Figure 3: Microbial Pattern in Amniotic Fluid among women with PROM and PPRM (the entire sample) at the Kenyatta National Hospital between May and August 2018. (N=50) (n = %).

As indicated in table 2 below, Bacterial vaginosis (positive, using the Nugent's criteriadescribed in in section 3.10.2) was more common in PPRM (38%) than in PROM (24%)

This is however not significant (p val= 0.365) Samples with PROM commonly had altered vaginal flora (66.67%) than PPRM (41.38%).

Table 2: Microbial Pattern (Bacterial vaginosis as per the Nugent’s criteria) in amniotic fluid amongst women with PROM and PPROM at KNH between May and August 2018.

Bacterial Vaginosis Pattern	PPROM N = 29 n (%)	PROM 21 n (%)	N=	RR(95% CI)	P value
Altered Vaginal Flora	12(41.4)	14(66.7)		0.62(0.36-1.06)	0.093
Positive	11(38)	05(23.8)		1.59(0.67 -3.81)	0.365
Negative	06(20.7)	02(9.52)		2.17(0.51-9.22)	0.441

Abbreviations: PROM, Premature rupture of membranes, PPROM, Preterm premature rupture of membranes, RR, Relative Risk, CI, Confidence interval, KNH, Kenyatta National Hospital.

The microbial pattern in figure 4 below indicates that candida species was the most common organism isolated in patients with PPROM (17.2%), followed by Group B streptococcus (13.8%) and E.coli (10.3%), 44.8% had no growth.

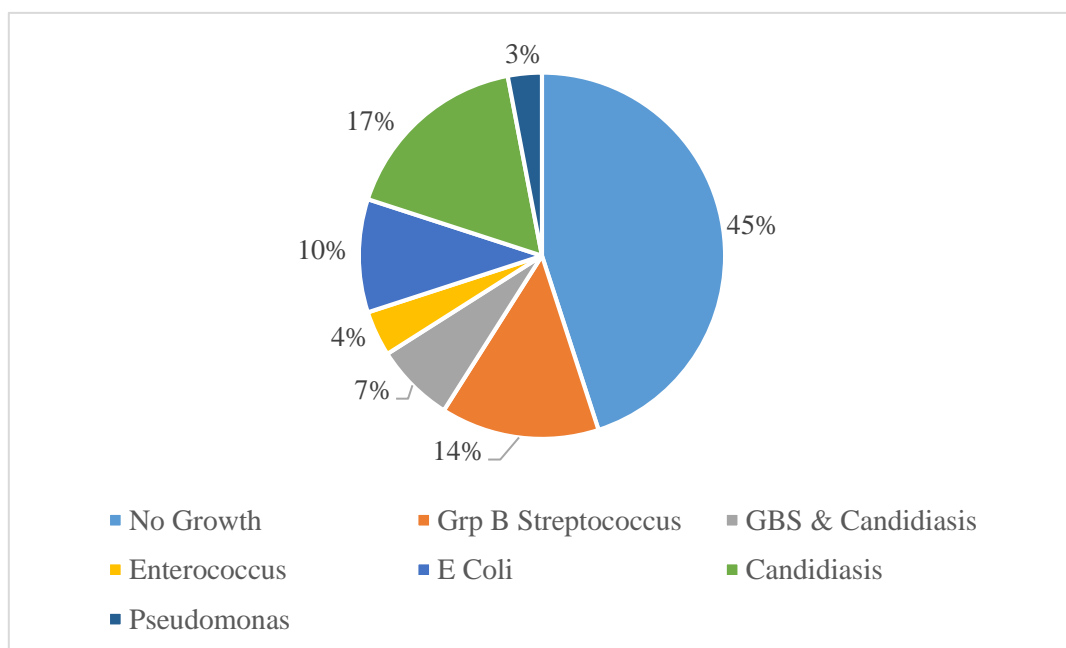


Figure 4:Microbial Pattern in Amniotic Fluid among women with PPROM at the Kenyatta National Hospital between May and August 2018. (N=29) (n = %)

Figure 5 indicates that *candida* species was the most common organism isolated in patients with PROM (19.05%), followed by *Group B streptococcus* (14.29%), *E.coli* (4.762%), *E. faecalis* (4.762%), and 42.86% had no growth.

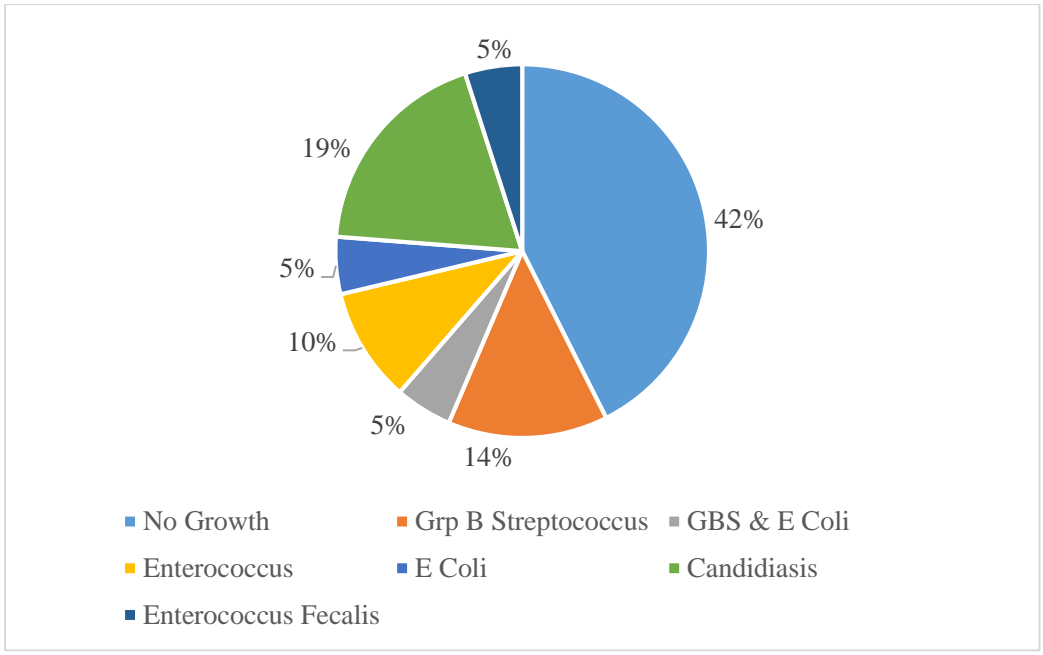


Figure 5:Microbial Pattern in Amniotic Fluid among women with PROM at the Kenyatta National Hospital between May and August 2018. (N = 21) (n= %)

Table 3 indicates that *Group B streptococcus* (n = 10) was the most common bacterial isolate, sensitive to other Penicillins 80%, Cephalosporin's 80%, Beta lactamase inhibitors 80%, Azithromycin 20%, Erythromycin 20%, and resistant to Amoxicillin 0%. *E. coli* (n = 5) was also isolated, sensitive to Cephalosporin 100%, other Penicillins 80%, Beta lactamase inhibitors 100% , resistant to Amoxicillin 0% and Erythromycin 0%. No sensitivity patterns were performed for *candidiasis*.

Table 3: Amniotic fluid antibiotic sensitivity amongst patients with PROM and PPRM at KNH between May and August 2018.

	Enterococcus (%) (n=3)	Candida (%) (n=0)	Group B Strep (%) (n=10)	Pseudomonas (%) (n=1)	E.Coli (%) (n=5)	E-Faecalis (%) (n=1)
Amoxicillin	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
Amoxiclav	1(33)	0(00)	0(00)	0(00)	0(00)	0(00)
Cephalosporins	1(33)	0(00)	8(80)	1(100)	5(100)	1(100)
B Lactamase	1(33)	0(00)	8(80)	1(100)	5(100)	0(00)
Cabapenems	1(33)	0(00)	3(30)	1(100)	2(40)	0(00)
Aminoglycosides	2 (67)	0(00)	7(70)	1(100)	5(100)	0(00)
Quinolones	2 (67)	0(00)	5(50)	1(100)	4(80)	0(00)
Cotrimoxazole	0(00)	0(00)	0(00)	0(00)	3(60)	0(00)
Glycoptedides	1(33)	0(00)	0(00)	0(00)	0(00)	0(00)
Penicilin	2 (67)	0(00)	8(80)	0(00)	4(80)	1(100)
Levofloxacin	1(33)	0(00)	0(00)	0(00)	0(00)	1(100)
Azithromycin	0(00)	0(00)	2(20)	0(00)	0(00)	0(00)
Clindamycin	0(00)	0(00)	2(20)	0(00)	0(00)	0(00)
Erythrmycin	0(00)	0(00)	2(20)	0(00)	0(00)	0(00)
Vancomycin	1(33)	0(00)	5(50)	0(00)	0(00)	1(100)
Tetracycline	0(00)	0(00)	3(30)	0(00)	0(00)	0(00)
Gentamycin	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)

Histological examination of the placenta (table 4 below) found maternal inflammation (histological chorioamnionitis) was more common in mothers with PPRM (52%) than those with PROM (14.3%), (RR 4, 95% C.I 1.34 – 11.9, p val. 0.008). Foetal inflammatory response, i.e funisitis, was only found in the samples taken from mothers with PPRM 8 (27.6%).

Table 4: Histological profile of the placenta amongst women with PROM and PPRM at KNH between May and August 2018.

Histological findings	PPROM (N = 29) n (%)	PROM (N= 21) n (%)	RR (95% CI)	P value
Chorioamnionitis	15 (51.7)	3 (14.3)	4 (1.34 – 11.9)	0.008
Funisitis	8 (27.6)	0 (0)	-	-
Cord vascular haemorrhage	0 (0)	2 (9.5)	-	-
Placental infarct	4 (13.8)	4 (19)	0.7(0.20-2.59)	0.705

Abbreviations: PROM, Premature rupture of membranes, PPRM, Preterm premature rupture of membranes, RR, Relative Risk , CI, Confidence interval, KNH, Kenyatta National Hospital.

In figure 6 below, the histological findings presented were inflammation of the umbilical cord-funisitis- (A) and chorioamnionitis (B). The other histological findings were umbilical cord vascular haemorrhage and placental infarct. The frequency distributions of these findings among women with PPRM were compared with those with PROM in table 4.

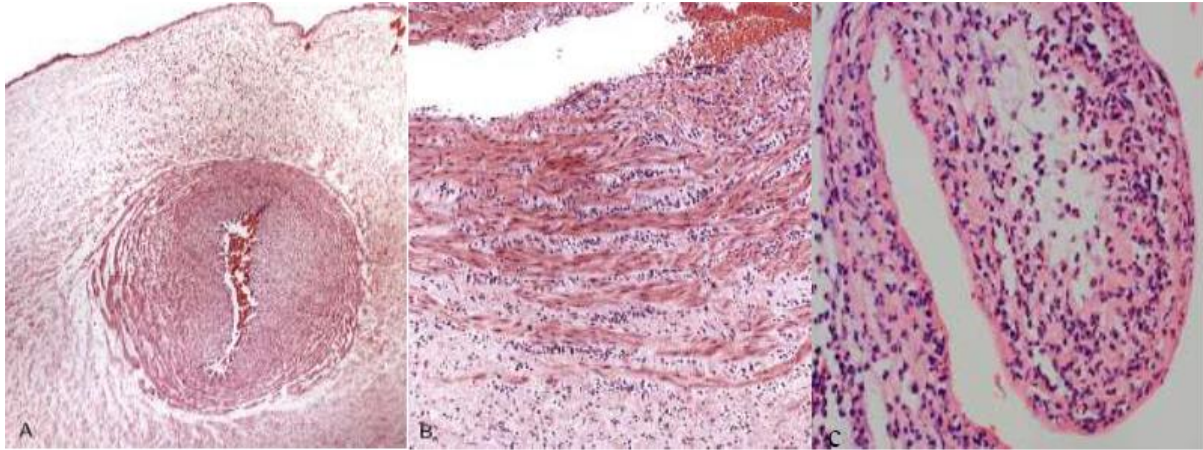


Figure 6: Histopathological images of the placental tissue: **a**, indicates features of umbilical cord vasculitis with funisitis at low power X10, **b**, shows infiltration of neutrophils at high power X40, indicating funisitis, **c**, indicates features of acute chorioamnionitis, with neutrophils infiltrating the membranes at high power field X40

Adverse maternal and neonatal outcomes (table 5 below), were more common in patients with PPRM than PROM. Amongst the patients with PPRM, 17.2% of the neonates had respiratory distress syndrome and 3.4% of the cases were neonatal deaths, while among women with PROM, there were no cases of respiratory distress syndrome and neonatal deaths. Among both populations, there were no cases of neonatal sepsis, as the participants were started on antibiotics after the diagnosis was confirmed. Post-partum haemorrhage was also more common in PPRM (6.9%), while only 4.7% of cases were found in mothers with PROM, but this difference was not statically significant.

Table 5: Adverse Maternal and Neonatal Outcomes amongst women with PROM, PPRM at KNH between May and August 2018.

Complication	PPROM(N = 29) n (%)	PROM(N=21) n (%)	RR(95% CI)	P value
Respiratory distress syndrome	5 (17.2)	0 (0)	-	-
Neonatal Sepsis	0 (0)	0 (0)	-	-
Neonatal deaths	1 (3.5)	0 (0)	-	-
Postpartum haemorrhage	2 (6.9)	1 (4.8)	1.48	1.000

Abbreviations: PROM, Premature rupture of membranes, PPRM, Preterm premature rupture of membranes, RR, Relative Risk , CI, Confidence interval, KNH, Kenyatta National Hospital.

6.0: DISCUSSION

The leading microbial agents isolated in both women with PROM and PPRM were *candidiasis*, *bacterial vaginosis*, *GBS* and *E.coli*, all occurring with different and low frequencies. *E.faecalis* was isolated only in women with PROM. Chorioamnionitis was found more commonly in women with PPRM than in those with PROM. Funisitis, cord vascular haemorrhage and placental infarct were identified in both groups, but again with different frequencies. The main adverse maternal and neonatal outcomes, all with very low frequency in this study were, respiratory distress syndrome, neonatal death and post-partum haemorrhage, but there was no neonatal sepsis. Low sensitivity patterns for mostly used antibiotics viz amoxicillin and erythromycin were documented, while sensitivity patterns for the other non-first line antibiotics were above 80%.

The low isolate yield in this study may be explained by the use of antibiotics among these participants before amniotic fluid and high vaginal samples were collected. However, routine application of the protocol for antibiotic use was instituted immediately upon clinical diagnosis of PROM and PPRM. At the same time, subclinical chorioamnionitis may be explained by the use of antibiotics as per the hospital protocol, which may explain absence of maternal and neonatal sepsis.

A study by Tum *et al* in a different county in Kenya, reported amniotic fluid cultures that isolated *C. albicans*, *T. vaginalis* and *S. aureus*, which were all sensitive to Augmentin, Ceftriaxone, Amoxicillin, Ampiclox and Levofloxacin (11). This variance may be due to the difference in geographical regions. A case control study by Onyango *et al* at KNH in 2009, identified *E. Coli* as the most common bacterial isolate accounting for 66–70% in

PROM, which frequency is much higher than that reported in this study(17). Other isolates were *Staphylococcus aerus* and *Streptococcus viridans*, which were not isolated in our study.

In a similar study by Arias *et al*, almost half of the study population exhibited acute histological chorioamnionitis (31). In Ethiopia, PROM and PPRM were associated with higher incidences of maternal and perinatal morbidity, with 1 in 10 mothers having sepsis and ¼ of babies requiring NICU admission and incurring poor Apgar scores(2), compared to our study which had low frequency of these adverse outcomes.

Post-partum haemorrhage has been associated with PROM/PPROM, particularly due to premature placental separation(23).Elsewhere, chorioamnionitis has been postulated to adversely affect uterine musculature, which would further predispose these patients to PPH (19).This study, had 3 cases of PPH. However, it was not possible to establish an objective statistical association due to the limited sample size.

Our study was unable to distinguish vaginal flora from potential isolates from amniotic fluid, however, histopathological evidence of chorioamnionitis provided evidence of infection related to chorioamnionitis as a strength. Furthermore, this multidisciplinary study is the first of its kind in the department of Obstetrics and Gynaecology, (UON), and placental studies have never been done in relation to PROM/PPROM locally.

7.0: CONCLUSION AND RECOMMENDATIONS

7.1 CONCLUSION

The common microbes isolated in our set up were *Group B Streptococcus*, *E. coli*, *Candidiasis* and *Bacterial vaginosis*(diagnosed as per the Nugent's criteria).Sensitivity patterns have demonstrated increased antibiotic resistance to these organisms. Chorioamnionitis was four times more common in PPRM than PROM. Although this may be difficult to establish, histological diagnosis of PROM and PPRM is a better indicator of sub clinical chorioamnionitis than amniotic fluid cultures done alone. Antibiotic therapy reduces incidence of adverse maternal and neonatal outcomes.

7.2 RECOMMENDATIONS

Findings from this study recommend update guidelines be put in place to initiate antimicrobial cover for patients with PROM and PPRM, with culture sensitive drugs recommended as first line treatment, while awaiting amniotic fluid culture results. It also provides baseline data for further research, to establish the associations between histological chorioamnionitis and adverse maternal outcomes further.

8.0 STUDY TIMELINE

	June 2017	July 2017	Nov 2017	Dec 2017	Jan 2018	Feb 2018	March 2018	April 2018	May 2018	June 2018	July 2018	Aug 2018	Sept 2018
Concept development													
Proposal development													
Ethical approval							DOCTOR'S STRIKE	DOCTOR'S STRIKE					
Data collection													
Data analysis													
Results presentation													

9.0 BUDGET AND JUSTIFICATION

Item	Description	Amount in (Ksh)
Personnel	2 research assistants allowances @Ksh 30,000 /month	180,000
	Data clerk/statistician@ 30,000Ksh	30,000
Supplies	Draft proposals printing:60pages, 3 copies @Ksh 10shs per page	1,800
	Final proposal printing: 60 pages, 3 copies @10Kshs per page	1,800
	Questionnaires printing ,13 pages, @10 Ksh per page	1,300
	Questionnaires photocopying, 13 pages, 148 copies @ 2 Ksh per page	3,848
	Lab form, 2 pages printing @ 10 Ksh per page	10
	Lab form photocopying, 2 pages , 148 copies @ 2 Ksh per page	592
	Airtime @ 5000Kshs	5,000
	Training of research assistants/ transport reimbursement	10,000
Materials	Lab costs	250,000
	Swabs, specimen containers, preservatives	35,000
Transport costs	-	5,000
KNH/UON	Submission to ERC	4,000
ERC	(twice)	
Miscellaneous costs	-	35,000
Subtotal		563,350
Total		563,350

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11.0 ANNEXES

ANNEX 1: LETTER TO ETHICS AND REVIEW COMMITTEE

Dr Angela Anzeze (MBChB)

H58/80841/2015,

P.O.BOX 3002 – 00506,

NAIROBI.

To,
The Chairperson,
Ethics, Research and Standards Committee,
Kenyatta National Hospital and University of Nairobi,
P.O. Box 20723,
NAIROBI

Dear Sir,

RE: SUBMISSION OF MASTERS DEGREE RESEARCH PROPOSAL FOR APPROVAL

I wish to submit my research proposal for review and approval by your committee. I am currently a 3rd year student pursuing a Master's Degree in Obstetrics and Gynaecology at the University of Nairobi, College of Health Sciences.

Yours Sincerely,

Dr. Angela Anzeze,

Resident,

Department of Obstetrics and Gynaecology,

College of Health Sciences

University of Nairobi

ANNEX 2: QUESTIONNAIRE

Microbial Pattern in Amniotic Fluid and Histology of the Smooth Chorion in Women with Premature Rupture of Membranes and Preterm Premature Rupture Of Membranes at the Kenyatta National Hospital

Date:

Serial No.

Section 1: Interview Process. English Version (To Be Filled with the Assistance of the Principal Investigator in Cases where an Explanation is required)

Tick in the Box Provided

Section A: Sociodemographic Data

1. Serial number

2. Age (Years)

3. Year of Birth

4. Educational background

1) Primary

2) Secondary

3) College

4) None

5) Don't know

5. What is your religion?

1) Catholic

2) Protestant

3) Muslim

4) Others Specify -----

5. Employment history

1) Employed

2) Unemployed

3) Self employed

6. Marital status

1) Single

2) Married

(Monogomous)

3) Married

(Polygamous)

4) Divorced

5) Separated

6) Widowed

7. Do you currently smoke cigarettes' or use traditional tobacco?

1) YES

2) NO

8. If the answer to question 7 above is yes, indicate the number of sticks per day -----
and total number of years since you started smoking.....

9. Do you currently drink alcohol?

1) YES

2) NO

10. If the answer to question 9 is yes, indicate the type of drink, -----
quantity ----- and specify how often you drink-----

11. Have you had any abdominal injury in this pregnancy?

1) YES

2) NO

12. If the answer to question 11 above is yes, specify the events of the injury -----

SECTION B: PAST OBSTETRIC HISTORY

13. How many pregnancies have you ever had?

14. What was the outcome of the pregnancies? Indicate number)

1) Abortions

2) Stillbirths

3) Livebirths (Term)

4) Livebirths (Preterm)

15. History of leakage of amniotic fluid in previous pregnancy?

(Leakage of amniotic fluid before delivery)

1) YES

2) NO

16. If the answer to question 15 is yes, at what gestation did it occur? -----

17. When was your last delivery? Indicate month and year.....

18. Any complications at last delivery

1) Bleeding

2) Infection (specify)

3) Preterm birth

4) Hypertension

5) Others (specify)

19. History of previous Caesarean section

1) YES

2) NO

20. If yes to question 19 above, what was the indication?

1) Bleeding in pregnancy

2) Previous Caesarean section

3) Infection (Chorioamnionitis)

4) Postdates (41 completed weeks)

SECTION C: HISTORY OF INDEX PREGNANCY

21. LNMP (1st day of the last normal period)

22. Gestation in weeks

23. Antenatal clinic attendance

1) YES

2) NO

24. If the answer to question 23 is yes:

Specify where

1) Dispensary

2) District hospital

3) Provincial hospital

4) National hospital

25. Number of antenatal visits so far

26. How many weeks were you on your 1st visit?

27. Antenatal profile (indicate date each test was done)

1) HIV test result

Positive

Negative

Not tested

2) HepBSAg result

Positive

Negative

Not tested

3) VDRL

Positive

Negative

Not tested

4) Haemoglobin level

5) Urinalysis

6) Blood group

7) Blood sugar levels

28. Drainage of liquor – describe number of pads used daily, colour, smell -----

29. Duration of membrane rupture prior to admission?

1. Hours

2. Days

30. Duration from rupture of membranes to delivery?

1. Hours

2. Days

31. Have you had drainage of liquor before in this pregnancy?

1) YES

2) NO

32. If yes, at how many weeks of gestation did it occur?

SECTION D: PAST MEDICAL HISTORY

33. Have you had any illness prior to this pregnancy?

1) YES

2) NO

34. If yes to above, specify which illness -----

35. Are you currently on long term medication?

1. YES

2. NO

SECTION 1 INTERVIEW PROCESS: KISWAHILI VERSION

SECTION A: TABIA YA IDADI

1. Nambari mfululizo

2. Miaka

3. Elimu

1) Shule ya msingi

2) Shule ya upili

3) Chuo kikuu

4) Hakuna

5) Haujui

4. Ajira

1) Umejiriwa

2) Huja ajiriwa

3) Umejajiri

5. Dini

1) Katholiki

2) Protestant

3) Muislamu

6. Hali ya ndoa (weka alama)

1) Haujaolewa

2) Umeolewa

(mke mmoja)

3) Umeolewa

(Wake wengi)

4. Ndoa kutengwa

4) Talaka

5) Mjane

7. Umewahi vuta sigara au tumbaku

1) NDIO

2) LA

8. Kama ndio, sigara ngapi kwa siku? -----

9. Unakunya pombe?

1) NDIO

2) LA

10. Umewahi pata jeraha hivi karibuni?

1) NDIO

2) LA

11. Kama ndio, ajali gani ulipata? (kuwa maalum)

SECTION B: HISTORIA YA MIMBA

12. Umepata mimba ngapi kwa maisha yako? -----

13. Ulipata shida yeyote katika mimba hizo?

1) Mimba kutoka

2) Kuzaliwa bado

3) Kuzaliwa kwa kuishi (kufikisha siku)

4) Kuzaliwa kwa kuishi (kabla kufikisha siku)

14. Umesha pata shida ya maji ya mimba kutoka kabla ya kuzaa (kwa mimba zilizopita)

• NDIO

• LA

15. Kama ndio, mimba ilikuwa na mienzi mingapi? -----

16. Ulizaa mwisho lini? (mwaka na mwezi) -----

17. Ulipata matatizo wakati ulizaa mwisho?

1) Vujadamu

2) Maabukizi (kuwa maalum)

3) kuzaliwa mapema

4) Shinikizo la damu

e) Nyingine (kuwa maalum)

18. Umewahi pata upasuaji wa mimba?

1) NDIO

2) LA

19. Kama ni ndio, sababu la kufanya upasuaji ilikuwa ni?

SECTION C: MIMBA YA SAA HIZI

20. Siku la kwanza la damu ya mwezi ya mwisho -----

21. Mimba ina miezi ngapi ?

22. Ulienda kliniki ya wajawazito?

1) NDIO

2) LA

KAMA ni NDIO

23. Ulienda wapi? -----

24. Ulienda mara ngapi?

25. Ulianza kliniki mara ya kwanza ukiwa na miezi ngapi?

26. .Vipimo muhimu vya damu kwa mimba

a) Matokeo vya virusi vya ukimwi

b) Matokeo ya homa ya manjano (hepatitis B)-----

b) Matokeo ya hemoglobin -----

c) Matokeo ya kaswende (VDRL) -----

d) Matoke ya mkojo-----

e) Aina ya damu -----

f) Sukari -----

27. Maji ya mimba (umetumia pedi ngapi, rangi, harufu) -----

28. Muda ya maji ya mimba kutoka

1. Saa

2. Siku

29. Muda ya maji ya mimba kutoka hadi kuzaa

1. Saa

2.Siku

30. Umewahi kuwa na shida hii mbeleni, kwa hii mimba?

1. NDIO

2. LA

31. Kama ndio, mimba ilikuwa miezi ngapi?

32. Umetokwa na damu wakati wa huu ujauzito? (baada ya miezi saba)

1. NDIO

2. LA

33. Umepata ugonjwa wa sukari katika hii mimba?

1. NDIO

2. LA

34. Umepata shinikizo la damu katika hii mimba ?

1. NDIO

2. LA

SECTION D: HISTORIA YA UGONJWA

35. Umeumwa na ugonjwa yeyote kabla hii mimba?

1. NDIO

2. LA

36. Kama ndio, ugonjwa gani?

37. Unameza dawa yoyote kwa muda mrefu?

1. NDIO

2. LA

SECTION 2: EXAMINATION (PERFORMED BY THE PRINCIPLE INVESTIGATOR/ RESEARCH ASSISTANT)

TICK IN THE BOX PROVIDED

- 1). Fundal height -----
- 2). Foetal heart rate and regularity -----
- 3) Diagnosis of prom (to be filled by the principle investigator)
- a) Speculum examination findings -----

b) pooling/Valsalva-----

4) Any evidence of chorioamnionitis

1) Fever

2) Tachycardia

3) Others specify.....

5). Investigations done

1) Ultrasound

2) Cervical swab

3) Total blood count

4) Urine analysis

6).Indicate the findings of the above investigations -----

7) Antibiotics given: indicate dose

1) Amoxicillin

2) Erythromycin

3) Augmentin

4) Cephalosporins (specify)

5) Others

8) Steroid given

1) Yes

2) No.

9) If yes, indicate the dose given and duration-----

GROSS EXAMINATION OF THE PLACENTA:

10. Weight (grams) -----

11. Diameter (cm) -----

12. Presence of developmental abnormalities

1) YES specify -----

2) NO

13. Presence of visible lesions

1. YES Specify -----

2. NO

14). Presence of haematomas and thrombi

1. YES

2. NO

FETAL OUTCOMES

15) Mode of delivery-----

1) Spontaneous vertex delivery

2) Caesarean Section

16. Apgar score at five minutes -----

17. Birth weight.....

18. Foetal admission to new born unit -----

19. Indication for 18 above.....

1) Neonatal sepsis

2) Respiratory distress syndrome

3) Low birth weight less than 1.8 Kg

4) Others Specify -----

20. Number of days in the ward up to discharge/death

MATERNAL OUTCOMES

21. Mode of delivery

22. Complications at delivery

1) Retained placenta

2) Postpartum haemorrhage

3) Others

4) None

23. Complications in puerperium.

1) Fever

2) Foul smell of lochia

3) Others

4) None

LAB FORM 1: FOR AMNIOTIC FLUID SPECIMEN

Microbial pattern in amniotic fluid and histology of the smooth chorion in women with premature rupture of membranes and preterm premature rupture of membranes at KNH.

Patient identification number:

Age:

LNMP:

Gestational age:

Parity:

Clinical summary (including recent use of antibiotics) and diagnosis:

Specimen collected: AMNIOTIC FLUID

Date collected:

Specimen source:

Collected by: (Name and signature)

Department collected from:

Investigation required: Microscopy and culture only.

Requesting clinician (Name, signature and contacts):

LAB FORM 2: PLACENTA SPECIMEN ONLY

Microbial patterns in amniotic fluid and histology of the smooth chorion in women with premature rupture of membranes and preterm premature rupture of membranes at KNH.

Patient identification number:

Age:

LNMP:

Gestational age:

Parity:

Clinical summary and diagnosis:

Specimen collected: SMOOTH CHORION OF THE PLACENTA

Date collected:

Specimen source:

Collected by: (Name and signature)

Department collected from:

Investigation required: Microscopy and culture only.

Requesting clinician (Name, signature and contacts)

LAB FORM 3: GROSS EXAMINATION

<u>GENERAL</u> <u>CONDITION</u> indicate if (unfixed, fixed/intact, fragmented)	
<u>MEMBRANES</u>	
Condition (indicate if intact, ragged, fragmented/slimy, mucoid)	
Colour (Meconium stained)	
Transparency (Translucent or opaque)	
Membranous insertion (marginal, circumvallate or circummarginate)	
Point of rupture, distance to end of disc;	
Other (amnion nodosum or any other lesions)	
<u>CORD</u>	
Appearance, coiling; (indicate if intact, shredded, fragmented/colour/hypo coiled, hyper coiled or normal coiled)	
Insertion, distance to margin: indicate if central, eccentric, marginal, furcate, velamentous, interpositional)	
Length and diameter	
Knot (indicate if true or false if present)	

Vessels number (2 or 3)	
Other; indicate if there is (thrombosis, rupture, perivascular haemorrhage)	
<u>DISC</u>	
Trimmed weight	
Diameter and thickness	
Shape (lobes, succenturiate, membranacea, ring, fenestrated)	
Foetal surface (describe any abnormalities within the surface vessels, amnion and chorion)	
Maternal surface (indicate if intact, fragmented, ragged/adherent clot, calcifications or fibrin - % of surface area)	
Cut surface (indicate if there are any infarcts, thrombi, fibrin, tumours and any other lesions - % of surface area)	
Other features noted	

LAB FORM 4 (PLACENTAL HISTOLOGY): Laboratory & Clinical Information

1	Lab No.	
2	Slide No.	
3	Date of Specimen Collection	
4	Age of Participant (months)	
5	Gestation by Date (weeks)	
6	Blood Pressure Status	
7	Birth Weight (Grams)	

SECTION B: Villous Microscopy Findings

Delayed villous maturity (only for 36 or more weeks' gestation)

(Enlarged distal villi with excessive stroma, hyper cellular villous trophoblast, central blood vessels, paucity of vasculosyncytial membranes)

Not Applicable Present

Accelerated villous maturity Absent

(In preterm; small/short hypermature villi for gestational period. Diffuse pattern of term-appearing villi with increased syncytial knots and intervillous fibrin, usually alternating with areas of villous paucity)

Presence of distal villous hypoplasia Yes No

(Paucity of villi in relation to the surrounding stem villi. The villi are thin and relatively elongated-appearing (lack of branching), and syncytial knots are increased).

a) Presence of villous oedema Yes No

b) If present, indicate percentage of villi affected as

A (< 25%) B (25-50%) C (>50% -75%) D (>75%)

a) Presence of villous necrosis Yes No

b) If present, indicate percentage of villi affected as

A (< 25%) B (25-50%) C (>50% -75%) D (>75%)

a) Syncytial Knots present absent

b) If present, indicate percentage of villi affected as

A (< 30%) B (30-60%) C (>60%-80%) D (>80%)}

c) Determine whether increased or decreased for gestational age

Increased Decreased

a) Thickening of villous basement membrane Present Absent

b) If present, indicate percentage of villi affected as

A (< 25%) B (25-50%) C (>50% -75%) D (>75%)}

a) Presence of villous stromal fibrosis Present Absent

b) If present, indicate percentage of villi affected as

A (< 25%) B (25-50%) C (>50% -75%) D (>75%)}

SECTION C: Inflammation and Fibrin deposition

a) Presence of Villitis Present Absent

b) If present, indicate percentage of villi affected as

A (< 25%) B (25-50%) C (>50% -75%) D (>75%)}

a) Presence of Intervillositis Present Absent

b) If present, indicate the proportion of intervillous area affected as

A (< 25%) B (25-50%) C (>50% -75%) D (>75%)}

a) Presence of Fibrin deposition Present Absent

b) If present, indicate the pattern and proportion of villi/Intervillous area affected)

Intravillous Intervillous

A (< 25%) B (25-50%) C (>50% -75%) D (>75%)}

Presence of foetal inflammatory response Present Absent

Presence of massive histolytic intervillitis Present Absent

SECTION D: Villous Vascular Findings

Villous vascularity Increased Not increased

(Criteria: 10 villi in 10 fields with 10 or more blood vessels)

a) Basal vessel wall abnormality Present Absent

b) If Basal vessel wall abnormality is present, which one?

Muscular wall hypertrophy Fibrinoid Necrosis Atheromatous changes

Foetal thrombotic vasculopathy Present Absent

SECTION E: Villous Trophoblast Apoptosis (Cleaved Caspase 3 Immunohistochemistry)

1. Quality of staining Good Poor

2. No. of positively staining cells out of 300 cells _____

3. Villous Trophoblast Apoptotic Rate (%) _____

CONCLUSION/DIAGNOSIS;

Signature of Consultant /Student:

.....

Date of Report:

.....

ANNEX 3: CONSENT INFORMATION

STUDY TITLE: MICROBIAL PATTERNS IN AMNIOTIC FLUID AND HISTOLOGY OF THE SMOOTH CHORIONIN WOMEN WITH PROM AND PPROM AT KNH'

Investigator

I, DR. ANGELA M. ANZEZE am a postgraduate student at the University of Nairobi Obstetrics and Gynaecology department. I am conducting the study, as part fulfilment for the award of the degree of Master of Medicine in Obstetrics and Gynaecology by the University of Nairobi. **Contacts:0738288843 resident in Obstetrics and Gynaecology** at the University of Nairobi, email angelaanzeze@yahoo.com, postal address **P.O.BOX 3002 – 00506, Nairobi.**

Lead supervisor,

PROFESSOR SHADRACK OJWANG, full professor of the department of Obstetrics and Gynaecology, University of Nairobi. **Contacts: 0722512283. Email: ojwangsbo@gmail.com . Postal address, University of Nairobi College of health sciences P.O.BOX 19676 code 00202.**

PURPOSE OF THE STUDY

The study aims to determine if there is an association between the microbial patterns, placental changes and gestational age in patients with drainage of amniotic fluid before labour (premature rupture of membranes and preterm premature rupture of membranes).

STUDY PROCEDURE:

You will be asked questions about your age, number of children, details of antenatal clinic attendance, whether you smoke, if you currently on any medication or recently taken, any other illness you have had during the current pregnancy, duration and characteristics of the drainage of amniotic fluid, and any other symptoms associated i.e. fever, pain. We will then look at your clinic card and delivery records to see if there is previous history that may predispose to premature drainage of amniotic fluid other than infection. We will examine you fully. The examination includes confirming if there is amniotic fluid leakage by inserting an instrument (speculum) into your vagina. The amniotic fluid will be collected and taken to the

lab for analysis. (Microscopy) After delivery, you will be examined again and part of the placenta will be taken to the lab for analysis.

BENEFITS:

The study participants may not directly benefit from the study, but the findings of the study will provide a policy with focused guidelines on treatment, that will benefit women with the same conditions in future.

RECRUITMENT AND CONSENT

Study personnel will explain the research procedures to you in either Kiswahili or English language, provide written information when appropriate and obtain written informed consent, prior to initiation of any study procedures.

POTENTIAL RISKS

The study procedures pose no danger to you or your baby. Study staff are trained health care workers. We acknowledge that answering certain personal questions may be overwhelming and stressful. There will be no extra cost to you for participating in the study.

There will be no direct monetary benefits to any participant in this study however if needed prompt referrals, interventions and treatments will be done as appropriate.

CONFIDENTIALITY

We will not use your name or initials in the questionnaires. The information you give us will not be used for any other purpose apart from the study

MINORS

All pregnant women 14 years and above will be allowed to participate in the study. In Kenya, Pregnant women between 14 – 18 years are legally allowed to give consent. (Emancipated minors are pregnant women below the age of 18 years who got pregnant out of will.)

VOLUNTARINESS OF PARTICIPATION AND WITHDRAWAL FROM THE STUDY

Participation is voluntary and you are free to decline the study or to withdraw from the study at any time. Declining to give consent or withdraw from participation will not influence your management in any way.

FOLLOW UP

No follow up is required after participation in the study. However routine check-ups at the postnatal clinics will be advised.

ETHICAL APPROVAL

This study has been reviewed and approved by the KNH/UON Ethics and Research Committee. Shall you need any further clarification regarding this study please feel free to contact the principal researcher:

Dr. Angela Anzeze on **0738288843** resident in **Obstetrics and Gynaecology** at the University of Nairobi, email angelaanzeze@yahoo.com, postal address **P.O.BOX 3002 – 00506, Nairobi**. Or, the lead supervisor of the study **Professor Shadrack Ojwang**, full Professor at the University of Nairobi, department of Obstetrics and Gynaecology, on **0722512283**. Email: ojwangsbo@gmail.com . Postal address, **University of Nairobi College of health sciences P.O.BOX 19676 code 00202**.

Or

The Secretary, KNH-ERC

Tel, 020-2726300 ext. 44102. Email: uonknh_erc@uonbi.ac.ke

CONSENT FORM.

I confirm that I have exhaustively explained the study to the participant and sought voluntary informed consent from her.

Signature research assistant/principle investigator.....

Initials.....Date.....

I have been explained to about the study and I accept to participate. I have not been coerced or enticed in any way.

Initials of participant.....

Participant's signature/Thumb

print.....Date.....

Witness initials.....Date.....

STUDY TITLE: MICROBIAL PATTERN IN AMNIOTIC FLUID AND HISTOLOGY OF THE SMOOTH CHORIONIN WOMEN WITH PROM AND PPROM AT KNH'

KISWAHILI CONSENT INFORMATION (Nakala ya itikio)

Tunakuuliza ujitolee kwa hiari ili ushiriki katika utafiti huu. Utafiti huu utajumuisha wale wanaotafuta matibabu katika hospitali ya Kenyatta.

Ikiwa utaamua kuhusishwa katika utafiti huu, utaulizwa kuweka sahihi katika nakala hii au kuweka alama ya kidole mbele ya shahidi. Tutakupa nakala ya fomu hii. Nakala hii ya itikio huenda ikawa na maneno mengine ambayo huelewi, tafadhali uliza tukuelezee chochote ambacho huenda ukakosa kuelewa.

LENGO LA UTAFITI:

Lengo letu ni kujaribu kufumbua ni kwa nini hili tatizo hutokea na vile ambavyo tunaweza kupunguza visa kama hizo kutokea tena.

KUSHIRIKI KWAKO NI KWA HIARI:

Kabla ya kujua kuhusu vipimo vya kuandikishwa na vya kufuatiliwa, ni muhimu ujue yafuatayo;

- Sio lazima kuwa katika utafiti huu ikiwa hutaki.
- Unaweza kuamua usifanyiwe vipimo vya kuandikishwa na vya kufuatiliwa, au kusimamisha vipimo vya kuandikishwa na vya kufuatiliwa wakati wowote, bila kupoteza huduma zako za matibabu za kawaida.
- Unaweza kuulizwa ikiwa unashiriki kwa tafiti zingine.
- Hata ikiwa umehitimu kujiunga na utafiti, sio lazima kujiunga na huu utafiti.

MATEMBEZI YA UTAFITI NA TARATIBU ZA UTAFITI

Taratibu za kuandikishwa zitaanza leo, baada ya kusoma, kujadili, na kuweka sahihi au alama ya kidole kwa nakala hii.

Katika utafiti huu, utaulizwa maswali kuhusu afya yako na mfanyikazi wa utafiti. Maswali ambayo utaulizwa na mfanyikzai wa utafiti ni kuhusu:

- Umri wako, kazi unayojikimu nayo na hali yako ya maisha kwa jumla. Kama ulienda kliniki ya uzazi, ulienda wapi, ulianza lini, ulienda mara ngapi, hali yako ya afya kwa wakati huu na wakati ukienda kliniki.

Kisha tutaangalia kadi yako ya kliniki na faili ya kuzaa ili tuangalie kama kulikuwa na shida yoyote ambayo inaeza sababisha maji ya mtoto kutoka kabla kuzaliwa.

Tutakupima baada ya hapo, na maji itachukuliwa kupimwa kwenye maabara.

TATIZO NA/AU KUKOSA STAREHE

Huenda ukawa na hofu au wasiwasi unapoongea kuhusu hali yako, mashauri utakayo pokea kutoka kwa mfanyikazi wa utafiti yatakusaidia kuelewa shida hii zaidi. Washauri waliohitimu watakuweco wakati wote wa utafiti na watakusaidia kukabiliana na hisia au maswali ambayo unaweza kuwa nayo.

FAIDA

Huenda ukakosa kupata faida ya moja kwa moja kwa kushiriki katika utafiti huu. Utapata maelezo ya jinsi kuzuia jambo kama hili kutokea wakati mwingine. Ikiwa utahitaji matibabu zaidi, mtafiti, mfanyikazi wa utafiti atakuelekeza kwa mhudumu wa afya kwa wodi ama kwa kliniki za baada ya kuzaa. Kushiriki kwako huenda kukachangia kuimarika kwa matibabu kwa wamama wajawazito.

GHARAMA KWAKO

Hakuna gharama kwako kwa kushiriki katika utafiti huu.

Hakuna malipo yoyote utapewa kwa kukubali kuingia kwa huu utafiti

USIRI:

Juhudi zitafanywa kuweka maelezo yako ya kibinafsi kwa usiri. Hata hivyo, usiri kabisa hauwezi kuhakikishiwa. Maelezo yako ya kibinafsi yanaweza kufichuliwa ikiwa yatahitajika kwa sheria. Linganisho kati ya jina lako na hiyo nambari spesheli itawekwa mahali salama kwa kliniki pekee. Uchapishaji wowote wa utafiti huu hautatumia jina lako au kukutambua wewe mwenyewe.

Rekodi zako za utafiti huenda zikapitiwa na wafanyikazi wa utafiti na wawakilishi wa Kamati ya Maadili ya Utafiti ya Hospitali ya kitaifa ya Kenyatta na Chuo Kikuu cha Nairobi.

Shida au maswali: Ikiwa una maswali kuhusu haki zako kama mshiriki wa utafiti, yafaa uwasiliane na mtafiti mkuu **Dr. Angela Anzezekwa** nambari ya simu **0738288843**, barua pepe: **angelaanzeze@yahoo.com**, sanduku la posta **3002- 00506**, Nairobi. Au msamizi mkuu **Pofessor Shadrack Ojwang**, nambari ya simu **0722512283**, barua pepe: **ojwangsbo@gmail.com**, sanduku la posta, **University of Nairobi college of health sciences P.O.BOX 19676 code 00202.**

Au**Karani** wa kamati ya maadili ya utafiti ya **Hospitali ya Kitaifa ya Kenyatta na Chuo Kikuu cha Nairobi** Sanduku la Posta **19676-00202, Nairobi**, Nambari ya simu: **0202-272-6300 Ext 44355**; barua pepe:uonknh_erc@uonbi.ac.ke

CONSENT FORM (Kiswahili version)

Kauli ya itikio na sahihi: Nimesoma nakala hii ya itikio au imesomwa kwangu. Nimejadili maelezo na mfanyikazi wa utafiti. Maswali yangu yamejibiwa. Nimeelewa uamuzi wangu ikiwa au sitaki kushiriki kwa utafiti huu ni kwa hiari. Nimeelewa ikiwa nitaamua kujiunga kwa utafiti, naweza kutoka wakati wowote. Kwa kuweka sahihi fomu hii, sipatiani haki zangu zozote nilizo nazo kama mshiriki wa utafiti.

_____	_____	_____
Jina la mshiriki (chapa)	Sahihi ya mshiriki/kidole gumba	Tarehe
_____	_____	_____
Mfanyikazi wa utafiti anaye Endeleza itikio (chapa)	Sahihi ya mfanyikazi wa utafiti	Tarehe
_____	_____	_____
Jina la shahidi	Sahihi ya shahidi	Tarehe

ANNEX 4: AMNIOTIC FLUID PROCESSING

The amniotic fluid was collected from the posterior fornix of the vagina, using a sterile syringe and placed into a sterile bottle. In cases where the patient was not actively draining, high vaginal swab samples were taken using a sterile swab for microscopy/culture and sensitivity. The amniotic fluid/HVS samples were kept in the fridge at 2 - 8 degrees Celsius, while awaiting transportation to the lab for analysis. Prior to microscopy, the specimen was centrifuged then a dry smear made on a slide. The smear was fixed using heat then gram stain done. For the gram stain, the smear was covered with crystal violet for 1 minute, then washed with water.

The smear was covered again with gram's iodine for 1 minute, then washed with tap water. Thereafter, the smear was decolourised using 50% acetone and 50% alcohol then washed with water. The smear was covered with a neutral stain for 2 minutes, air dried, fixed with oil immersion and observed in the national model 163 microscope at power 100. Culture was done to isolate the organisms. Specific culture media was used for each organism as follows:

Group B Streptococcus - Blood agar

E. Coli – Blood agar

Neisseria Gonorrhoea – Thayer Martin agar

Candidiasis species - Sabouraud Dextrose agar

Trichomonas Vaginalis – It was identified using wet preparation of the sample

Bacterial Vaginosis – It was identified using gram stain of the sample by calculating the bacterial vaginosis score

ANNEX 5: EXAMINATION OF THE PLACENTA

Specimen from the smooth chorion of the placenta was taken and submitted for histology examination. The fresh placenta was sectioned for histological examination using a sharp blade. Sections of the placenta were taken to include the smooth chorion. Placenta awaiting examination was initially stored in individual, labelled containers containing 10% formalin and refrigerated in a fresh state at 4 degrees Celsius. Fixative volume was sufficient to surround and immerse the placenta, which was stored in a container that is large and flat, to preserve the shape of the placenta. Haematoxylin – eosin staining was used.

The research assistant and principal investigator examined the placenta immediately after delivery, with great care to avoid lacerations. It was examined in the following order: membranes, cord, foetal surface and maternal surface. Any abnormalities were noted down and the doctor on duty was informed. The membranes were examined for completeness, insertion, decidual necrosis, oedema, extra – amniotic pregnancy, retro membranous haemorrhage, meconium staining and transparency.

The cord was examined for completeness, point of insertion, length, presence of knots, torsion, strictures, number of vessels and haematomas. The foetal surface of the placenta was examined for: colour, opacity, cysts (number and size), amnion nodosum, and thrombosis of the foetal vessels. The maternal surface was examined for: completeness, normal fissures, lacerations (extent), depressed areas, retro placental haemorrhage (size and distance from

margin). Using a digital scale, the placental weight was measured. The placenta was placed on a green towel on a flat well-lit surface. Measurements of the disk diameter (in two dimensions) and placental thickness was obtained using a ruler. The umbilical cord length was measured. Standard descriptions of the maternal surface, foetal surface, membranes and cord was made.

Labels were placed on the ruler. Using a digital camera, two photographs of the maternal surface were taken. Two photographs of the foetal surface were taken. Serial sections (5 mm) of the placenta were made using a dissection knife. Additional pictures were taken of the serial dissection surfaces. The umbilical cord was photographed and dissected in 10 mm sections, with dissection surfaces photographed. Standard descriptions of the maternal surface, foetal surface, membranes and cord were made.

Standard sampling was performed of the following structures into appropriately labelled histopathology cassettes:

Cassette 1: a 5 mm piece of cord in addition to focal lesions identified on the cord.

Cassette 2: a 5 mm piece of the foetal surface of the placenta.

Cassette 3: a 5mm piece of the most normal looking placental parenchyma

Cassette 4: a 5mm piece of abnormal looking placental parenchyma

Cassette 5: a 5mm piece of membrane roll.

These were immersed into the fixative (10% neutral buffered formal saline) and submitted to the University of Nairobi's Anatomic Pathology Core laboratory for tissue processing and embedding into paraffin wax using standard histopathology processing procedures. From each specimen, one Haematoxylin and Eosin stained section and one Gram stained section

were taken. Where indicated, further special stains were performed. These were examined by the study pathologist and documented using a standardized synoptic report.

The remaining fresh placental tissue were discarded using standard procedures. Formalin fixed paraffin embedded tissue specimens were archived in the histopathology laboratory for a minimum period of 5 years.

Interpretation of Laboratory Results

Weekly multidisciplinary case discussions were convened where clinical and laboratory information was presented, and a final diagnosis issued based on integrated clinical and laboratory features as described by the Amsterdam placental pathology diagnostic criteria. Participants (quorum) for the multidisciplinary meeting were the principle investigators, pathologist and the research assistants, chaired and convened by the principle investigator.

Tissue processing and staining of the placenta

Placental tissue for processing was taken from the formalin bottle and processed as follows:

1. The tissue was cut into small sizes that fit in a standard cassette.
2. The tissue was fixed in 10% formalin to preserve tissue elements.
3. Dehydration – removal of water from tissues to allow complete processing. This was achieved by taking tissues through ascending grades of alcohol (30%, 50%, 75%, 95% and absolute)
4. Clearing – The alcohol was removed from the tissue by passing the tissues through chloroform.

5. Impregnation – The tissues were saturated with molten wax to remove the clearing agent.
6. Tissue was embedded in molten paraffin wax for microtomy.
7. Stained with haematoxylin and eosin:
 - Bring sections to water.
 - Stained in haematoxylin and eosin for 7 minutes.
 - Rinsed in water.
 - Differentiated in 1% acid alcohol for 3 seconds.
 - Rinsed in water.
 - Blue in Scott's tap water – 3 dips.
 - Rinsed in water.
 - Stained in eosin 0.5% for 5 minutes.
 - Dehydrated in ascending grades of alcohol
 - Cleared in 3 changes of xylene
 - Mounted using DPX mountant.
 - Microscopy – expected results
 - Nucleus – blue
 - Cytoplasm – pink.
 - Examined under a high power field light microscope, model 163.

