
# PREVALENCE OF PLACENTAL BED BACTERIAL INFECTIONS AMONG PRETERM BIRTHS AT KENYATTA NATIONAL HOSPITAL.

**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF DEGREE OF MASTER OF MEDICINE (MMed) IN OBSTETRICS AND GYNAECOLOGY, UNIVERSITY OF NAIROBI**

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# DECLARATION

This dissertation is my original work done with guidance of my supervisors and has not been presented for the award of any degree in any other university.

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# DEDICATION

I would like to dedicate this work to my wife Florence and my daughters Chloe and Crystal for their understanding and support throughout my studies and work on this project.

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# LIST OF ABBREVIATION

| Abbreviation | Meaning |
|---|---|
| KNH | Kenyatta National Hospital |
| WHO | World Health Organization |
| PTB | Preterm Birth |
| PTL | Preterm Labor |
| PPROM | Preterm Premature Rupture of Membranes |
| SDG | Sustainable Development Goal |
| ACOG | American College of Obstetricians and Gynecologists. |
| KDHS | Kenya Demographic and Health Survey |
| IL | Interleukin |
| TNF | Tumor Necrosis Factor |
| G-CSF | Granulocyte-colony stimulating factor |
| Spp | Species |
| CA | Chorioamnionitis |
| BV | Bacterial Vaginosis |
| UoN | University of Nairobi |
| BMI | Body Mass Index |
| FIRS | Fetal Inflammatory Response Syndrome |
| IUFD | Intrauterine Fetal Death |
| LBW | Low Birth Weight |
| CRH | Corticotropin Releasing Hormone |
| PCR | Polymerase Chain Reaction |

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# OPERATIONAL DEFINITIONS

**Preterm Birth:** Any birth before 37 completed weeks of gestation

**Preterm Labor:** Onset of labor before 37 completed weeks.

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

**Placenta bed**: Describes the maternal-fetal interface, i.e., the area the placenta attaches itself to the uterus.

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

**Histopathology**: Is the diagnosis and study of diseases of the tissues and involves examining tissues and/or cells under a microscope.

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

**Chronic deciduitis**: Chronic inflammation of the decidua.

# ABSTRACT

**Introduction:** Major cause of cause of death globally in children below the age of 5 years is attributed to prematurity. Intrauterine infection during pregnancy contributes significantly to preterm labor, PPROM and finally preterm birth. Approximately 40% cases of spontaneous preterm labor and birth are attributed to infections. The bacteria infecting the placental bed thus have the potential of causing adverse pregnancy outcomes like preterm premature rupture of membranes, chorioamnionitis, preterm labor and birth with resultant neonatal morbidity and mortality.

**Study Objective:** To determine the prevalence of bacterial infections at the placental bed in patients with preterm births at KNH through placental bed bacterial culture and histopathology.

**Methodology:** It was a descriptive cross-sectional study, that involved 116 pregnant women who presented for delivery between 28 and 37weeks' gestation at KNH obstetric unit. Informed consent was obtained. Pretested questionnaire was used to collect demographic and obstetric data.

Immediately upon delivery, the placenta was placed in a sterilized container and refrigerated at a temperature of 2-8 $^{0}$C at KNH Morgue. Using CHAMPS protocol for minimally invasive tissue sampling (MITS) Standard Operating Procedure (SOP) protocol v1.1 November 2017, placental cuts were made within 24 hours of specimen collection and submitted to microbiology laboratory for culture and sensitivity analysis. The rest of the placenta was fixed in 10% formalin, standard cuts made into cassettes and submitted to histology laboratory where they were processed into slides. Histological diagnosis was then made using Amsterdam Criteria.

**Results:** From a sample of 116 placentas, the prevalence of placental bed bacterial infection was found to be 47.4%. The most commonly isolated microbes include *E.coli(27.3%), Staphylococcus haemolyticus(7.3%),Klebsiella pneumonia(10.9%), Enterococcus faecalis(9.1%)* and *Acinetobacter baumanni(7.3%).* Nitrofurantoin and ciprofloxacin were the most sensitive antibiotics. Histological chorioamnionitis was present in 40.5% of placentas. However, it was found in 40% of placentas that also had positive bacteria culture compared to 41% of placentas that had negative bacteria culture and was negative in 60% of placentas that had positive bacteria culture. There was absent bacterial culture growth and histological chorioamnionitis in 59% of the placentas. Other histological profiles identified include maternal vascular malperfusion, funisitis, fetal vascular malperfusion, Chorangiosis, fetal thrombotic vasculopathy and delayed villous maturation.

**Conclusion and Recommendations:** There is need to update our guidelines to culture sensitive antimicrobials for treatment of patients presenting with preterm labor and PPROM. Molecular techniques of microbe isolation are recommended to supplement culture.

**Key words: Preterm birth, placental bed, bacterial culture, histopathology**

# CHAPTER 1: INTRODUCTION

## 1.1 Background

The global major cause of death in children under the age of 5 years is prematurity. Prematures are estimated at 5 million annually and about 1 million deaths occur in children annually and these are related to complications of preterm birth(1).

Approximately 12% of births in the lower-level income countries are prematures with an average mortality rate of 90% occurring within the first few days compared with 9% preterm babies born in high-income countries, where the mortality rate is about 10%. Majority who survive are exposed to long-term disabilities that include learning, hearing and visual impairments(1).

Preterm births are associated with high financial burden to health systems as well as individual families on top of psychological hardships. The most common complications associated with preterm births include infections and/or sepsis, IVH, RDS, NEC, cerebral palsy, hypoxic ischemic encephalopathy, feeding difficulties, seizures, visual and hearing problems(2). It was estimated that in 2001, US$ 5.8 billion was utilized in caring for neonates delivered prematurely in the United States of America(3).
Approximately 40% of preterm births result from intrauterine infections(4)(5). Histologic CA is commonest in lower gestation deliveries at 66% for births between 20 to 24 weeks compared with 16% at 34 weeks(6). There exist similarities between intraamniotic and lower genital tract micro-organisms. Preterm labor that is microbial-induced is normally mediated via an inflammatory process(7).

Most preterm births occur spontaneously and in many cases the cause is unknown. However, some are due to provider initiative such as early labor induction or caesarean section either due to medical or non-medical grounds(2). Preterm birth generally presents clinically as spontaneous premature labor at 40–50% when membranes not ruptured, 3-40% in pre-labor ROM, and 20% following medical or surgical

intervention secondary to maternal or fetal indication(8). Common causes of preterm birth include infections (intrauterine or extra uterine), multiple pregnancies, chronic conditions like diabetes and hypertension, antepartum hemorrhage, hormonal disruptions and sometimes no cause is identified(4).

In a study by Mwanyumba F et al on placental inflammation and perinatal outcome carried out between 2003-06 in Kenya, a prevalence of 19.6% on acute placental inflammation was found. There was independent association between acute placental inflammation and preterm low birth weight (9).

Jonathan L. Hecht et al in 2008 in the US conducted a study on histology of placentas of early gestations below 28 weeks. From a total of 947 placentas, CA accounted for the commonest finding with inflammation of the chorionic plate at 43% vs cord at 19%, while neutrophil infiltration into fetal vessels of the plate stood at 30%. Poor utero-placental perfusion associated morphological features that include increased syncytial knots, infarcts and decidual hemorrhage were present in approximately 20% of placentas(10).

Mueller-Heubach E et al in 1990 in a study on histologic chorioamnionitis and preterm delivery, 1843 placentae were evaluated for histologic CA following delivery. Seventy-four percent of preterm and 15% of term deliveries had severe CA. For patients who had PROM, the prevalence of CA was 42% in preterm compared to 15% of term deliveries(11).

Fetal inflammatory response syndrome (FIRS) develops when most of the fetuses are exposed to CA and is a known risk factor contributing to perinatal morbidity and mortality(12).

This study aims at describing the histopathological changes and bacterial population at the placental basal plate of women with preterm births.

**1.2 Problem Statement**

Preterm births account for up to 70% of all mortalities within the perinatal period. The burden is more in those born under 32 weeks of gestation and weigh below 1500g(13). Prevalence of preterm births at KNH in 2017 was 20.2%(14), while in 2018 it was reported as 18.3%(15).

Infections, particularly chorioamnionitis is a major cause of PTB. There is evidence implicating infections close to 40% of PTB, and this includes intra-amniotic and vaginal infections. Intra-amniotic infections are more frequent in the earlier gestation age pregnancies(16). Chorioamnionitis is categorized as either clinical or subclinical/histologic. Clinical CA entails tachycardia, maternal fever, uterine tenderness, PPROM and leukocytosis while subclinical CA is characterized by inflammation of the placenta, amnion and chorion(17). There is evidence that implicates occult intra-uterine infection in PTL and delivery(18).

Histological diagnosis of CA looks at inflammatory cells within chorion and amniotic membranes, umbilical cord, and the placental disc. The diagnosis varies among different studies to some degree due to different criteria used in the assessment of histological CA(19). Interventions that would focus on infection causing microorganisms and the body inflammatory response would go a long way in mitigating and reducing premature deliveries.

By doing bacterial culture and antimicrobial sensitivity of the bacteria infecting the placental bed, this study will be key in the choice of antimicrobials for use in KNH for patients with PPROM and neonates who end up been admitted at the neonatal unit following preterm births.

# CHAPTER 2: LITERATURE REVIEW

## 2.1Preterm birth definition:

WHO defines preterm births as babies born alive before 37 weeks are completed. It further sub-categorizes them as Extremely preterm (<28 weeks), Very preterm (28-32 weeks), Moderate preterm (32-34 weeks) and Late preterm (34-37 weeks)(1).

## 2.2 Term birth definition

ACOG Committee Opinion number 579 defines term pregnancy to be between 37 and 42 weeks. The subcategories of term birth include Early term (37 0/7 to 38 6/7), Full term (39 0/7 to 40 6/7), Late term (41 0/7 to 41 6/7) and Post term (>42 0/7)(20).

## 2.3Burden of Preterm Birth

SDG 3 target 2, seeks an end to deaths of newborns and under 5 years children by the year 2030. Also aims at reduction of mortality in neonates to about 12 per 1000 live births and under-5 mortalities to about 25 per 1000 live births**(**21). Preterm births accounted for about 10.6% of all live births in 2014, of which, 81.1% came from the sub-Saharan African countries(2).

Approximately 14.9 million births were preterm in 2010 and this accounted for about 11·1% of all livebirths globally(22). In 2015, 5.9m deaths were reported in children below 5 years of age of which 2.7m were among the neonates. Most of the deaths were from preterm birth complications at 1.055 million(23). According to 2014 KDHS, the under-5 mortality is 52 deaths per 1000 live births, while the infant mortality is 39 deaths per 1000 livebirths. The neonatal mortality is 22 deaths per 1000 live births(28).

In Brazil, the rate of PTB was found to be 11.5% from a study by Do Carmo Leal M et al that looked at the prevalence and risk factors related to PTB in Brazil in 2016(24) while in Ghana, the PTB rate was

found to be 9.3% from a study by K. Nkyerkyer et al on singleton PTB in Korle Bu Teaching Hospital, Accra Ghana (2006)(25). The rate of preterm delivery from singleton live births was 16.8% from a study by Azeez Butali et al in 2016 on characteristics and factors associated with PTB in a tertiary center in Lagos Nigeria(26) while a study on PTB in rural Malawi by Chikondi Ntonya et al in 2005 found the PTB rate to be 20.3%(27).

In KNH, preterm birth prevalence was found to be 18.3% from a study by Peter Wagura et al in 2013. It was a cross-sectional descriptive study with a total of 322 participants. There was association between preterm birth and twin pregnancy, age above 20 years, parity above 4 ,UTI, APH, prolonged ROM and hypertension in pregnancy(15).

**2.4 Pathogenesis of spontaneous preterm birth**

Spontaneous preterm birth which refers to births that follow spontaneous PTL and PPROM(29)can result from either of the following four processes, singularly or in combination: Premature activation of the maternal or fetal hypothalamic-pituitary-adrenal axis, exaggerated inflammatory response/infection, abruption (decidual hemorrhage) or pathological uterine overstretching. This can cause effacement of the cervix and may be initiated a long way prior to preterm labor or PPROM is clinically evident(30). Risk factors for spontaneous PTB include black race, previous PTB, low maternal BMI and periodontal disease. The strongest predictors of spontaneous PTB include shortened length of the cervix and increased cervical-vaginal fetal fibronectin(29).

Infection leads to preterm delivery through four main mechanisms: 1. Choriodecidual space bacterial invasion via exotoxins and endotoxins which in turn activate fetal membranes and decidua to produce cytokines which include TNF-α, IL-1α, IL-1β, IL-6, IL-8, G –CSF. 2.These in turn stimulate production of prostaglandins and initiate neutrophil chemotaxis, infiltration, and activation. The hallmark being

production and release of matrix metalloproteases and other bioactive substances. Production of prostaglandins initiate uterine contractions and membrane rupture occurs from degradation of the chorioamniotic membranes by the metalloproteases which also cause cervical softening by way of collagen remodeling. 3.Within the chorionic tissue are prostaglandin dehydrogenases that inactivate prostaglandins and thus prevent myometrial contraction. In presence of chorionic infection, the levels of this enzyme decrease and consequently lead to myometrial contraction and preterm birth. 4. With CA and FIRS, there is increased fetal production of CRH, then corticotropin and eventual cortisol from the fetal adrenals which results in increased production of prostaglandins. Infected fetus also produces cytokines which also leads to accelerated preterm birth(13). Maternal sepsis can also trigger the innate immune response and eventually result in uterine contractions when granulocytes and monocytes are activated(31). This process is inhibited by IL-10 and lipoxins which act as anti-inflammatory immunomodulators and therefore, a balance should be attained between the two processes in order for contractions to occur(32)(33).

Patients with placental bed infections may present with chorioamnionitis. According to ACOG, chorioamnionitis refers to intraamniotic infection which may occur in conjunction with infection of the chorion, amnion, decidua or the fetus(34). As much as the etiology of spontaneous preterm labor is multipronged, infection is a major cause accounting for up to 40% of the cases(35). Bacteria can infect fetal tissues via hematogenous spread, however ,commonest source is from oral infections(36)(37) and the second being ascending infection that originate from the vagina and cervical canal. It is these bacteria that lead to chorioamnionitis which is a major cause of preterm delivery(38). Other routes that bacterial invasion into uterus may occur include translocation from abdomen via the fallopian tubes or iatrogenically by a contaminated needle while doing procedures like amniocentesis or chorionic villus sampling(13).

Bacteria most isolated from patients with both clinical and histologic CA are the *Mycoplasma hominis* and *Ureaplasma urealyticum.* This is especially in the earlier gestation.(39). *Mycoplasma hominis* and *Ureaplasma urealyticum* are also known causes of spontaneous PTL and PPROM through stimulating production of cytokines, prostaglandins and matrix metalloproteinase(39). Commonly isolated organisms in patients diagnosed with preterm labor and whose membranes are intact include *Mycoplasma hominis, Ureaplasma urealyticum, Gardnerella vaginalis, Bacteroides* and *Peptostreptococcus.* Group B streptococci and *Escherichia coli* are occasionally found in the patients with ruptured membranes. All these organisms are from the genital tract.(13)(40)(41).

A study that looked at the microbiome found at the placental tissues showed higher incidence of *Lactobacillus iners, Gardnerella vaginalis* and *Sneathia sanguinegens* in the fetal membranes and higher incidence of *Acinetobacter* and *Enterobacteriaceae* in the placental tissues(38). Bacterial vaginosis which refers to reduction of lactobacillus which forms the vaginal normal flora and the proliferation of other bacteria including *Gardnerella vaginalis, Bacteroides spp ,Peptostreptococcus, Mobiluncus spp, Ureaplasma urealyticum, Mycoplasma hominis, Sneathia, Leptotrichia, Atopobium, Prevotella, and BV-associated bacterium 1 (BVAB1) to BVAB3*(42)(43)(18).BV is associated with increment in levels of mucinase, elastase and sialidase in the vagina and cervix. However, without ascension of these organisms into the uterus, preterm labor would not occur. Therefore, BV is just a marker of intrauterine colonization with similar organisms(13). The prevalence of BV among prenatal women in KNH in a study by Kuruga Martha W et al in unpublished MMed thesis at UoN in the year 2012 was found to be 26%(44). Hillier S et al,1995, in a study on the association between BV and preterm delivery of LBW infant found 16% of 10,397 women had BV. Multivariate analysis showed association between BV and preterm delivery of LBW infant. Vaginal Bacteroides and Mycoplasma hominis had the greatest risk of delivering a LBW infant(45).

Infection is commonest in preterm births <30 weeks' gestation as compared to late preterm as evidenced by histology of fetal membranes at delivery(13). Vanessa Queiros da Mota et al in their study on placental bacterial culture and histological CA conducted in 2013 found 26.9% of placentas (101/376) had histological CA of which 27.7% (28/101) of the placentas yielded positive bacterial cultures. Twenty seven percent of the placentas with a positive yield bacterium culture largely comprised Gram-positive and Gram-negative bacilli. Negative histology had a corresponding negative culture in (230/275) with the balance (45/275) being pathogenic bacteria. Concordance of histology and culture results was found in 70% of examined placentas, whereby, 61.1% were negative and 7.4% were positive cases (46).

Andrew B. Onderdonk et al in 2008 conducted a study on detection of bacteria in placental tissues obtained from extremely low gestational neonates. By use of both culture and PCR, they found 68% positive culture in vaginal deliveries in comparison to cesarean sections at 41%. Breakdown of the positive culture results yielded 33% aerobic vs 21% anerobic bacteria and 9% *Mycoplasma* and *Ureaplasma. Staphylococcus spp, Corynebacterium spp* and BV associated organisms were most frequently recovered(47).

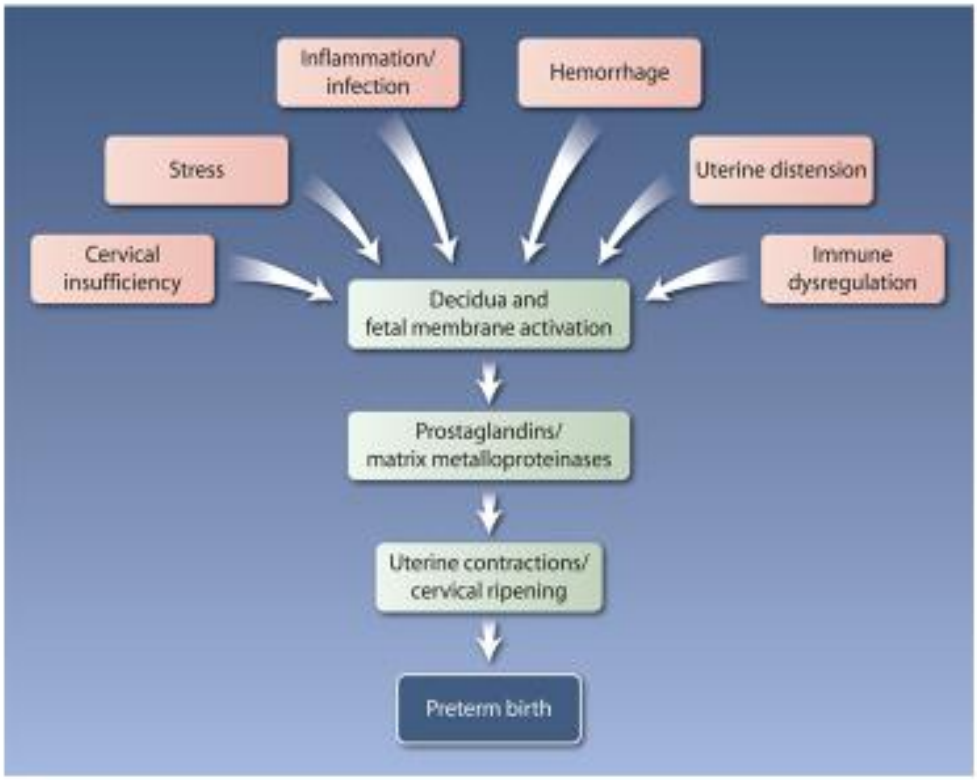
In a retrospective study by Bhola K et al on placental cultures in the era of antibiotic use, 26% (106/412) had histological inflammation. Four-point six percent (12/259) of the cultured placentas yielded positive bacteria culture, of which 75% (9/12) had acute inflammation on histology and 42% (5/12) had prior exposure to antibiotics. The commonest bacteria cultured were the Group B streptococcus and E. coli(48).

In a systematic review on bacterial etiological agents of intra-amniotic infections and PTB in pregnant women by George L. Mendz et al in 2013, 349 (46%) out of a total of 761 women with PTB had intra-amniotic infection. Among the women who had intra-amniotic infections, bacteria belonging to 5 phyla and 16 orders were isolated. Mycoplasmatales (59%) and Lactobacillales 25%) were the frequent

isolates. Treatment with antibiotics showed statistically significant prolongation of pregnancy in patients who had PTL and intact membranes and reduction in the number of delivered neonates in patients who had PPROM within 48 hours(16).

In a study by Nadeen Edmondson et al on the prevalence of chronic deciduitis in cases of preterm labor without clinical CA conducted in Mount Sinai Hospital Canada in 2010, chronic deciduitis was found in 41% cases vs 15% controls compared with histologic acute CA that was found in 46% of cases vs 18% of controls. In their conclusion, chronic deciduitis had a link with PTL(49). Molly J. Stout et al, 2013, in their study on identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestation, found 27% of all placenta basal plates had Gram positive and negative intracellular bacteria. Preterm deliveries accounted for 35% of the total patients. Placental basal plates from both term and preterm gestations showed no difference. Fifty-four percent of spontaneous premature deliveries below 28 weeks had intracellular bacteria in the placental basal plates compared to 26% seen in the spontaneous term deliveries. Intracellular bacteria were present in some of the placentas with negative pathological and clinical chorioamnionitis(50). Owino A et al conducted a study on gross presentation and histomorphological changes of placentae in patients presenting with intrauterine fetal death at KNH published in the East African Medical Journal in 2014. Histological CA was significantly present among membranes from stillbirths. Acute CA and abscess were mainly present. Other placental abnormalities were also significantly present in the stillbirth group including infarcts, peri-villous deposition of fibrin and infiltration with leukocytes. However, since no autopsy was done on the fetus, there was limitation as to the cause of stillbirth(51).

**Fig. 1. Pathways to PTB.The pathways to preterm labor and PTB are multifactorial and complex.**




**Craig E. Rubens et al., Sci Transl Med 2014;6:262sr5**



*Figure 1: Pathways to PTB*

## 2.5 Conceptual framework

### 2.5.1Narrative

Several common pathways can lead to PTB. Placental bed bacterial infections can arise from oral infections through hematogenous spread or ascending cervical-vaginal infections. Colonization of the placental bed with bacterial microorganisms can result to intrauterine infections, chorioamnionitis, funisitis, FIRS or even IUFD. The hallmark of this bacterial infection is PPROM and/or preterm labor and eventual preterm delivery/birth.

In this study, we explore the different organisms infecting the placental bed in patients that deliver preterm babies following onset and progression of preterm labor through placental bed bacterial culture and histopathology.

### 2.5.2 Diagrammatic Representation

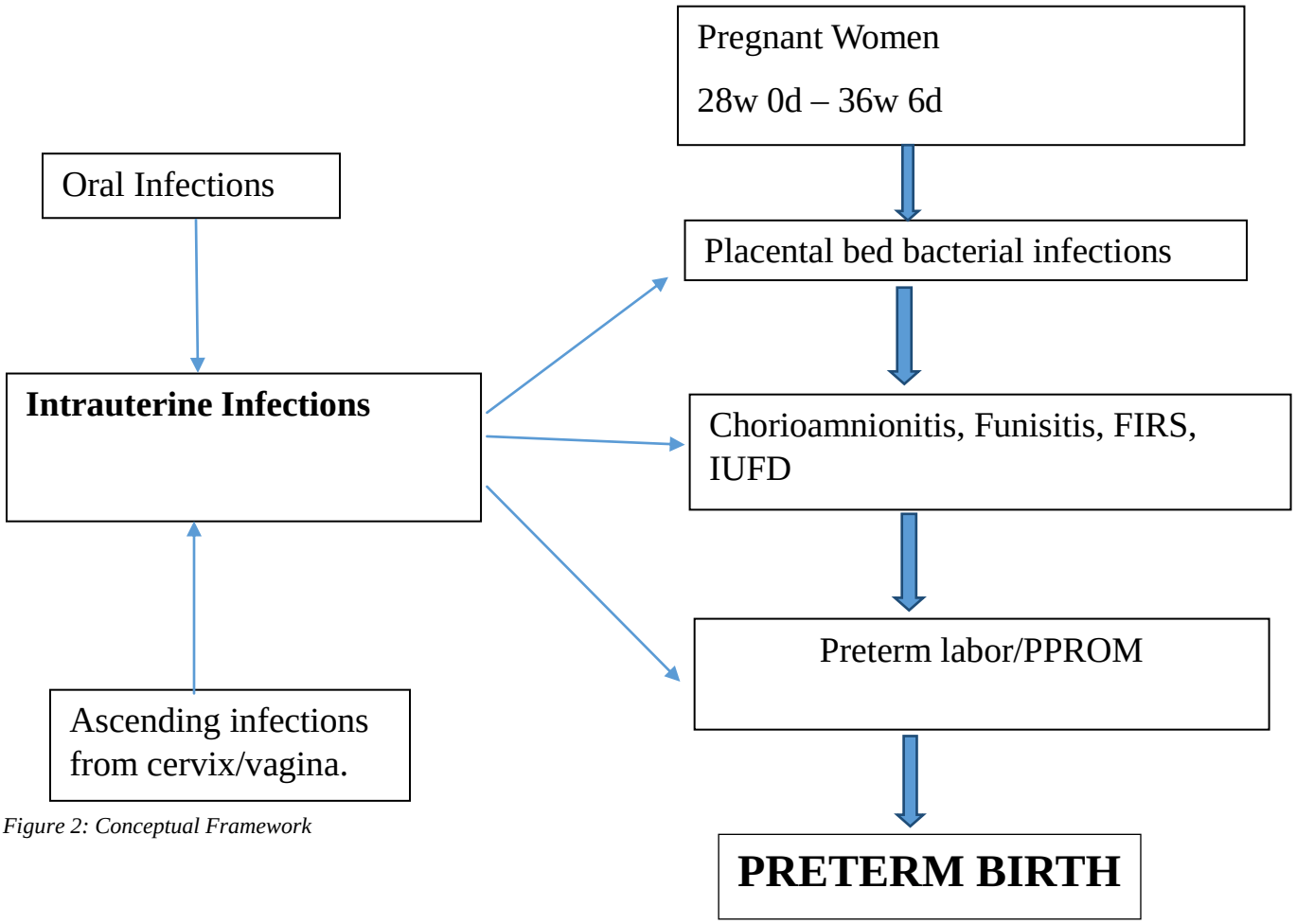



*Figure 2: Conceptual Framework*

## 2.6 Study Justification

Globally, perinatal morbidity and mortality is largely attributed to preterm births(41).

The prevalence of PTB in KNH as of 2018 was 18.3%(15).

There is a high burden of neonatal mortality in our country at 22 deaths per 1000 livebirths as per the 2014 KDHS(28).

Most of patients presenting at KNH with PPROM, PTL and proceed to preterm birth are treated empirically with antimicrobials. Ideally, antimicrobial sensitivity studies are crucial in the choice of antimicrobials to use.

Placental bacterial culture and histology studies offer valuable insights in the diagnosis and management of both mother and fetus. The choice of therapy would be greatly influenced by positive placental culture results(48).

Most patients presenting with PPROM are treated with β-lactamase antibiotics as first line. However, Ureaplasma spp are resistant to these antibiotics. These organisms have been a major cause of PPROM and PTL, the investigations for the same should be mandatory for all patients presenting with chorioamnionitis(41).

There is no known local data on the prevalence and actual isolate of specific bacteria infecting the placental bed. The findings of this study will therefore form a basis for formulation of policy on the approach to management of patients who present with PTL and deliver preterm babies.

## 2.7 Research Question

What is the prevalence of placental bed bacterial infections in patients who deliver preterm at KNH?

## 2.8 Study Objectives

### 2.8.1 Broad Objective

Determine the occurrence of bacterial infections at the placental bed in patients with preterm births at KNH.

### 2.8.2 Specific Objectives

1. Determine the prevalence of placental bed bacterial infections of patients who deliver preterm births.

2. Find out the antimicrobial sensitivity of the bacteria isolated from the placental bed tissues.

3. Establish the histopathological patterns of placental bed tissue in patients who deliver preterm births.

# CHAPTER 3: METHODOLOGY

### 3.1 Study Design

It was a descriptive cross-sectional study involving 116 participants.

### 3.2 Study Site

The study was based at the Obstetrics and Gynecology department in antenatal and labor wards and maternity theatres at Kenyatta National Hospital.

### 3.3 Study Setting

KNH is a public national teaching and referral hospital located within Nairobi, the capital city of Kenya, and has a total bed capacity of 2063. It has a wide catchment area serving residents of Nairobi and multiple other neighboring counties mainly Kiambu, Machakos and Kajiado. This is in addition to being the largest national referral facility and hence receives referrals from far and wide across the country.

On average, about 1100 babies are delivered at the Obstetrics and Gynecology department every month.

#### 3.3.1 Factors that make the site suitable.

1. It is the largest referral hospital in Kenya thus receives many patients from different parts of the country with obstetric complications including preterm labor for management.
2. The hospital has a wide pool of medical specialists for multidisciplinary management of patients with preterm labor and birth.
3. The hospital has a dedicated Newborn Unit with specialists for management of preterm births.
4. The hospital has two dedicated maternity theatres for handling both elective and emergency surgical cases.
5. The hospital also has other departments including radiology and pathology that are key in our study.
6. The hospital been located within the city of Nairobi is easily accessible to patients.

### 3.4 Study population

The study involved mothers who delivered prematurely at KNH between 28 weeks and 37 weeks' gestation and met the eligibility criteria within the study period.

### 3.5 Eligibility Criteria

#### 3.5.1 Inclusion criteria

Patients admitted with PTL and intact membranes between 28 weeks 0 days and 36 weeks' 6 days gestation by dates or by first ultrasound and proceeded to deliver a preterm neonate.

#### 3.5.2 Exclusion criteria

Antepartum hemorrhage

Pregnancy Induced Hypertension

Polyhydramnios

IUFDs

Twin gestation

Diabetes

HIV positive

### 3.6 Sample Size calculation

Fischer's formula for sample size calculation was used(52).

$$n = \frac{Z^2 x\ P(1-P)}{d^2}$$

Where,

$n$ = Desired sample size

$Z$ = value for standard normal distribution corresponding to desired confidence level

of 95%: $Z = 1.96$

$P$ = expected true proportion (Proportion from prior similar study).

$d$ = desired precision: margin of error 5% (0.05)

The study by Vanessa Q M et al in 2013 on correlation between placental bacterial culture results and histological chorioamnionitis found 7.4% of placentas yielded both positive histology and culture results(46). Using a proportion of 7.4%, the sample size was calculated as follows:

$$n = \frac{1.96*1.96*0.074*0.926}{0.05*0.05}$$

n= 105

Attrition of 10% = 11

sample size for the study was 116 participants.

### 3.7 Sampling technique

Consecutive sampling method was employed for those mothers presenting with preterm labor who met the inclusion criteria for the study. Eligible participants were enrolled until the desired sample size was achieved.

### 3.8 Recruitment and Consent

Participants were recruited from labor ward at KNH. Informed consent was administered by the principal investigator or the trained study assistants.

The same participants were followed up until delivery at the delivery room in labor ward or maternity theatre if delivery through caesarian section, where the placenta was taken for the study.

History was taken by the principal investigator or trained research assistant.

### 3.9 Data Variables

#### 3.9.1 Independent variables

Bacterial infections

#### 3.9.2 Dependent variables

Preterm labor and birth

### 3.10 Data collection procedure

Sterility was observed for both abdominal and vaginal deliveries by use of sterilized gowns and patient drapes. Sterile surgical gloves were also used by the midwife or surgeon conducting the delivery. Vulvovaginal toilet using chlorhexidine (hibitane) solution was done prior to draping of the patient to reduce contamination in vaginal delivery. Delivery of the fetus and placenta then followed. The placenta was delivered on a sterile kidney dish.

Immediately after delivery, the entire placenta was placed in a sterile plastic container which had undergone prior sterilization using UV light. This was then transported in a cool box with icepacks to the KNH morgue where the placenta was then refrigerated at a temperature of 2-8 degrees Celsius. This was submitted for pathological examination.

Using CHAMPS protocol for minimally invasive tissue sampling (MITS) Standard Operating Procedure (SOP) protocol v1.1 November 2016(53)(54), placental cuts of the bed and membranes were made within 24 hours of specimen collection and submitted to microbiology laboratory. At the laboratory, direct aerobic incubation and cultures of the placental tissues were done at $37^0$C for 18-24 hours using sheep blood agar (SBA), chocolate blood agar (CBA), cysteine lactose electrolyte deficient (CLED) agar and Brain Heart Infusion (BHI) broth. If no growth was detected after the initial 18-24 hours' culture, aerobic subcultures were done from the BHI broth for another 18-24 hours at $37^0$C using SBA, CBA and CLED culture media.

For positive cultures, biochemical identification and antimicrobial sensitivity were performed using automated VITEK 2 machines. For identification (ID), GN83 and GP83 cards were used while antimicrobial sensitivity was done using AST GN and AST GP cards.

After samples for microbiology had been taken, gross examination, description, measurements, and photographs of the remaining placenta with membranes and umbilical cord was done. More details in ANNEX 5.

The placenta, membranes and cord were then fixed with 10% formalin solution for 24 hours before cuts were made for histological analysis.

Five standard cassettes were submitted from each placenta for histological analysis. The areas sampled from the placenta included: a cassette for membrane roll; two cassettes from umbilical cord, one from the maternal side and another from the fetal side; two cassettes from the placenta parenchyma. An extra

cassette was factored-in, in case of a gross pathology/abnormality either on the cord or parenchyma. The cassettes were then processed into slides.

Morphological examination for diagnosis using the Amsterdam criteria(55) was performed by the pathologist using H&E(hematoxylin and eosin) and Gram stains. An extract of the Amsterdam criteria is summarized as ANNEX 9.

### 3.11 Validity and Reliability

Quality of data collected was maintained throughout the study by ensuring the two research assistants were well trained by the principal investigator on the study methodology including getting an informed consent and the protocol to be used in the collection of placental tissue samples.

The principal investigator worked with the research assistants during the first week of the study to provide guidance and supervision to ensure the standards were adhered to.

Histological analysis of the samples was carried out by a pathologist who is also one of the supervisors for the study with some of the samples being analyzed by a different pathologist for quality assurance and control.

### 3.12 Data Management and Analysis

The data in the questionnaires was entered into Statistical Package for Social Sciences, SPSS version 23. Data cleaning and analysis was done.

The socio-demographic and obstetric characteristics were presented in tables.

Prevalence of placental bed bacterial infections was calculated based on the results of culture; with the number of patients whose placentae had the organisms being divided by the total number of patients with preterm birth.

Subgroup analysis of the patients whose placentas grew bacteria on culture were analyzed and specific bacterial colonies identified, expressed in proportions, and presented in form of tables and bar graphs.

Antimicrobial sensitivity results were expressed in proportions and presented in form of tables.

Sub-analysis was done for the placentae with the identified organisms that showed any histopathological changes and expressed in proportions and presented in form of tables and pie chart.

Data safety was guaranteed by only using the principal investigator and trained data collection assistants during the data collection phase of the study. During data entry and analysis, security of the data was guaranteed by using password protected computers with access restricted to the principal investigator, data collection assistants and the statistician. The study questionnaires were also anonymous and kept in a locked cabinet by the principal investigator with restricted access.

### 3.13 Study results Dissemination and Closure

The results of the study were presented to the Department of Obstetrics and Gynecology as a dissertation for filing in the University of Nairobi Library services.

The findings will then be published in peer reviewed journals and presented at conferences and continuous medical education (CME) events.

### 3.14 Ethical considerations

1. The study proposal was submitted to the KNH/UoN Ethics and Research Committee for ethical approval before commencing the study and approval granted (P950/11/2019).

2. Confidentiality was maintained by use of unique identifiers on every questionnaire instead of patients' names and hospital in-patient numbers. Collected data could only accessed by the principal investigator, research assistants, statistician, and the supervisors.

3. The participants had the right to withdraw from the study; however, their management would continue as per the usual standard of care.

### 3.15 Study Strengths

1. The study is first of its kind in Kenya and region

2. The study incorporates both culture and histology which validates presence of bacteria at the placenta as a cause of infection and not just a contaminant.

### 3.16 Study Limitations.

1.Use of culture methods compared to molecular techniques has been shown to underestimate the burden of microbial species that cause preterm labor(41).

2. Some bacteria like *Mycoplasma* and *Ureaplasma* are difficult to culture

# CHAPTER 4: RESULTS

Deliveries within the study duration totaled 1256. Out of this number the preterm deliveries totaled 194. A total of 61 patients did not meet the inclusion criteria, which was broken down as follows: Preeclampsia/Eclampsia 33, antepartum hemorrhage 5, HIV positive 5, IUFD 9, twin pregnancy 9. A total of 17 patients who delivered preterm within the study period were missed. A total of 116 patients which was our sample size were recruited into our study. Informed written consent was given and a questionnaire filled. We then followed the patient to delivery whereby the placenta was collected for analysis.

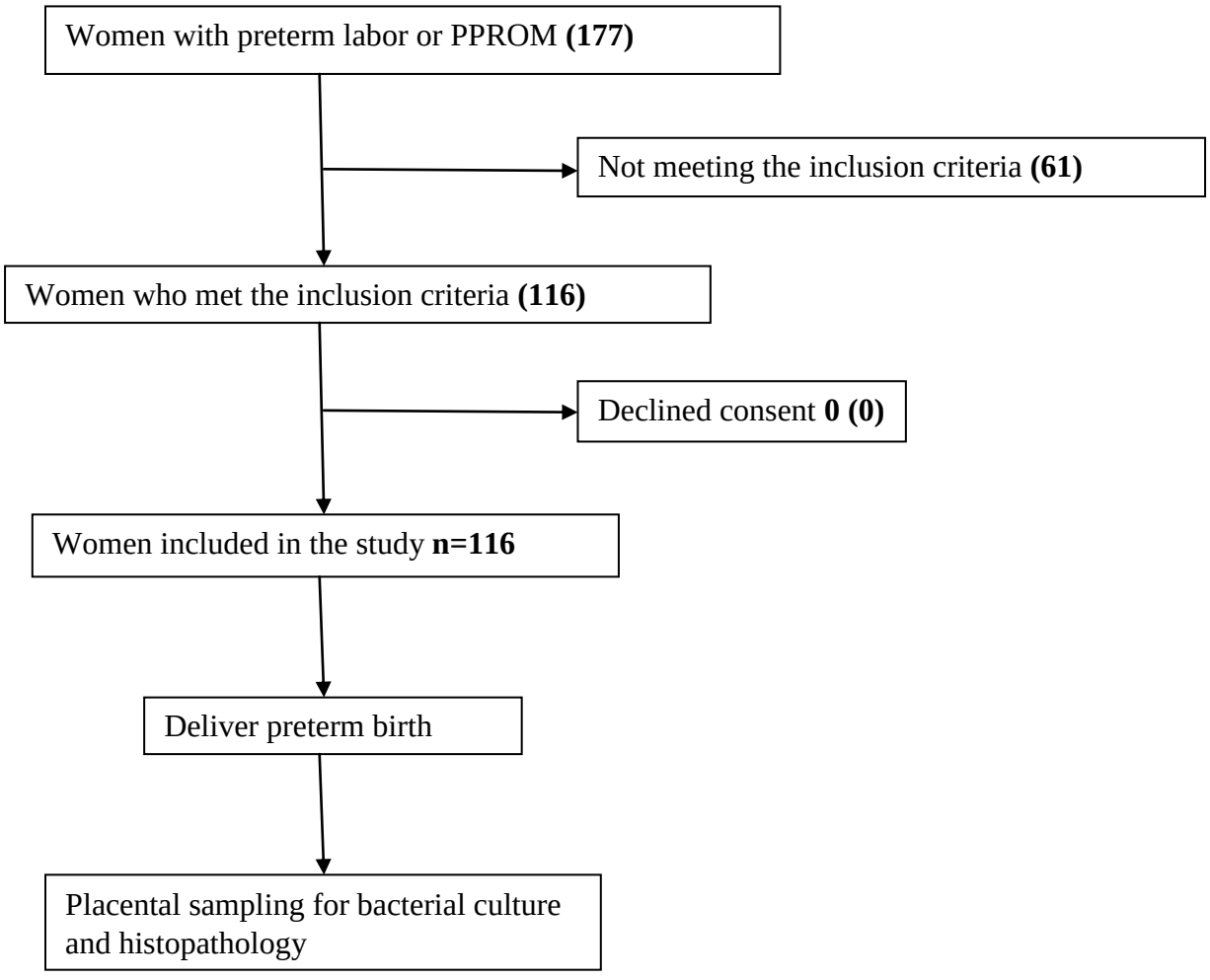



*Figure 3: Study flow chart*

**Socio-demographic Characteristics**

The mean age was 25.8(SD 5.1) years, while the median age was 25.0(IQR 22-29) years. The minimum age was 16 years, while the maximum age was 39 years. Majority of the patients were married at 75%, had attained secondary level education at 48.3%, they were Christians at 94% and unemployed at 52.6% as shown in table 1 below.

*Table 1: Socio-demographic characteristics.*

| Characteristic | Frequency | Percent |
|---|---|---|
| **Age** | | |
| <18 | 2 | 1.7 |
| 18-25 | 58 | 50.0 |
| 26-30 | 36 | 31.0 |
| 31-35 | 14 | 12.1 |
| 36-40 | 6 | 5.2 |
| **Marital status** | | |
| Single | 27 | 23.3 |
| Divorced | 2 | 1.7 |
| Married | 87 | 75.0 |
| **Education** | | |
| None | 5 | 4.3 |
| Primary | 22 | 19.0 |
| Secondary | 56 | 48.3 |
| Tertiary | 33 | 28.4 |
| **Religion** | | |
| Christian | 109 | 94.0 |
| Muslim | 4 | 3.4 |
| Hindu | 2 | 1.7 |
| **Employment status** | | |
| Unemployed | 61 | 52.6 |
| Self-employed | 34 | 29.3 |
| Employed | 21 | 18.1 |

## Obstetric Characteristics

Majority of the patients were in their second and third pregnancies at 51.7% and fell in the moderate to late preterm category at 78.4%. All the patients were attending ANC clinics, but majority had attended less than 4 visits at 53.4% and only 19.8% had treatment for UTI (urinary tract infection) during the current pregnancy as shown in table 2 below.

*Table 2: Obstetric characteristics.*

| Characteristic | Frequency | Percent |
|---|---|---|
| **Gravidity** | | |
| Primigravida | 45 | 38.8 |
| Gravida 2-3 | 60 | 51.7 |
| Gravida 4-5 | 10 | 8.6 |
| Gravida >5 | 1 | 0.9 |
| **Gestation** | | |
| 28-32 weeks | 25 | 21.6 |
| 33-37 weeks | 91 | 78.4 |
| **Number of times ANC attended** | | |
| <4 | 62 | 53.4 |
| ≥4 | 54 | 46.6 |
| **UTI in this pregnancy** | | |
| Yes | 23 | 19.8 |
| No | 93 | 80.2 |

**Prevalence of bacterial infections in the placental bed of patients who delivered preterm births.**

Prevalence of placental bed bacterial infections was calculated based on the results of culture; with the number of patients whose placenta had the organisms being divided by the total number of patients with preterm birth as shown below:

$$Prevalence = \frac{Patients\ with\ positive\ placenta\ culture\ results}{Total\ patients\ who\ had\ preterm\ delivery}$$

$$Prevalence = \frac{55}{116} * 100$$

$$= \mathbf{47.4\%}$$

The prevalence of placental bed bacterial infections for patients who had preterm delivery was found to be 47.4%.

**Spectrum of bacteria isolates cultured from the placental bed tissues**

Twenty different bacteria were identified from the culture. *Escherichia coli* were the most cultured microorganisms at 27.3% (15/55). Other bacteria that were also commonly cultured include *Klebsiella pneumonia* 10.9% (6/55), *Enterococcus faecalis* 9.1% (5/55), *Staphylococcus haemolyticus* 7.3% (4/55) and *Acinetobacter baumanni* 7.3% (4/55) as shown in table 3 below.

*Table 3: Spectrum of bacteria isolates cultured from the placental bed tissues.*

| | Frequency | Percent |
|---|---|---|
| Staphylococcus haemolyticus | 4 | 7.3 |
| Staphylococcus hominis | 2 | 3.6 |
| Staphylococcus epidermidis | 2 | 3.6 |
| Staphylococcus aureus | 1 | 1.8 |
| Staphylococcus sciuri | 1 | 1.8 |
| Staphylococcus saprophyticus | 1 | 1.8 |
| Staphylococcus lentus | 1 | 1.8 |

| | | |
|---|---|---|
| Staphylococcus warneri | 1 | 1.8 |
| Klebsiella oxytoca | 2 | 3.6 |
| Klebsiella pneumoniae | 6 | 10.9 |
| Enterobacter aerogenes | 3 | 5.5 |
| Enterobacter cloacae | 2 | 3.6 |
| Enterococcus faecalis | 5 | 9.1 |
| Enterococcus faecium | 1 | 1.8 |
| Stenotrophomonas maltophilia | 1 | 1.8 |
| Sphingomonas paucimobilis | 1 | 1.8 |
| Acinetobacter baumanni | 4 | 7.3 |
| Escherichia coli | 15 | 27.3 |
| Raoultella ornithinolytica | 1 | 1.8 |
| Serratia fonticola | 1 | 1.8 |

When the different bacteria were placed in their respective groups, *Escherichia coli* were the commonest cultured at *27.3%* (15/55). The other bacteria most cultured include *Staphylococcus spp* 23.6% (13/55), *Klebsiella spp* 14.5%, *Enterococcus spp* 10.9% (6/55), *Enterobacter spp* 9.1% (5/55) and *Acinetobacter baumanni* 7.3% (4/55) shown in table 4 and figure 3 below

*Table 4: Spectrum of bacteria isolates cultured from the placental bed*

| | **Frequency** | **Percent** |
|---|---|---|
| Staphylococcus spp | 13 | 23.6 |
| Klebsiella spp | 8 | 14.5 |
| Enterobacter spp | 5 | 9.1 |
| Enterococcus spp | 6 | 10.9 |
| Stenotrophomonas maltophilia | 1 | 1.8 |
| Sphingomonas paucimobilis | 1 | 1.8 |
| Acinetobacter baumanni | 4 | 7.3 |
| Escherichia coli | 15 | 27.3 |
| Raoultella ornithinolytica | 1 | 1.8 |
| Serratia fonticola | 1 | 1.8 |

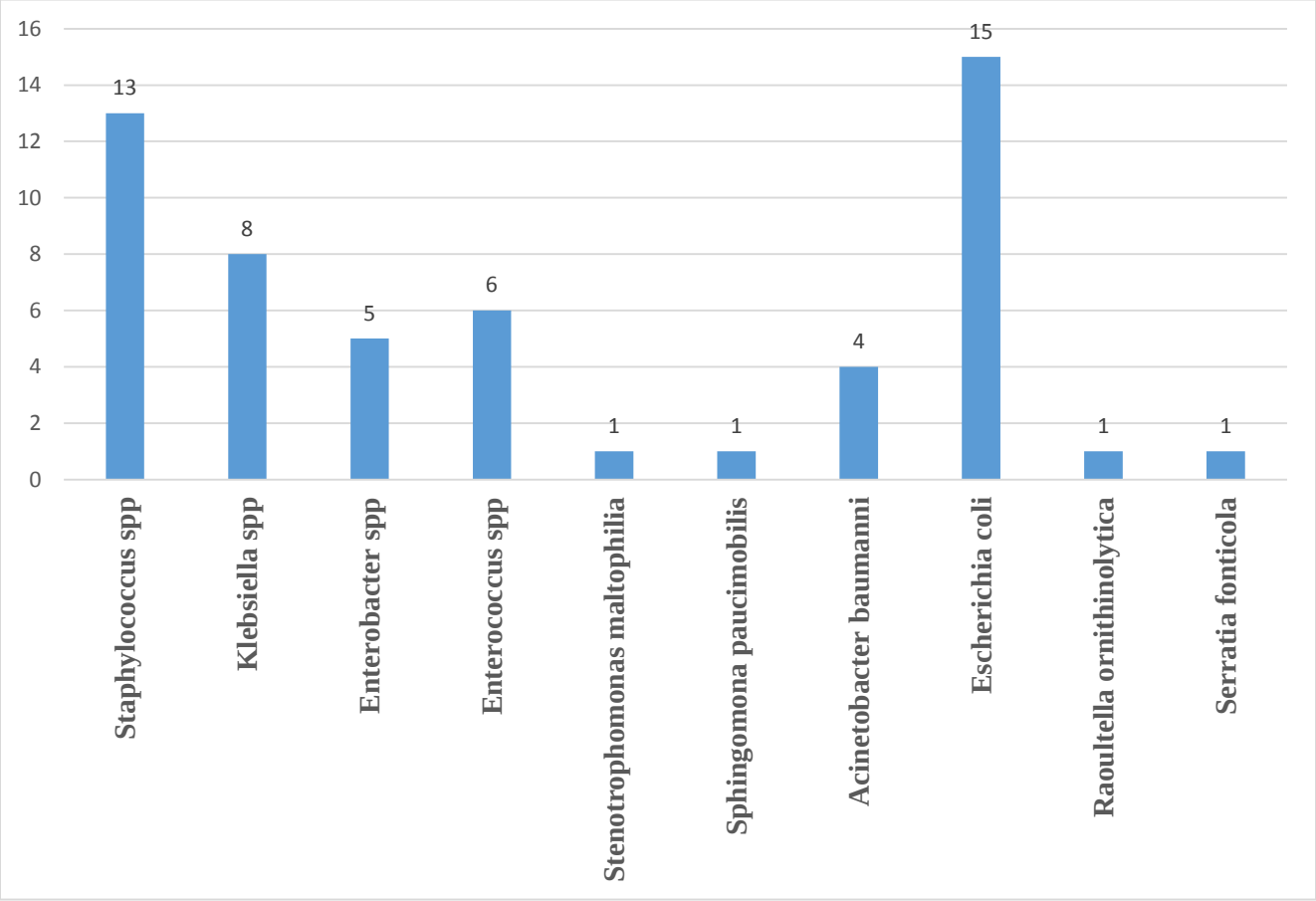



*Figure 4:Spectrum of bacteria isolates cultured from the placental bed tissues*

**Antimicrobial sensitivity of the bacteria isolated from the placental bed tissues**

Antimicrobial sensitivity was done for selected 5 different bacteria that were most cultured and identified. Selected 7 antibiotics that are commonly used at KNH were analyzed.

Nitrofurantoin was the antibiotic most sensitive followed by ciprofloxacin and ceftriaxone respectively as shown in table 5 below:

*Table 5: Bacteria that were most frequently isolated and were sensitive to selected antibiotics.*

| | NUMBER OF PATIENTS | AMOXICILIN/CLAVULI | CEFUROXIME | CEFTRIAXONE | CIPROFLOXACIN | NITROFURANTOIN | ERYTHROMYCIN | CLINDAMYCIN |
|---|---|---|---|---|---|---|---|---|
| Staphylococcus haemolyticus | 4 | | | | | 1 | 2 | 2 |
| Klebsiella pneumonia spp pneumonia | 5 | 1 | 1 | 3 | 5 | 3 | | |
| Acinetobacter baumanni | 4 | | | 2 | 4 | | | |
| Escherichia coli | 15 | 7 | 5 | 8 | 15 | 10 | | |
| Enterococcus faecalis | 5 | | | | | 3 | | |

**Histopathological patterns of placental bed tissue in patients who delivered preterm birth.**

Chorioamnionitis was the most common pathology. This was followed by maternal vascular malperfusion and fetal thrombotic vasculopathy as shown in table 6 and figure 4 below.

*Table 6: Histological profile of the placentas.*

| | **Frequency (n=110)** | **Percent** |
|---|---|---|
| Histological chorioamnionitis | 47 | 42.7 |
| Funisitis | 6 | 5.5 |
| Fetal vascular malperfusion | 2 | 1.8 |
| Delayed villous maturation | 4 | 3.6 |
| Maternal vascular malperfusion | 38 | 34.5 |
| Chorangiosis | 3 | 2.7 |
| Fetal thrombotic vasculopathy | 10 | 9.1 |

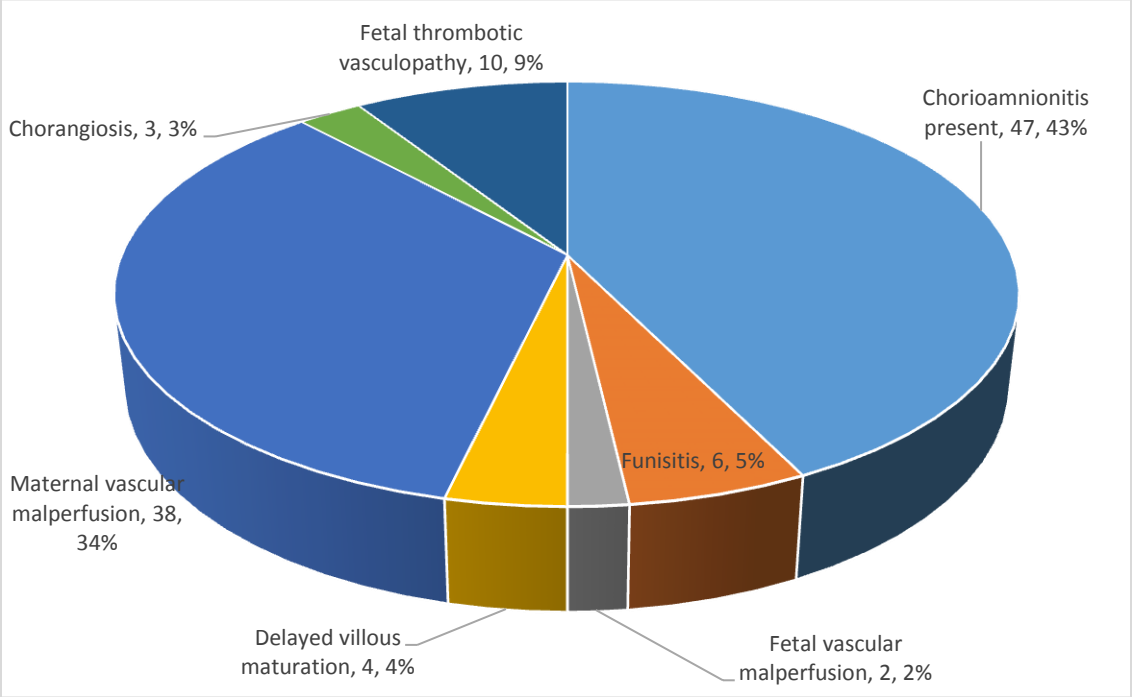
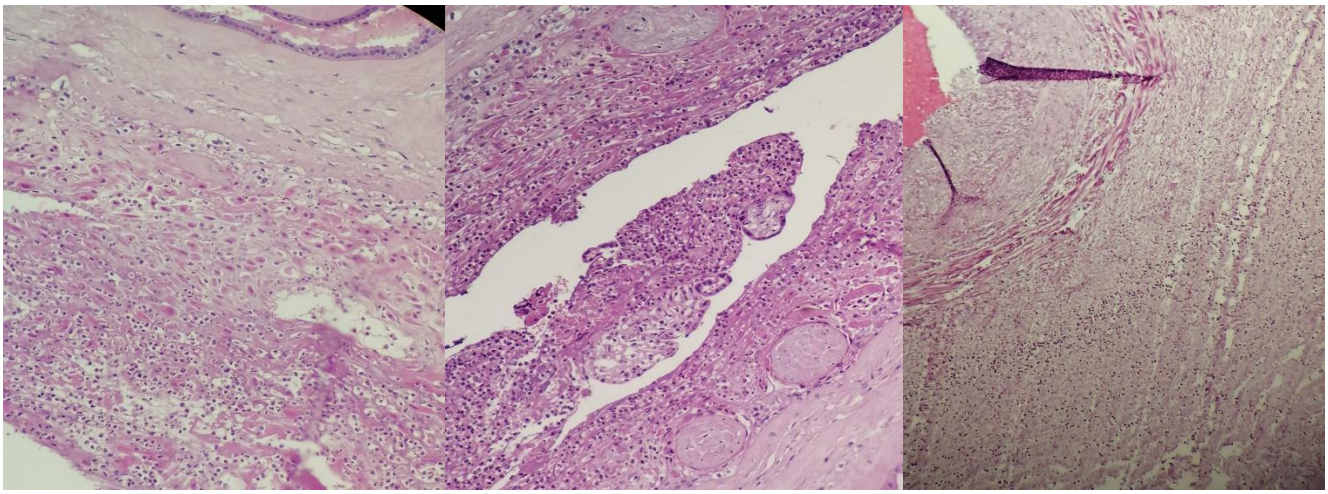



*Figure 5: Pie chart showing the histopathological patterns of placental bed tissue*



**A** **B** **C**

*Figure 6: Histopathological images of the placental tissue*

A and B shows features of chorioamnionitis while C shows funisitis with perivascular neutrophilic infiltrates.

**Placenta bacterial culture results compared with histological findings**

Histological chorioamnionitis was present in 40.5% (47/116) of the placentas. However, it was found in 40% (22/55) of placentas that also had positive bacteria culture and 41% (25/61) of placentas that had negative bacteria culture. It was negative in 60% (33/55) of placentas that had positive bacteria culture. There was absent bacterial culture growth and histological chorioamnionitis in 59% (36/61) of the placentas as shown in table 7 below:

*Table 7: Placenta bacterial culture results compared with histological findings*

| | Histological Chorioamnionitis present | | | |
|---|---|---|---|---|
| Growth | Yes n (%) | No n (%) | Total | p-value |
| Yes | 22 (40.0) | 33 (60.0) | 55 (100.0) | 0.914 |
| No | 25 (41) | 36 (59.0) | 61 (100.0) | |

# CHAPTER 5: DISCUSSION

This study looked at the bacterial culture and histopathology of placentas for patients who had preterm delivery at KNH. Positive bacterial culture was found in 47.4% (55/116) of the placentas. This result compares to the findings in the study by Andrew B. Onderdonk et al who reported a 51% bacteria culture positive result from 696 placentas amongst patients who delivered extremely preterm neonates(56). Vanessa Queiros da Mota et al found a lower rate of 19.4% (73/376) among the positive bacteria culture results.

Molly J. Stout et al found 27% of placentas examined by morphological techniques had intracellular bacteria within the basal plate(50). This shows the placenta basal plate could be colonized by microbes that are difficult to culture but which could easily be identified by morphological techniques. Only aerobic bacterial culture was done in this study. The commonest isolates included *Escherichia coli, Staphylococcus spp, Klebsiella spp, Enterococcus spp, Enterobacter spp* and *Acinetobacter baumanni.*

Bhola K et al were able to culture *Group B Streptococcus, Bacteroides, Enterobacter cloacae, Escherichia coli, Staphylococcus aureus, Morganella morganii* and *coliform spp*(48). Andrew B. Onderdonk et al mainly isolated *Staphylococcus spp, Corynebacterium spp* and BV associated bacteria(56). George L. Mendz et al in 2013 using molecular techniques isolated mainly microbes in the order *mycoplasmatales* and *lactobacillales*(16) while Doyle RM et al in 2017 using molecular techniques mainly isolated *Acinetobacter spp* and *Enterobacteriaceae spp*(38). *Staphylococcus epidermidis* cultured in two placentas in our study were probable contaminants.

Low sensitivity for *Erythromycin* and *Amoxicillin/clavulinic acid* which are the antibiotics mainly used as first line in management of patients with PPROM was noted. However, *Nitrofurantoin, Ciprofloxacin* and *Ceftriaxone* were the three antibiotics noted to have the highest sensitivity to the bacteria cultured.

Histological profile of the placentas examined revealed the following in order of frequency: histological chorioamnionitis, maternal vascular malperfusion, fetal thrombotic vasculopathy, funisitis, delayed villous maturation, Chorangiosis and fetal vascular malperfusion.

Histological chorioamnionitis was found in 40.5% of placentas in our study and this compares to Jonathan L Hecht et al,2008 in USA who reported histological chorioamnionitis at 43%(10). This also compares to Nadeen Edmondson et al, 2010 who found the prevalence of histologic chorioamnionitis to be 46% and chronic deciduitis to be 41% (49). This also compares to Mueller-Heubach E et al, 1990 who found a prevalence of histologic chorioamnionitis at 42% in preterm deliveries(11). However, lower rates of histological chorioamnionitis were reported by Bhola K et al,2008 at 26%(48) and Mwanyumba F et al at 19.6%. (9).

Vanessa Queiros da Mota et al found a diagnosis of histological chorioamnionitis in 26.9%. In the placentas that had histological chorioamnionitis, 27.7% had positive bacteria culture compared to 40% in our study(57). However, histological CA was negative in 16% of the placentas that had positive bacterial culture compared to 60% in our study. Possible explanations for negative histological results with positive culture results include early stages of bacterial infection where histological changes of inflammation have not occurred and sampling bias since histological specimens are representative of a larger specimen. A focal and segmental inflammation can easily be missed during sampling of the specimen.

Histological chorioamnionitis was found in 41% of placentas that had negative bacterial culture which compares to 40% in Vanessa Queiros da Mota et al study. This could be explained by prior antibiotic prophylaxis that could have hindered bacterial growth. Secondly, some fastidious bacteria like Mycoplasma are difficult to culture while Chlamydia trachomatis are strict intracellular microbes.

# CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

## CONCLUSION

The commonest bacteria isolated in our study were *Escherichia coli, Staphylococcus spp, Klebsiella spp, Enterococcus spp, Enterobacter spp* and *Acinetobacter baumanni*.

The prevalence of bacteria infecting the placenta isolated through culture was 47.4%.

Histological chorioamnionitis prevalence was found to be 40.5%.

Antimicrobial sensitivity results showed increased resistance to the common antibiotics routinely in use in our setup.

However, nitrofurantoin and ceftriaxone which are readily available have been shown to be overly sensitive.

## RECOMMENDATIONS

The prevalence of placental bacterial infections could be higher when molecular techniques like PCR are used since some bacteria like mycoplasma are difficult to culture. Future studies are recommended that would incorporate molecular techniques of bacteria isolation.

This study only cultured aerobic bacteria. The spectrum of bacteria isolates could be more when anaerobic bacteria culture is factored in. Future studies that would culture both aerobic and anaerobic bacteria are recommended.

Nitrofurantoin which showed good sensitivity against the cultured microbes is recommended for prophylaxis and treatment of patients with PTL and/or PPROM.

# STUDY TIMELINES

| | **Activity** | **Duration** | **Timeline** |
|---|---|---|---|
| 1. | Proposal Development | 3 months | June –Aug 2019 |
| 2. | Internal Marking | 2 months | Sept-Oct 2019 |
| 3. | Ethical Approval | 3 months | Nov-Jan 2020 |
| 4. | Data Collection | 2 months | Feb-March2020 |
| 5. | Data Analysis | 1 month | April 2020 |
| 6. | Thesis write-up | 1 month | May 2020 |
| 7. | Manuscript Development | 2 months | June-July 2020 |

# BUDGET

| **Items** | **Unit Cost Ksh.** | **Units** | **Total Ksh.** |
|---|---|---|---|
| Research assistant allowances/ administration of questionnaires and consent | 26,000 | 2 | 52,000 |
| Stationery & Flash drives | 1,000 | 4 | 4,000 |
| Printing / Photocopy | 10 | 1000 | 10,000 |
| Binding | 500 | 4 | 2,000 |
| Communication/ Airtime | 2,000 | 4 | 8,000 |
| Data entry charges | 5000 | 1 | 5,000 |
| Data analysis/ statistician | 30,000 | 1 | 30,000 |
| Histology lab reagents and consumables | 41,350 | 1 | 41,350 |
| Histology lab fees | 81,200 | 1 | 81,200 |
| Microbiology lab reagents | 14,384 | 1 | 14,384 |
| Microbiology lab fees | 58,000 | 1 | 58,000 |
| Morgue consumables | 13,384 | 1 | 13,384 |
| Cool box and icepacks | 3,000 | 1 | 3,000 |
| Miscellaneous | 10,000 | 1 | 10,000 |
| **TOTAL** | | | **332,318** |

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# ANNEX 1: QUESTIONNAIRE FORM (ENGLISH)

**STUDY TITTLE: PREVALENCE OF PLACENTAL BACTERIAL INFECTIONS AND RELATED PLACENTAL TISSUE HISTOPATHOLOGY AMONG PRETERM BIRTHS AT KENYATTA NATIONAL HOSPITAL**

**Serial number ……**

**Interview process: English version**

1. **Section A: Sociodemographic Data**

Age (Years) …… Parity …… Gravidity…….

Gestation by dates (LNMP) ……

Gestation by 1st ultrasound……. Date u/s done……. Estimated gestation age at the time ……..

a) Level of Education

None ☐

Primary ☐

Secondary ☐

Tertiary ☐

b) What is your religion?

Christian ☐

Muslim ☐

Hindu ☐

Others Specify ----------

c) What is your marital status?

Single ☐ Divorced ☐ Married ☐

Separated ☐ Widowed ☐

d) What is your employment status

Unemployed ☐ Self- employed ☐ Employed ☐

**2. Past Obstetric History**

a) When was your last delivery?.....

b) Have you undergone cesarean section before? Yes ☐ No ☐

If yes, how many times ……..

c) In the previous pregnancies, did you suffer from any of the following:

Hypertension in pregnancy Yes ☐ No ☐

Diabetes mellitus Yes ☐ ☐ No

Antepartum hemorrhage Yes ☐ ☐ No

Post-partum hemorrhage Yes ☐ ☐ No

Puerperal sepsis Yes ☐ ☐ No

d)Have you been treated for preterm labor before? ☐ Yes ☐ No

If yes, what was the outcome of the pregnancy?

Stillbirth ☐

Preterm delivery ☐

Term delivery ☐

### 3. History of the Current Pregnancy

a) Have you been attending your antenatal clinic? Yes ☐ No ☐

If yes, what is the level of the facility

Dispensary ☐

Sub County hospital ☐

County Referral hospital ☐

National Teaching/Referral hospital ☐

b) How many times did you attend…….

c) Have you had an antenatal profile done? Yes ☐ No ☐

If yes what were the test results of the following

HIV..…… VDRL…… Blood group……. Hb……

d) Were you started on any medication during this pregnancy? Yes ☐ No ☐

If yes, which ones …….

e) Have you been treated for a urinary tract infection in this pregnancy? Yes No

f) Do you suffer from diabetes mellitus? Yes ☐ No ☐

g) Do you suffer from any other medical disorders or are you on any chronic treatment?

……

h) What is the duration of time you experienced on/off lower abdominal pains radiating to the back (in hours) ……..

i)Drainage of liquor-describe number of pads used daily, color, smell……

……

j) Where were you when the membranes ruptured: At home In hospital

k) Duration of time from rupture of membranes to delivery Hours…….Days…….

l) Have you had drainage of liquor before in this pregnancy? Yes……... No…….

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

m)Have you been given antenatal corticosteroids for maturing the fetal lungs? ☐ ☐

Yes No

n)Have you consumed alcohol in the current pregnancy?

Yes ☐ No ☐

o)If yes, what kind of alcoholic drink do you take?..... how many times in a week ……

and how many glasses/bottles per drinking session……..

p)Have you been using tobacco in this pregnancy?

Yes ☐ ☐ No

If the answer to the question m above is yes, indicate the number of sticks per day ---------- and the number of years you have been smoking……..

# ANNEX 2: QUESTIONNAIRE (KISWAHILI VERSION).

**STUDY TITTLE: PREVALENCE OF PLACENTAL BACTERIAL INFECTIONS AND RELATED PLACENTAL TISSUE HISTOPATHOLOGY AMONG PRETERM BIRTHS AT KENYATTA NATIONAL HOSPITAL.**

**INTERWIEW PROCESS: KISWAHILI VERSION**

**NAMBARI MFULULIZO……**

**1.Section A: Tabia ya idadi**

Umri(Miaka)…… Nambari ya mimba……..

Mimba ina wiki ngapi kulingana na siku ya kwanza ya damu ya mwezi ya mwisho?.....

Mimba ina wiki ngapi kulingana na ultrasound ya kwanza?.....

**a) Elimu**

Hakuna ☐

Shule ya msingi ☐

Shule ya upili ☐

Chuo Kikuu/Kolejia ☐

**b) Dini**

Mkristo ☐

Muislamu ☐

Kihindi ☐

Dini aina nyingine……

**c) Hali ya ndoa**

Haujaolewa ☐ Umeolewa ☐ Talaka ☐

Ndoa kutengwa ☐ Mjane ☐

**d) Ajira**

Umeajiriwa ☐ Hujaajiriwa ☐ Umejiajiri ☐

**2.Historia ya Mimba**

a) Ulizaa mwisho lini?.....

b) Umewahi pata upasuaji wa mimba? Ndio ☐ La ☐

Kama ni ndio, umepasuliwa mimba mara ngapi?.....

**c) Ulipata matatizo wakati ulizaa mwisho?**

Shinikizo la damu Ndio ☐ La ☐

Kisukari Ndio ☐ La ☐

Vuja damu ukiwa na mimba Ndio ☐ La ☐

Vuja damu baada ya kujifungua Ndio ☐ La ☐

Maambukizi (kuwa maalum) Ndio La

**d)Umewahi tibiwa uchungu wa mwana kabla ya masiku kufika**? Ndio La

Kama ni ndio, ulijifungua nini?

Kuzaliwa bado

Kuzaliwa kwa kuishi (kabla kufikisha siku)

Kuzaliwa kwa kuishi (Kufikisha siku)

**3.Mimba ya saa hizi**

**a) Ulienda kliniki ya wajawazito?** Ndio La

Kama ni ndio, kiwango gani?

Zahanati

Hospitali Kaunti ndogo

Hospitali ya Kaunti

Hospitali ya Rufaa

**b) Ulienda kliniki mara ngapi?**.....

**c) Umepiwa maabara vipimo muhimu za ujauzito?** Ndio La

Kama ni ndio, majibu ya vipimo zifuatazo:

Virusi vya ukimwi……. Matokeo ya kaswende(VDRL)……

Aina ya damu…… Kiwango ya damu……

e) **Ulianzishwa matibabu yoyote katika ujauzito?** ☐ Ndio ☐ La

Kama ni ndio, matibabu gani ulipata…….

e) Umetibiwa ugonjwa kwenye mkojo katika hii mimba? Ndio ☐ ☐

La ☐ ☐

f) Uko na ugonjwa wa kisukari? Ndio La

g) Unaugua ugonjwa mwingine wowote ama kuna dawa zozote umetumia kwa muda mrefu?.....

h) Kwa muda wa masaa mangapi ulihisi uchungu kwa tumbo unao enea kwa mgongo?

……

i) Maji ya mimba (umetumia pedi ngapi, rangi, harufu)

……

……

j) Ulikuwa wapi wakati maji ilipasuka? Nyumbani……. Hospitalini……

k) Muda ya maji ya mimba kutoka hadi kuzaa

Saa……Siku…….

l)Umewahi vunja maji tena kwa hii mimba hapo mbeleni? Ndio ☐ ☐ La

Kama ndio, mimba ilikuwa wiki ngapi?.....

m)Umepewa madawa ya kusaidia mapafu ya mtoto kukomaa? Ndio ☐ La ☐

n) Umekunywa pombe ukiwa na hii mimba? Ndio ☐ La ☐

o) Kama ndio, ni pombe aina gani?.....mara ngapi kwa wiki……..

Chupa/glasi ngapi kwa kila kikao…….

p) Unavuta sigara ukiwa na hii mimba? Ndio ☐ La ☐

Kama ndio, unavuta sigara ngapi kwa siku……Umevuta sigara miaka ngapi……

# ANNEX 3: CONSENT INFORMATION (ENGLISH)

**STUDY TITTLE: PREVALENCE OF PLACENTAL BACTERIAL INFECTIONS AND RELATED PLACENTAL TISSUE HISTOPATHOLOGY AMONG PRETERM BIRTHS AT KENYATTA NATIONAL HOSPITAL.**

I, **DR. KIBUNJA JOHN VICTOR KARANJA** am a postgraduate student at the University of Nairobi Obstetrics and Gynaecology department. I am conducting the above-named study in partial fulfilment for the award of the degree of Master of Medicine in Obstetrics and Gynaecology by the University of Nairobi.

My cellphone number is **0723 593130**

Email address **vibuj2001@gmail.com,**

Postal address **P.O.BOX 28129– 00200, Nairobi.**

Lead supervisor:

**Dr Wanyoike Gichuhi**, Senior Lecturer at the Department of Obstetrics and Gynaecology, University of Nairobi. **Contacts: 0722 522234.** Email address: **drjoewanyoike@yahoo.co.uk**

**AIM OF THE STUDY**

The study aims to determine the prevalence of bacterial infections and the associated histopathogical changes in placentae of mothers who go into preterm labor and proceed to deliver before reaching term gestation.

**STUDY PROCEDURE**:

You will be asked a few questions regarding your age, the number of your children/number of times you have been pregnant, level of education, employment status, history of your previous pregnancies if any as well as your current pregnancy and antenatal clinic attendance, whether you smoke, if your currently on any medication or recently taken, any other illness you have had during the current pregnancy, the number of hours you have been in labor, treatment given prior to delivery, and associated symptoms e.g.

rupture of fetal membranes .We will review your antenatal card to see if there is previous history that may put you at a risk of preterm labor and delivery other than infection. Following delivery, we shall use the placenta for laboratory tests and examination (bacterial culture, antimicrobial sensitivity, and histopathology studies).

**BENEFITS**

The study participants may not directly benefit from this study, however the findings from the study will guide the use of antibiotics to target these organisms on the mothers who deliver before term, during their puerperium as well as on the preterm babies once they are admitted in the newborn unit.

**RECRUITMENT AND CONSENT**

The researcher and his assistants will explain the research procedure to you, provide written information when appropriate and obtain written informed consent, before starting the study.

**POTENTIAL RISKS**

This study does not pose any danger to you or your baby since we will use the placenta after you deliver. You will receive all the necessary care that a mother who comes for delivery is entitled to. There will be no additional costs for you to incur for participating in this study. There will be no direct monetary benefits after participating in the study.

**CONFIDENTALITY**

Your name, hospital number or initials will not be used to identify you in any way. Instead, only a serial number will be used. The information contained in the questionnaires will only be used by the principal investigator and research assistants.

**MINORS**

All pregnant women can participate in the study. In Kenya, Pregnant women below 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 WITHRDAWAL FROM THE STUDY**

Your participation in this study is voluntary. You will not be forced to answer any questions you are not comfortable. You can also withdraw from this study at any point without any negative impact to the kind of quality health care services that you are entitled to.

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

**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……..

# ANNEX 4: CONSENT (KISWAHILI VERSION)

**STUDY TITTLE: PREVALENCE OF PLACENTAL BACTERIAL INFECTIONS AND RELATED PLACENTAL TISSUE HISTOPATHOLOGY AMONG PRETERM BIRTHS AT KENYATTA NATIONAL HOSPITAL.**

**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 mfanyikazi 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 ama uchungu wa uzazi kuanza kabla ya kufikisha siku.

Baada ya kujifungua, tutachukua ‘placenta’ na kuipeleka kwenye maabara ambapo utafiti zaidi utafanywa na wataalamu.

**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 watakuweko 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 akina mama 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. Kibunja John Victor Karanja**

Kwa nambari ya simu **0723 593130**,

Barua pepe**:vibuj2001@gmail.com,**

Sanduku la posta **28129-00200, Nairobi.**

Au msimamizi mkuu **Dr Wanyoike Gichuhi,**

Nambari ya simu **0722522234,**

Barua pepe**:drjoewanyoike@yahoo.co.uk,**

Sanduku la posta**, University of Nairobi, College of Health Sciences,**

**P.O.BOX 19676-00202 Nairobi.**

**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 5: DATA COLLECTION SHEET

**STUDY TITTLE: PREVALENCE OF PLACENTAL BACTERIAL INFECTIONS AND RELATED PLACENTAL TISSUE HISTOPATHOLOGY AMONG PRETERM BIRTHS AT KENYATTA NATIONAL HOSPITAL.**

Study Number: _____

Sex: F

Maternal age years…….

Gestation in weeks……..

Route of delivery: Vaginal ◯ Caesarean section ◯

Areas of infarction Yes ◯ No ◯

Areas of thrombosis: Yes ◯ No ◯

Site of cord insertion: Central ◯ Eccentric ◯ Marginal ◯ Velamentous ◯

Cord diameter: _____ mm

Cord length: _____ cm

Shape of the placenta: Discoid ◯ Annular ◯ Circular ◯ horseshoe ◯

Color of the membranes and chorionic plate

Maroon ◯ Green-brown ◯ Yellow-gray ◯

Areas of calcification: Yes ◯ No ◯

Cord colour; White ◯ dark brown ◯ black green ◯

Number of vessels in the cord; one ◯ two ◯ three ◯ > three ◯

Umbilical cord hemorrhages. Yes ◯ No ◯

Weight of the placenta (gms)……..

Diameter of the placenta (cms) in three dimensions; Greatest…… Major……. Minor……...

Thickness of the placenta (cms); Greatest…….. Minor……

**Histomorphology Placenta**

**Central sections 1 and 2**

1_____

2 _____

**Peripheral sections 1, 2, 3, 4, 5, 6**

**1**_____

**2**_____

**3**_____

**4**_____

**5**_____

**6**_____

Histomorphology, umbilical cord taken in two sections

1 _____

2 _____

Results of Giemsa staining

_____

# ANNEX 6: LAB REQUEST FORM 1

**LABORATORY REQUEST FORM 1: PLACENTA FOR HISTOPATHOLOGY**

**STUDY TITTLE: PREVALENCE OF PLACENTAL BACTERIAL INFECTIONS AND RELATED PLACENTAL TISSUE HISTOPATHOLOGY AMONG PRETERM BIRTHS AT KENYATTA NATIONAL HOSPITAL.**

Patient identification number:

Age:

LNMP:

Gestational age:

Parity:

Clinical summary and diagnosis:

Date collected:

Collected by: (Name and signature)

Department collected from:

Investigation required: Histology

Requesting clinician (Name, signature, and contacts)

# ANNEX 7: LAB REQUEST FORM 2

**LABORATORY REQUEST FORM 2: PLACENTA FOR MICROBIOLOGY**

**STUDY TITTLE: PREVALENCE OF PLACENTAL BACTERIAL INFECTIONS AND RELATED PLACENTAL TISSUE HISTOPATHOLOGY AMONG PRETERM BIRTHS AT KENYATTA NATIONAL HOSPITAL.**

Patient identification number:

Age:

LNMP:

Gestational age:

Parity:

Clinical summary and diagnosis:

Mode of delivery: SVD ◯ Cesarean section ◯

Date collected:

Collected by: (Name and signature)

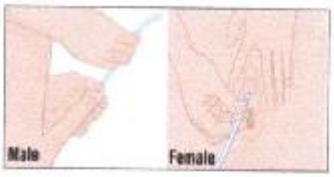
Investigation required: Microscopy, culture and sensitivity.

Requesting clinician (Name, signature, and contact)

# ANNEX 8: CHAMPS PROTOCOL



b. The assistant sticks one of the extra labels form the MITS specimen collection kit.
c. The assistant opens the urinary sterile catheter 8FR.
d. In males, the specialist holds the penis erect and inserts the bladder opening port of the catheter slowly into the urethra opening about 8-12 cm. Once the urine starts to flow, advance the catheter about 3 cm.




e. In females, the specialist separates the labia major and still holding the labia apart, inserts the bladder opening port of the catheter slowly into the urethra opening about 3 cm. Once the urine starts to flow, advance the catheter about 3 cm.
f. The assistant places the urinary drainage port of the catheter into the urine container.
g. The specialist collects all the urine that flows from the sterile tube.
h. The assistant fills the required information on urine in the body fluid section of the MITS specimen collection form.

**7.27 End of the procedure and completion the MITS specimen collection form**

a. The assistant and the specialist make sure that all the containers and jars are properly labeled and closed.
b. In the case of stillbirths, the assistant places the non-used bone marrow trephine in the MITS backup box.
c. The assistant and the specialist make sure that all non-used labels are also disposed in the biowaste container.
d. The assistant and the specialist make sure that used tools, as well as all non-used containers and jars (placental vials in infants and children, bone marrow vials in stillbirths and neonates, etc) should be disposed in a biowaste container.
e. Once the MITS specimen collection kit box is empty, the assistant and the specialist double check all the containers and jars and take all the cassettes and reallocate them in the MITS specimen collection kit box.
f. The assistant and the specialist write their names and sign the MITS specimen collection kit.
g. The MITS specimen collection form is put into the MITS specimen collection kit box.
h. The MITS specimen collection kit box containing all cryovials, containers, jars, cassettes and the MITS specimen collection form is sent to the local lab.
i. The assistant describes in the specimen collection form, by indication of the specialist, any additional comment to the procedure
j. The assistant writes in the specimen collection form the time in which the procedure has finished
k. The specialist and the assistant write their names in the specimen collection form and sign it

## 8.0 Actions to be done in case of excessive seepage or bleeding through the biopsy entry points

**8.1 In case of excessive seepage or bleeding though the biopsy entry points**

a. The assistant prepares the container with the Monsel's solution and a swab for its application, and opens the Mosel's solution jar
b. The MITS technician takes with the swab some Monsel's solution from the jar.
c. The MITS technician applies the Monsel's solution to the bleeding entry point
d. If necessary, take a gauze and roll it around the bleeding area to make pressure and reinforce the hemostatic effect of the Monsel's solution
e. Before delivering the body to the family remove the gauzes

## 9.0 Placenta Evaluation (for stillbirths)

**9.1 Take photos of the placental surfaces, including the photo card in the setting:**

i. Maternal surface
ii. Fetal surface

**9.2 Describe the umbilical cord**

a. Diameter of the cord. In the case of significant variations in diameter, provide the minimum and the maximum.
b. Length of the cord.
c. Site of insertion in relation to the center/margin of the placenta, determined by measuring the distance between the insertion site and the nearest placental margin
d. Presence of strictures
e. Appearance of the cord (hypocoiled or hypercoiled). Segmental or localized areas of hypercoiling should be recorded. Direction of coiling (handedness) should be noted if possible

**9.3 Describe the membranes**

a. Color/opacity and completeness
b. Record, if possible, the shortest distance between the site of rupture to the placental edge
c. If circumvallate or circummarginate, the percentage of the circumference involved should be noted

**9.4 Weigh the whole placenta**

a. Trim the extraplacental membranes and umbilical cord off the placenta. The placenta should be weighed only after trimming it
b. Weigh the placenta
c. Record its weight in the form. Record whether the placenta was fresh or fixed when measured
d. Any prior sampling of the placental parenchyma should also be documented (note that prior to the reception at the pathology lab, samples for microbiology of the placental parenchyma and membranes have been taken)
e. Any disruption of the basal plate should be noted (note that prior to the reception at the pathology lab, samples for microbiology of the placental parenchyma and membranes have been taken)

**9.5 Measure the placental disk (three dimensions)**

a. Maximal linear dimension (length)
b. Greatest dimension of the axis perpendicular to this linear measurement (width)
c. Mural minimal and maximal thickness

**9.6 Perform serial sections of the placenta**

a. Using a knife, the specialist serially sections the disc from the fetal to the maternal surface at 2 cm interval and examines each slice for parenchymal lesions (e.g. infarcts)

**9.7 Take photos of the placental sections, including the photo card in the setting**

a. All the placental sections are put on a clean surface and a photo is taken
b. If placental lesions are identified extra photos are taken

**9.8 Describe any lesions identified**

a. Any grossly identified lesions should be described
b. Estimate the percentage of the total parenchymal volume affected by the lesions, or measure of the two maximal dimensions of each lesion
c. The number of lesions of the same gross appearance should be counted and stated as being single or multiple
d. The location(s) of the lesions should be stated: central/paracentral or peripheral. Lesions that are microscopically different may appear similar in a gross examination.

**9.9 Sampling of Cord, Membranes, and Placental Disk**

m. Prepare at least 5 blocks:
   i. CASSETTE #15 and ALCOHOL JAR 16: include a roll of the extraplacental membranes obtained from the rupture edge to the placental margin, including part of the marginal parenchyma
   ii. CASSETTE #17 and ALCOHOL JAR 18: include 2 cross sections of the umbilical cord
      1. fetal end
      2. approximately 5 cm from the placental insertion end
   iii. CASSETTE #19 and ALCOHOL JAR#20: Two blocks, each containing a full thickness section of normal-appearing placenta parenchyma should be submitted. Full-thickness samples should be taken from within the central two-thirds of the disc and include one adjacent to the insertion site itself.
   If the transmural thickness is greater than the length of the cassette, divide the gross slice and submit it in two cassettes: the upper third (chorionic plate and subjacent tissue) and lower third (basal aspect) of the parenchyma. A full-thickness sample should be taken from close to the umbilical cord insertion site to document fetal vascular ectasia and fetal and/or maternal inflammatory response
   iv. CASSETTE #21 and ALCOHOL JAR#22: In case of placental lesions, additional blocks of the lesion/s (one of each type of lesion) should be sampled, with adjacent normal parenchyma if possible, in up to three additional blocks

**9.10 End of the procedure and completion of the placental collection form**

f. The assistant and the specialist make sure that all the cassettes are placed in a container with formalin
g. The assistant describes in the specimen collection form, by indication of the specialist, any additional comment to the procedure
h. The specialist and the assistant write their names in the specimen collection form and sign it
i. The remaining placenta can be discarded once the cassettes have been processed and the pathologist has made sure that the placental slides are adequate for histological evaluation and no extra sampling is needed

## 10.0 Safety

Wear standard PPE (gloves, labcoat, respiratory and eye protection). Dispose of needles and all waste generated during procedure in appropriate container as per laboratory protocols.

## 11.0 References

Khong TY, Mooney EE, Ariel I, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. Arch Pathol Lab Med. 2016; 140(7):698-713.

## 12.0 Appendix

**11.1 Specimen Collection Kit Components and Backup Box Components**
**11.2 Table of Formalin and Jar Designations**
**11.3 MITS Specimen Collection Form CRF_06.02.01**
**11.4 Job-Aid for Using the Supplied Labels**

# ANNEX 9: PLACENTAL PATHOLOGY REPORT FORM

SECTION A: 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

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

*(Enlarged distal villi with excessive stroma, hypercellular villous trophoblast, central blood vessels, paucity of vasculo-syncytial membranes)*

Not Applicable ☐ Absent ☐ Present ☐

2. Accelerated villous maturity Present ☐ Absent ☐

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

3. 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).*

4. a) Presence of villous edema Yes ☐ No ☐

b) If present, indicate percentage of villi affected as

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

5. 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%)}**

6. 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 ☐

7. 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%)}**

8. 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

1. 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%)}**

2. 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%)}**

3. 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 histiolytic intervillusitis Present ☐ Absent ☐

SECTION D: Villous Vascular Findings

1. Villous vascularity Increased ☐ Not increased ☐
   *(Criteria: 10 villi in 10 fields with 10 or more blood vessels)*

2. a) Basal vessel wall abnormalityPresent ☐ Absent ☐

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

Muscular wall hypertrophy ☐ Fibrinoid Necrosis ☐ Atheromatous changes ☐

3. Fetal thrombotic vasculopathy Present ☐ Absent ☐

**OTHER SIGNIFICANT FINDINGS**

**CONCLUSION/DIAGNOSIS.**

Signature of Consultant /Student:

……

Date of Report:

……