PREVALENCE OF MYCOPLASMA HOMINIS, UREAPLASMA UREALYTICUM AND UREAPLASMA PARVUM IN PLACENTAE OF MOTHERS WITH PRETERM DELIVERY AND THE ASSOCIATED NEONATAL OUTCOMES AT KENYATTA NATIONAL HOSPITAL, 2020. A DESCRIPTIVE CROSS SECTIONAL STUDY

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF THE DEGREE OF MASTER OF MEDICINE IN OBSTETRICS AND GYNAECOLOGY

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DEDICATION

I would like to dedicate this work to my wife Valentine and my son Ayden for their understanding and support throughout my studies and as I worked on this project.

LIST OF ABBREVIATIONS

Abbreviation	Meaning
ACOG	American College of Obstetricians and Gynaecologists
ANC	Antenatal Care
CHAMPS	Child Health and Mortality Prevention Surveillance
CLD	Chronic Lung Disease
CRP	C-Reactive Protein
DNA	Deoxyribonucleic Acid
FIRS	Fetal Inflammatory Response Syndrome
GBS	Group B Streptococci
HIV	Human Immunodeficiency Virus
KAVI	Kenya AIDS Vaccines Initiative
KDHS	Kenya Demographic Health Survey
KNH	Kenyatta National Hospital
MOH	Ministry of Health
NICU	Neonatal Intensive Care Unit
PCR	Polymerase Chain Reaction
PPROM	Preterm Premature Rupture of Membranes
RDS	Respiratory Distress Syndrome
UON	University of Nairobi
UTI	Urinary Tract Infections
WHO	World Health Organisation

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OPERATIONAL DEFINITIONS

Preterm Birth: Any live baby born before 37 completed weeks of gestation

Preterm Labour: Onset of labour before 37 completed weeks.

Polymerase Chain Reaction: A rapid procedure for in vitro enzymatic amplification of specific DNA sequences using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA

ABSTRACT

Background: The global preterm birth rate in 2014 was estimated at 10·6%, equivalent to 14·84 million live preterm births. 81·1% preterm births happened in Asia and sub-Saharan Africa.

Intrauterine infection during pregnancy accounts for about 40% of preterm births. *Mycoplasma hominis and Ureaplasmas* are frequently isolated from the placenta, amniotic fluid and cord blood and contribute towards clinical and histological chorioamnionitis. The prevalence *of M. hominis, U. urealyticum and U. Parvum* in preterm delivery within our setting remains largely unknown despite the poor preterm neonatal outcomes.

Broad Objective: To determine the prevalence of *Mycoplasma hominis Ureaplasma urealyticum and Ureaplasma parvum* in placentae following preterm labor and delivery and the neonatal outcomes associated with infection with these organisms.
Methodology: The study utilized a descriptive cross sectional study design, involving 125 pregnant women admitted with preterm labor between 28 weeks 0 days and 36 weeks, 6 days gestation at Kenyatta National Hospital. Informed consent was obtained. Pretested questionnaires for socio-demographic data.
Following delivery, placentae were immediately collected into sterile plastic containers and taken to the Kenyatta National Hospital mortuary in a cool box for refrigeration between 2^o and 8^o Celsius. Using CHAMPS protocol for (minimally invasive tissue sampling MITS) procedure Standard Operating Procedure (SOP) protocol v1.0 September 2016, placental cuts were made and frozen at -80^o Celsius at KAVI laboratory. Polymerase chain reaction for *M. hominis, U. urealyticum* and *U. Parvum* was done at KAVI laboratory within the College of Health Sciences, University of Nairobi.

Early neonatal outcomes were established for all the 125 newborns.

Results: The mean gestation age at delivery was 34.1 weeks. The mean neonatal weight was 2336 gm. There were 81.6% vaginal deliveries. Placental isolation of organisms was 63.2% M. hominis, 25.6% U. urealyticum and 53.2% U. parvum. 29.2% of the neonates were admitted into the newborn unit was Newborn admission and a mortality rate of 24.3%.
Conclusion and Recommendations: There was no significant difference in the burden of placental infection and early neonatal outcomes. Larger studies to confirm our findings, and investigate the infection with *M. hominis, U. urealyticum* and *U. parvum* among preterm neonates should be conducted.
Screening for these organisms before conception and offering targeted treatment may provide knowledge about future practices with an attempt to reduce global burden of morbidity and mortality associated with preterm delivery.
Key Words: Preterm Birth, *Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum,* Polymerase Chain Reaction

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Worldwide, it is estimated that about 15 million neonates are born before term, majority of whom are in the moderate to late preterm category. Each year, about 1 million deaths are reported due to complications of prematurity, representing 29% of neonatal deaths whereas in childhood, prematurity is the second leading cause of mortality after pneumonia. Majority of these are in Sub-Saharan Africa and South Asia, with Preterm births contributing about 60% of deliveries and 80% of all mortalities from preterm birth complications(1)(2)(3).

WHO defines a preterm birth a baby born alive before completion of 37 weeks gestation.

Based on the gestation age, preterm births can further be sub-classified as:

a) Extreme preterm (less than 28 weeks)
b) Very preterm (28-32 weeks) and
c) Moderate to late preterm (32 to 37 weeks).

Preterm babies are born when they are not physically ready to face the extrauterine life, thus a great health concern to them. Some of the challenges include chronic lung disease, visual and hearing impairment, cerebral palsy as well as intellectual deficits. This poses a further challenge to their immediate families as well as the community(4).

The global preterm birth rate in 2014 was estimated at 10·6%, equivalent to 14·84 million live preterm births. Out of these, 81·1% preterm births happened in Asia and sub-Saharan Africa(5).

According to 2010 Global disease burden, complications of preterm birth account for 3.1% of disease burden. Despite technological and medical advancements, preterm birth is on the rise worldwide. In Japan, the rate increased from 4.1% in the year 1980 to 5.9% by 2010. In South Korea, the rate increased from 4.5% in 2003 to 6.5% in 2013(6). In the UK in 2012, 7.3% (52,000) of all live births were preterm, 75% of whom were as a result of preterm labor.

The survival rates of preterm births has increased over time, however there is no significant change in the number of those living with complications of preterm delivery(7).

The KDHS 2014 reported a decrease in the infant mortality rate to 30/1000 live births from 52/1000 live births in 2008-2009, and a decline in the neonatal mortality rate from 31 per 1,000 live births to 22 per 1,000 live births in the period 2008-2014.

Intrauterine infection during pregnancy is associated with about 40% of all preterm births with the commonest organisms isolated from placenta, amniotic fluid as well as cord blood being *Ureaplasma urealyticum and Mycoplasma hominis*(7)(8)(9). The main source of infection is thought to be ascending bacteria from the lower genital tract. In the uterus, these bacteria colonise and cause inflammation of the fetal membranes resulting in chorioamnionitis(10). Other routes of infection are haematogenous spread, retrograde movement through the fallopian tubes and direct introduction of microorganisms during invasive procedures such as chorionic villous sampling and amniocentesis(11). A number of studies have shown a positive relation of periodontal disease and preterm birth and low birth weight. Haematogenous spread of microorganisms and or inflammatory mediators arising from periodontal disease to the uterus may play a role in causing preterm labor (12). Studies on mice have shown that dental infection with *Porphyromonas gingivalis* was able to cause preterm birth in 2 days by way of up-regulation of inflammatory mediators especially galectin-3. Other elevated markers are IL-8, TNF-α and COX-2 in the placenta, amniotic fluid and in blood(13).

The presence of these microorganisms is a major contributing factor to preterm delivery, with an estimated 25-40% preterm births associated with infection(14).

Spontaneous preterm labor and subsequent preterm birth contributes significantly to perinatal morbidity and mortality, however, most cases of preterm delivery remain unknown(15)(16). A strong correlation between infection of the placenta with mycoplasmas and chorioamnionitis and subsequent

still birth has been found prompting recommendation to have all complicated pregnancies be subjected to microbiological and histological studies(17).

According to the American College of Obstetricians and Gynaecologists 2018, 12% of all live births are premature, with preterm labor contributing to about 50%. (2018). Among women presenting with preterm labor and/or preterm premature rupture of membranes, *Ureaplasma spp*. are the organisms most commonly isolated from cultures of amniotic fluid and infected placentas. The longer the duration of rupture of fetal membranes, the higher the likelihood of isolation. Some of the risk factors for preterm delivery include race, social economic status and social stressors(16)(18)(19)(20). Although chorioamnionitis is frequently associated with bacterial infections, some cases, do not have any demonstrable microbial invasion, thus the term sterile chorioamnionitis(21). Among patients with PPROM, amniotic fluid and maternal blood analysis has been found to have more leucocytes in infections with genital mycoplasmas than with other microbes. Similarly, the C-reactive protein has been noted to be higher. *Mycoplasmas hominis* has been associated with more intense inflammatory responses especially IL-4 whereas *Ureaplasma urealyticum* infection has no correlation with elevations in any of the cytokines The same study also showed that all the women with preterm premature rupture of membranes had either *M. hominis* or *U. urealyticum*(22).

In men, *M. genitalium* and *Ureaplasma urealyticum* has been found to cause non gonococcal urethritis, epididymo-orchitis, and prostatitis while in women causing cervicitis, endometritis and pelvic inflammatory disease. *M. hominis* has been isolated in some cases of pyelonephritis (23).

1.2 Pathophysiology of preterm delivery

Preterm delivery results from several mechanisms including abnormal hormone metabolism especially progesterone, utero-placental ischemia and infection of the uterine environment.

Progesterone is the main hormone responsible for maintaining a pregnancy. Whereas it causes expression of beta-adrenergic receptors that promote uterine relaxation, estrogen on the other hand cause expression of alpha-adrenergic receptors that are associated with uterine contractions. Congenital or acquired coagulopathies are associated with negative effects on the placental microcirculation leading to an increased risk of spontaneous abortions, IUGR, intrauterine fetal death, preeclampsia, thrombo-embolic disease and placental abruption(24)(25).

Reduction of the protective lactobacilli population in the vagina alters the pH favoring an overgrowth of other commensals including Mycoplasma and Ureaplasma spp. Genital mycoplasma esp. *M. hominis* have been implicated in causing pelvic inflammatory disease and ectopic pregnancy(15). *Gadnerella vaginalis* is commonly associated with bacterial vaginosis and which can lead to preterm labor. Group B Streptococci (*S. agalactiae*) as a vaginal commensal is seen in about 30% of women and has been linked with early onset neonatal sepsis(26)(8).
Ureaplasmas have been reported to produce IgA cleaving enzymes which enables then to escape the host defense system and thus maintain genital tract colonization. These enzymes as well as the lipoprotein multiple banded antigen are among the virulence factors. The latter causes inflammatory responses through increased transcription of toll like receptor genes and production of TNFα(27).

Chorioamnionitis which refers to the presence of active infection in the amniotic cavity and which results in maternal inflammatory changes is a polymicrobial process which is difficult to diagnose without invasive tests, and is mainly dependent on clinical signs and symptoms. Complications include preterm birth, postpartum haemorrhage, endomyometritis and surgical site infections. GBS and mycoplasma have frequently been associated with chorioamnionitis and subsequently preterm births(28).

Intrauterine infection leads to development of chorioamnionitis, funisitis and fetal inflammatory syndrome (FIRS). Consequently, there is increased production of IL-1, IL-6 IL-8 and TNFα, prostaglandins, matrix metalloproteins. Together with cortisol generated from activation of fetal hypothalamo-pituitary adrenal axis the labor process is triggered(24)(29).

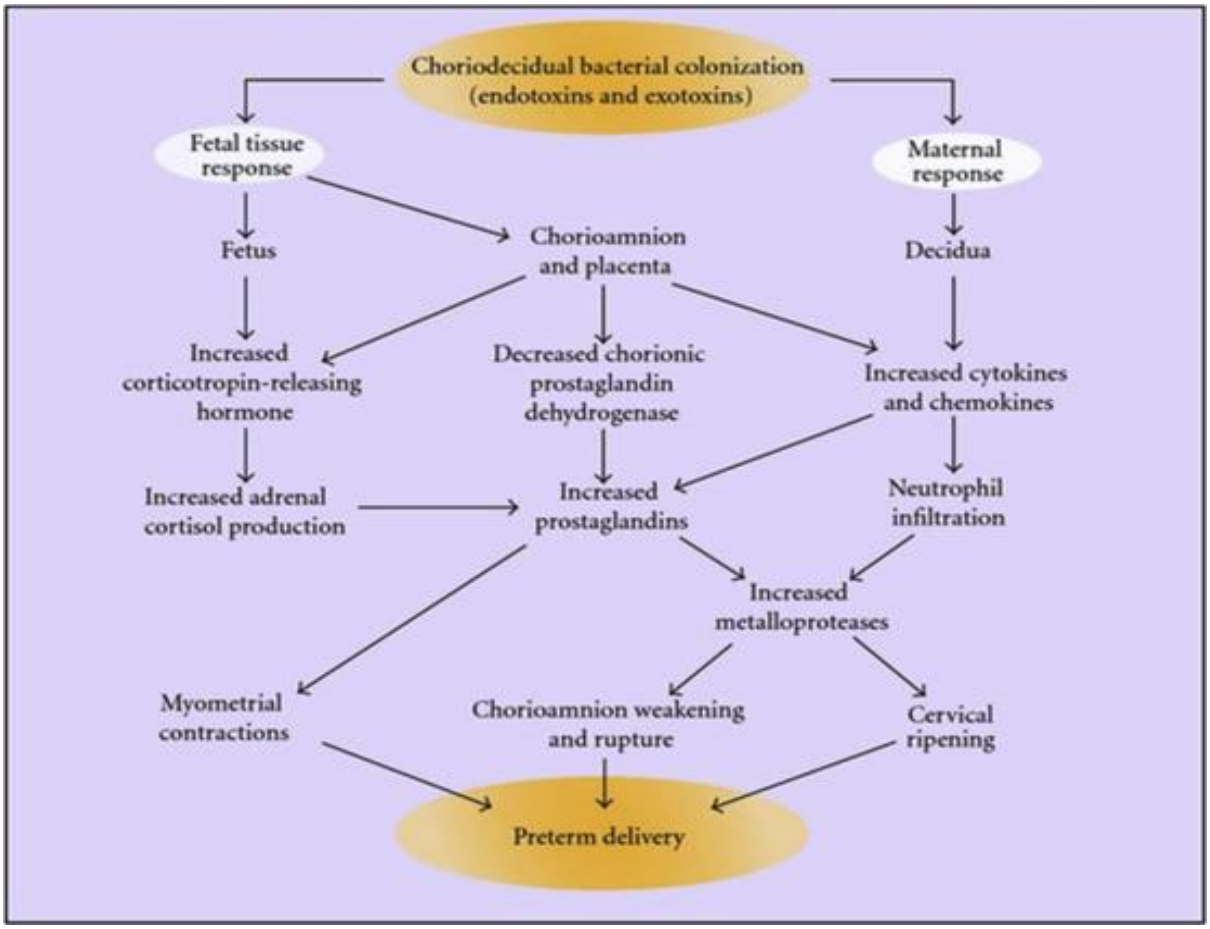

Figure 1: Pathophysiology of preterm labor from Sciencedirect.com

1.3 Problem Statement

Globally, about 15 million babies are born preterm and over 1 million die within the first four weeks of life. The survivors are at an increased risk of suffering from chronic lung disease, pneumonia, cerebral palsy, as well as dying. *Mycoplasma hominis* and *Ureaplasma urealyticum* are the organisms commonly isolated in patients with clinical and histological chorioamnionitis and subsequently preterm labor. Most diagnostic laboratories are moving towards using rapid diagnostic tests and PCR for 16SrRNA. By determining the prevalence of these organisms in preterm birth and the associated neonatal outcomes, we hope that the results will guide the use of antibiotics to target these organisms on the mothers during puerperium as well as on the preterm babies once they are admitted in our newborn unit.

CHAPTER 2: LITERATURE REVIEW

2.1 Description of *Ureaplasma spp* and *Mycoplasma hominis*

These organisms are small fastidious bacteria that belong to the class mollicutes and family of mycoplasmataceae. They are characterized by the absence of a cell wall and have reduced biosynthetic activities. They are also the smallest self-replicating micro-organisms in both cell and genome size. The absence of a cell wall hinders gram staining and predisposes them to dehydration. This limits them to a parasitic relation with their hosts.(30)(31).

They are usually found in the genito-urinary tract where they cause sexually transmitted infections, infertility as well as poor pregnancy outcomes including preterm labor, neonatal morbidity and mortality.

2.2 Isolation of the Organisms

Mycoplasma and *Ureaplasma spp*. have strongly been associated with preterm labor.

The detection of these organisms is done serologically or in vitro isolation. DNA PCR serves as the most reliable method for isolation of these organisms as well as species identification(32).

According to the Preterm Birth Study, which involved collection of cord blood for cultures in neonates aged between 23 – 32 weeks of gestation, *Ureaplasma urealyticum* and/or *Mycoplasma hominis* were found positive in 23.4% of cord blood cultures. Out of these, *Ureaplasma urealyticum* dominated at 52%, while *Mycoplasma hominis* was 26%. Both organisms were cultured simultaneously in individual cord bloods of 22% of the study population(16).

Ondari, in 2010 looked at factors associated with preterm birth at KNH and found the prevalence of spontaneous preterm delivery at 8.7%, with urinary tract infections and hypertension in pregnancy as the commonest events leading to delivery.

Wagura in 2014 found that the rate of preterm birth in Kenyatta National Hospital, Kenya at 18.3%, and among the factors contributing to this include urinary tract infections, preterm premature rupture of membranes, hypertension in pregnancy and antepartum haemorrhage(33).

Ureaplasma spp. are the organisms that have been found to be the commonest in all preterm births associated with infections(1)(8)(21).

Mycoplasma hominis can exist as normal vaginal floral in which case they cause no symptoms or disease or exist as pathogens with a potential of causing adverse pregnancy outcomes like preterm premature rupture of membranes, chorioamnionitis, preterm labor and birth and neonatal morbidity and mortality(13)(15).

One in every 3 preterm deliveries is related to infection of the amniotic cavity. *M. hominis* is often times isolated from the amniotic fluid and fetal membranes and ureaplasmas frequently from cord blood of preterm births.

Odendaal et al, in 2002 assessed the association of *M. hominis* on preterm labor in primigravidae, and multigravidae at risk with a history of previous midtrimester abortion or preterm labor between 16 and 26 weeks of gestation who were on follow up at Tygerberg Hospital, Western Cape South Africa. Endocervical swabs were taken at the first antenatal visit and cultures for *M. hominis*. The pregnancy outcomes for culture positive women was compared to those with negative cultures. 21% of the study population had *M. hominis* positive cultures, 40% of whom had preterm delivery. *U. urealyticum* was cultured in 96% of the group with *M. hominis*. On the other hand, out of the 79% with culture negative results, only 28% had preterm delivery, therefore showing an association of *M. hominis* and preterm delivery(34).

Marian et al assessed the presence of Mycoplasma and Ureaplasma spp. DNA in cord blood cultures using absolute quantification techniques. From 158 samples, 9% had the DNA for both organisms. They further found no association in the microbial load of amniotic fluid and umbilical blood.

Furthermore, there was no impact on the short term morbidity in neonates whose cord blood had these organisms(18).

Cheserem, in 1990 did a case control study looking at the role of sexually transmitted diseases in intrauterine fetal death in Kenyatta National Hospital. High vaginal swabs were taken and cultured in special liquid media. The isolation rate for *Mycoplasma hominis* and *Ureaplasma urealyticum* was similar in both the cases as well as controls at 34% and 32%, therefore not showing any significant association of infection with these organisms and intrauterine fetal death.

Shahin et al, in 2003 looked at the presence of *Mycoplasma hominis* and *Ureaplasma urealyticum* among infertile women aged between the 17 and 45 years in Tehran city in Iran, by taking endocervical swabs for DNA extraction then PCR. Of the 377 women sampled, 30.7% were colonized by genital mycoplasmas. *U. urealyticum* was more common than *M. hominis* at 22.5% and 14.8% respectively. Higher prevalence of these organisms have been associated with poverty, higher number of sexual partners as well as oral contraceptive, which was not the case here(32). They went ahead to compare the rates of isolation as well as sensitivities of culture versus PCR. Out of 312 endocervical swabs, 111 were positive for both culture and PCR. 102/111 samples were positive for PCR thus a sensitivity of 91.8%, while culture results were positive in 59/111 samples thus a sensitivity of 53.1%. Therefore PCR is superior in isolating these organisms even when dead unlike standard microbial culture methods(35).

Culture of these organisms takes time, up to five days as well as requiring special culture media and culture conditions. Cunningham et al compared culture versus real time PCR for detecting these organisms in genitourinary specimens of urine and swabs. Mycoplasmas were first cultured in arginine broth while Ureaplasma in U9 broth, at 35 degrees and checked for a color change. This suggested change into alkaline pH. Subsequently, they were sub-cultured in anaerobic environment in A7 agar plates. Fried egg appearance of the colonies confirmed presence of Mycoplasma. Ureaplasma was highly suspected if there were small irregular to circular colonies with a red zone around the colonies.

Addition of 0.167M CO (NH2)2 and MnCl2 in water to cause a golden brown staining on the colonies, confirmed the Ureaplasma spp. DNA extraction was done with special isolation kits. PCR for Detecting Mycoplasma hominis had sensitivity of and a specificity of 90.5% and 99.2% respectively. Ureaplasma PCR detection had a sensitivity and specificity of 96.5% and 93.6%. They found comparable results in both cultures and PCR, however the latter had the advantage of taking a shorter time of 3 hours unlike 5 days with cultures. PCR was superior in detection and species differentiation of the organisms unlike with cultures(36).

The commonest pathogens isolated from infected placentas and amniotic fluid are the *Ureaplasma spp*. The risk of fetal transmission is inversely proportional to the gestation age. Stein et al investigated the presence of genital mycoplasmas (ureaplasma and mycoplasmas) as well as other bacteria and evaluated how they are related to morbidity and mortality of pregnancy, and he concluded that these organisms infect the placenta and cause chorioamnionitis, which is also associated with preterm birth and fetal death. Of the 82 placenta specimens that were cultured, 27% had genital mycoplasmas isolated, and 21% had other bacteria. *Ureaplasma urealyticum* was also the organism mostly isolated among genital mycoplasmas. In the placentae that had these organisms isolated, 69% had histological evidence of chorioamnionitis(17).

Following haematogenous spread of organisms into the placenta, the placental villi are involved in the pathology and thus the focus of histological examination. On the other hand, ascending infections cause pathological changes on the fetal membranes (amnion and chorion) and umbilical cord. The severity of chorioamnionitis depends on the level of maternal leukocytic infiltration, with involvement of the chorion and amnion being the most severe. Histologically, acute and sub-acute chorioamnionitis is represented my maternal neutrophilic infiltration and partly fetal neutrophils. Chronic chorioamnionitis is not common, and is histologically seen with a marked reduction of the inflammatory cells, with only a few lymphocytes. There is associated thickening of the chorioamniotic

membrane as well as fibrosis of the same. The cells that invade the umbilical cord in chronic chorioamnionitis are mainly fetal in origin(37).

2.3 Diagnosis of preterm labor

According to ACOG, preterm labor is diagnosed when there are uterine contractions between 20 and 37 weeks of gestation and which lead to cervical dilatation and effacement. Tocolysis of labor to allow administration of antenatal corticosteroids is advocated in order to reduce neonatal morbidity.

2.4 Role of treatment of mycoplasma and ureaplasma positive cervical/vaginal swabs

Jason et al found that the prevalence of *Mycoplasma genitalium* among the HIV infected women in Johannesburg, South Africa to be 7.4%, with a higher association with low CD 4 counts and high viral loads. They also noted that cervicitis due to this organism is comparable to other bacteria causing sexually transmitted infections(38). There was no reported mutation following treatment with quinolones or macrolides.

Bowes Jr et al, found that for women with intact membranes, there was no role of antibiotics in reducing the incidence of preterm birth. In any case, the antibiotics may mask intrauterine infection and even increase the risk of developing cerebral palsy especially for those who had erythromycin with or without amoxicillin/clavulanic acid(39).

ACOG does not support the use of antibiotics for patients with preterm labor and intact membranes. However in PPROM, antibiotics and antenatal corticosteroids are indicated.

Payne et al in 2016 were able to demonstrate a higher vagina colonization of Ureaplasma spp. esp. U. parvum in the vaginal fluids of preterm births compared to term deliveries at 77 and 36% respectively(40). Kikhney et al were able to isolate Ureaplasma spp. in the fetal membranes and placental tissue of 35.8% preterm births compared to none in the term group, suggesting that the Ureaplasma spp. contribute significantly to chorioamnionitis and preterm delivery(41).

Vouga et al in 2014 in Switzerland analysed the treatment of pregnant women who were presented with symptoms and signs of preterm labor. Vaginal swabs that were cultured positive for these organisms and treated with clindamycin showed reduced rates of preterm delivery, fewer incidences of chorioamnionitis as well as reduced NICU admissions and reduced hospital stay compared to women who had culture positive and no treatment given(31).

2.5 Outcome at day 7 among preterm babies

Tank et al in 2019 audited antibiotic prescribing practices for neonatal sepsis as well as the outcomes in the newborn unit of Kenyatta National Hospital. The perinatal risk factors that were identified included prolonged rupture of membranes, chorioamnionitis, prolonged labour and low birth weight. 320 neonates were recruited into the study, with 160 of them being preterm. Therapeutic doses of penicillin and gentamicin were started upon admission in 96.9% 88% respectively as per the Kenyan guidelines 2016. At 48 hours of admission, the condition of 53.62% was noted to have clinically improved and 12.32% had worsened. At 72 hours out of the 258 who were present, 65.2% improved clinically while deterioration was noted in 12.4%. At this time, blood culture was done only in 3.9% and CRP in 6.2% of the neonates.

By the 7th day of life, the overall mortality was 25%. They noted that mortality was quite high among preterm neonates at 43.8%, with most of the deaths (55%) occurring in the first 48 hours from delivery(42).

2.6 Conceptual framework

2.6.1 Narrative

Ureaplasma spp. and *Mycoplasma hominis* are common commensals of the lower genital tract of women. *M. hominis* is found in about 20-50% of asymptomatic sexually active women while the Ureaplasmas have been identified in 40-80% of women(43).

During pregnancy they cause adverse outcomes including PPROM and preterm labor, chorioamnionitis as well as puerperal sepsis(15). Ascent of these organisms into the uterus or iatrogenic inoculation brings them to the choriodecidual space, chorioamnion plane and finally into the amniotic sac causing chorioamnionitis and FIRS. Inflammatory reaction in both the maternal and fetal compartments leads to generation of chemokines, cytokines, production and release of prostaglandins followed by cervical ripening, rupture of membranes and progression into labor, both at term and preterm gestations. For preterm gestations, there is significant increase in the risk of neonatal morbidity and mortality. FIRS may result in damage to the cerebral white matter which if severe enough can cause cerebral palsy, neuro-developmental delays and neurological deficits. Maternal sequelae include hemorrhage, puerperal sepsis; wound infections and endomyometritis (28)(44)(45).

2.6.2 Diagrammatic Representation

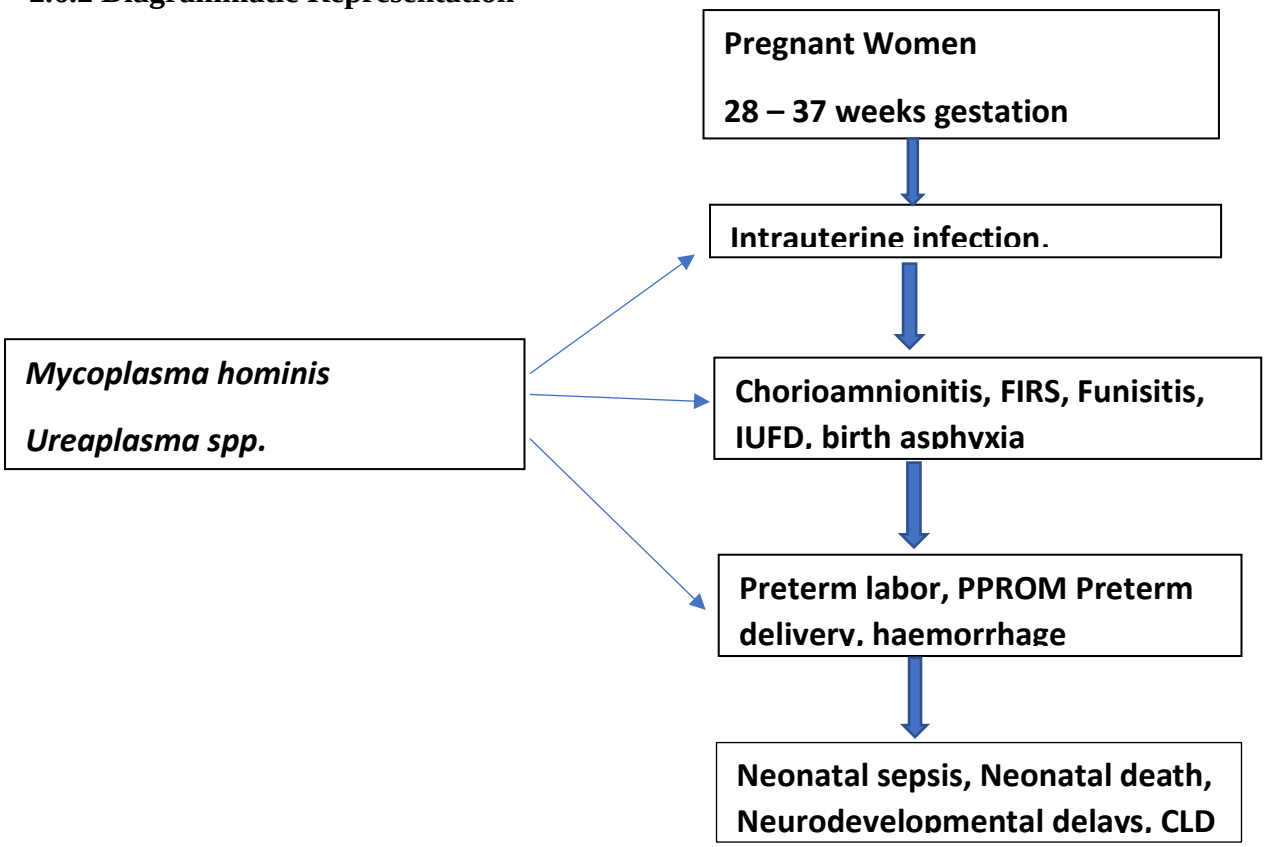

Figure 2: Conceptual framework

2.6.3 Justification of the Study

Preterm birth has for a long time remained a global concern. While the rates of survival have improved with technological advancements, the long term complications and socioeconomic impact has remained the same. Intrauterine infection contributes immensely to preterm labor, PPROM and subsequent preterm birth, with *M. hominis* and *Ureaplasma spp*. being the most commonly isolated organisms. Isolation of the organisms using culture requires special media and takes time, as much as 2 to 5 days. Additionally, culture methods do not distinguish the two species of Ureaplasma. Conversely, PCR offers a rapid diagnostic method with some machines giving results as early as 1 hour from the time of specimen submission to the laboratory as well as species differentiation. This therefore allows timely therapeutic intervention, thus reducing morbidity and mortality.

This study will also bridge the knowledge gap, since there are no local studies done to determine the prevalence of these organisms and the associated neonatal outcomes.

2.7 Research Questions

1. What is the prevalence of *Mycolasma hominis, Ureaplasma urealyticum* and *Ureaplasma parvum* in the placenta following preterm labor and delivery?
2. What are the neonatal outcomes following placental infection with *Mycoplasma hominis, Ureaplasma urealyticum* and *Ureaplasma parvum?*

2.7 Study Objectives

2.7.1 Broad Objective

To determine the prevalence of *Mycoplasma hominis Ureaplasma urealyticum and Ureaplasma parvum* in placentae following preterm labor and delivery and the neonatal outcomes associated with infection with these organisms.

2.7.2 Specific Objectives

1. To determine the prevalence of *M. hominis U. urealyticum and U. parvum* in placentae using polymerase chain reaction (PCR) following preterm labor and delivery at Kenyatta National Hospital.
2. To establish the neonatal outcomes associated with placental infection with *M. hominis U. urealyticum and U. parvum* in the first 7 days of life

CHAPTER 3: METHODOLOGY

3.1 Study design

The study utilized a descriptive cross-sectional study design.

3.2 Study Site and Setting:

This study was conducted at Kenyatta National Hospital, the largest public teaching and referral hospital in Kenya. It is located in Nairobi, the capital city of Kenya, and has a bed capacity of 1800. It attends to an annual average of 70,000 inpatients and 600,000 outpatients. It has a big catchment area with referrals coming from all over the country due to its wide range of specialist and super specialist services. Every month, about 1000 deliveries are conducted within the department of reproductive health; that is in labor ward and maternity theatres. There are 3 antenatal/post-natal wards and 2 maternity theatres.

3.3 Factors that make the site suitable:

1. It is the largest referral facility in the country and thus receives referrals of a large number of patients with preterm labor mainly for newborn care.
2. There exists a pool of specialists in various fields capable of managing preterm labor as well as its complications: obstetricians, paediatricians, neonatologists as well as pathologists and haematologists.
3. The hospital has a new born unit that has a capacity of 70 beds, and a NICU with a capacity of 6 beds.
4. Availability of specialized services including mechanical ventilation of preterm babies exchange transfusion of blood, administration of surfactant for patients with respiratory distress syndrome, parenteral nutrition among others.
5. The site is easily accessible within the capital city of Kenya.

6. Proximity to Kenya Aids Vaccine Initiative institute (KAVI) of clinical research within the College of Health Sciences, University of Nairobi, which has a modern laboratory with ability to do polymerase chain reaction for many organisms.

3.4 Factors that limit the suitability of the site:

1. KNH is still a relatively low resource setting however there is a positive trend towards acquiring modern equipment for its day to day running. The PCR kits will have to be sourced out of the country due to their unavailability locally.
2. Being a large referral hospital, the findings may not represent the statistics of all hospitals in the country

3.5 Study Population

Mothers admitted at Kenyatta National Hospital labor ward with preterm labor and who progressed to deliver before term whether referred from other health facilities or otherwise were included in the study.

3.6 Eligibility criteria

3.6.1 Inclusion Criteria

1. Mothers who developed preterm labor without PPROM, or who develop PPROM during preterm labor or during their antenatal ward admission and progress to deliver between 28weeks 0 days - 36 weeks 6 days by dates or by 1st ultrasound scan
2. Mothers at cervical dilatation equal to or more than 4cm.

3.6.2 Exclusion criteria

1. Planned preterm delivery due to medical disorders in pregnancy e.g pre-eclampsia with severe features, diabetes mellitus among others
2. HIV positive mothers
3. Multiple gestation, polyhydramnios, antepartum haemorrhage

3.7 Sample Size Calculation

Using P=9% as the expected prevalence of Mycoplasma and Ureaplasma, a finding by Marian et al et al (2014) in their study 'Microbial load of umbilical cord blood *Ureaplasma spp.* and *Mycoplasma hominis* in preterm pre-labor rupture of membranes' whereby they studied 158 women with singleton pregnancies at gestations of 24 weeks up to 36 weeks and 6 days and preterm pre-labour rupture of membranes and who progressed to deliver. *Ureaplasma* species and *Mycoplasma hominis* DNA was identified in 9% of the umbilical cord blood samples.

Sample size (N) was calculated using Fischer's formula as shown below

$N=Z\alpha^2\ p\ (1 - p) \div d^2$

Where,

Zα is standard normal variate (at 5% type 1 error ($p<0.05$) it is 1.96, and at 1% type 1 error ($p=<0.01$) it is 2.58). In majority of studies, the p value is considered significant if <0.05. This will be considered thus Zα will be 1.96 in the formula.

P is the expected proportion in the population based on previous studies.

d = acceptable margin of error, will be chosen as 0.05

Sample size $n = \frac{1.96^2 x\ 0.09(1-0.09)}{0.05^2}$

$= 125$

3.8 Sampling Technique:

Consecutive sampling method was employed for those mothers who presented in labor ward with preterm labor without rupture of membranes and those in antenatal wards who develop preterm labor and preterm premature rupture of membranes during the course of their hospital stay and progress into labor and delivery.

They were approached, informed of the study, objectives and significance of the study and requested to participate voluntarily. They were asked to give informed consent. They were further informed that they were free to withdraw from the study at any point without compromising the quality of health care being provided. Those who did not consent to participate in the study were excluded. Eligible participants were enrolled until the desired sample size was achieved.

3.9 Data Variables

The study variables assessed by the study were:

3.9.1 Independent variables:

Placental infections with *Mycoplasma hominis, Ureaplasma urealyticum and Ureaplasma parvum*

3.9.2 Dependent Variable

Preterm labor and delivery, Poor neonatal outcomes including respiratory distress syndrome, neonatal sepsis and neonatal death.

3.10 Data Collection Procedure

Informed consent was obtained, then the participants interviewed using pretested questionnaire to extract bio-data, clinical and reproductive health information. Data collection was done by the principal investigator with the help of research assistants, who were trained on confidentiality and data collection procedure.

Following preterm delivery, the entire placenta was immediately collected into a sterile container and taken to the Kenyatta National Hospital mortuary in a cool box for refrigeration between 2^{o} and 8^{o} Celsius. Using CHAMPS protocol for (minimally invasive tissue sampling MITS) procedure Standard Operating Procedure (SOP) protocol v1.0 September 2016, placental cuts were made within 24 hours of specimen collection and kept in cryovials and frozen at -80^{o} Celsius at KAVI laboratory. The tissues were then thawed and manual DNA extraction done.

Tissue lysing buffer (ALT) and proteinase K was added to the placental tissue and left overnight to disrupt all the cellular components, then a lysing buffer (AL) added for 10 minutes followed by wash 1 and 2. Incubation was done in a water bath at 56^{o}c for 10 minutes prior to centrifuging. We used kit **U.parvum/U.urealyticum/M.hominis Real-TM Quant, a** multiplex Real Time PCR test for the quantitative detection of *Ureaplasma parvum, Ureaplasma urealyticum and Mycoplasma hominis* in the urogenital swabs, urine, prostatic liquid and other biological materials. It has a sensitivity of 100% and a specificity of 100%. It had no cross-reactivity with other organisms (*Gardnerella vaginalis, Lactobacillus spp., Escherichia coli, Staphylococcus spp., Streptococcus spp., Candida albicans, Chlamydia trachomatis, Neisseria gonorrhoeae, Neisseria spp., Mycoplasma genitalium, Trichomonas vaginalis, Treponema pallidum, Toxoplasma gondii, HSV, CMV, HPV*). It was not observed to have any cross-reactivity with other pathogens.

For the neonates admitted in NBU, their files were reviewed to determine the diagnosis on admission, treatment given and outcome at 48 hours and on day 7 of life.

For those preterm neonates who were allowed home, their mothers were contacted through a mobile phone call to establish the wellness of their baby for the first seven days of life.

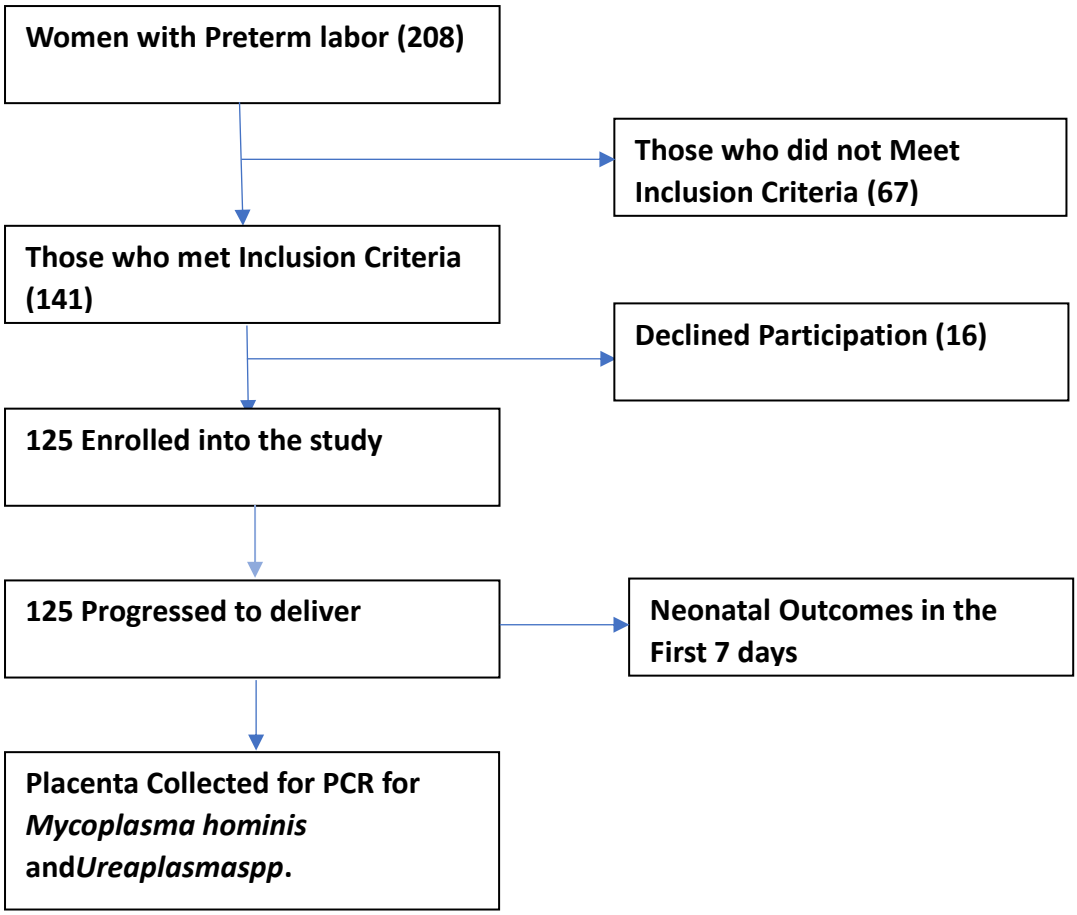

Figure 3: Study flow chart

3.11 Validity and Reliability

The principal investigator trained nurse research assistants to enable them administer the questionnaires as well as get informed consent from the study participants. One other research assistant from the laboratory was recruited to assist in preservation of the placenta and doing the placental cuts.

3.12Data Management and Analysis:

A research assistant was trained on confidentiality, questionnaire administration.

All questionnaires had nothing to directly identify the study participants, instead serial numbers were used. The filled forms were stored in a secure cabinet accessible only to the principal investigator and the research assistants.

Data was checked for completeness prior to entry and analysis done using Statistical Package for Social Sciences, SPSS version 23.

Mean, median and mode were used in describing continuous data that is age, parity, gestational age and presented in tables and pie charts.

Prevalence of *Mycoplasma hominis, Ureaplasma urealyticum and Ureaplasma parvum* was calculated based on the results of PCR, with the number of patients whose placentae had the organisms being divided by the total number of patients with preterm birth.

3.13 Study results Dissemination and Closure

The final results of the study were presented to the department of Obstetrics and Gynaecology and later published into a thesis for filing in the University of Nairobi Library services. The findings were then summarized for publishing and wider dissemination.

3.14 Ethical Considerations

The study proposal was submitted to the Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee (KNH-UoN ERC) for ethical approval before commencing the study. Permission was also sought from the University of Nairobi, Department of Obstetrics & Gynaecology and Kenyatta National Hospital administration before the study was commenced.

Particular ethical considerations included:

3.14.1 Benefits of the study:

1. Patients benefitted from enhanced diagnostic procedure as to the potential cause of preterm birth
2. The use of telephone interviews improved the quality of the study by getting information from post-partum mothers who did continue with follow up at Kenyatta National Hospital

3. The study fostered a continuity of care model of care, providing an example of how patients, particularly those who deliver preterm babies with acceptable birth weight for discharge can be followed up into the community.
4. The study also fostered the use of modern communication technologies in the follow-up of patients in addition to the traditional facility based face to face care model.

3.14.2 Risks of the study:

1. Mobile phone scams are very common in Kenya and use of phone interviews has challenges which may make the study participants feel vulnerable. To mitigate this, the study was thoroughly explained to the patient and consent to obtain information over the phone sought. They were also informed in detail that they would not be required to make any payments and that the interviewer would bear the entire costs of the phone call or any other communication that may be needed. There was also explicit explanation that the interview was not part of a reward or promotional scheme and that they were not required to divulge any details regarding mobile money transactions or passwords and that there was no form of monetary reward or expense from participating in the study.

2. Some of the questions asked were very sensitive and risked causing psychological distress. To avoid this occurrence, research assistants were trained beforehand on how to build rapport, administer the questionnaire humanely respond to patients found to be traumatized. During consent seeking, the sensitive nature of the study was explained to the participants.

3. The participants reserved the right of discontinuing with the study at any stage without any consequences to their quality of care.
4. Confidentiality was maintained by omitting the patients' names or other identifying details from the study. Serial numbers were used.

5. The data collected and generated was stored in password protected computers and remained in the custody of the principal investigator unless otherwise permitted.

3.15 Study Limitations

- There was a delay in transferring the placenta from labor ward to the mortuary for refrigeration at night. To ensure cooling of the placentae, a cool box containing 2 ice packs, was used and the specimen taken for refrigeration very early in the morning.
- Break down of the automatic DNA extraction machine which led us to manual extraction which was very tedious and time consuming.
- PCR is too sensitive and can pick contamination from the lower genital tract as well as non-viable organisms therefore increasing isolation of organisms. This would require correlation with placental histopathology features.

CHAPTER 4: RESULTS

A total of 125 women admitted at KNH labor ward with preterm labor and who qualified for the study were recruited. All the placentae were sampled and tested for the presence of *Mycoplasma hominis, Ureaplasma parvum* and *Ureaplasma urealyticum* using the kit *U. parvum/U. urealyticum/M. hominis* Real-TM Quant.

The respective neonates were followed for early neonatal outcomes up to day 7. 37 of them were admitted to the newborn unit immediately after delivery while the rest were allowed home.

The mean gestation age at delivery was 34.1 weeks (SD 2.3). 18.4% (23/125) mothers delivered through cesarean section while the rest 81.6% (102) had vaginal deliveries.

56% (70) of the newborns were male while 44% (55) female. The average weight at delivery was 2336.2 (SD 541.1) grams.

Table 1: Placental Infection with Mycoplasma hominis and Ureaplasma spp. in preterm births.

	Frequency n (%)	
Organism	**Positive**	**Negative**
Mycoplasma hominis	79 (63.2)	46 (36.8)
Ureaplasma urealyticum	32 (25.6)	93 (74.4)
Ureaplasma parvum	66 (52.8)	59 (47.2)

Placental infection with the all the three organisms was noted in 12.8% of the placentae.

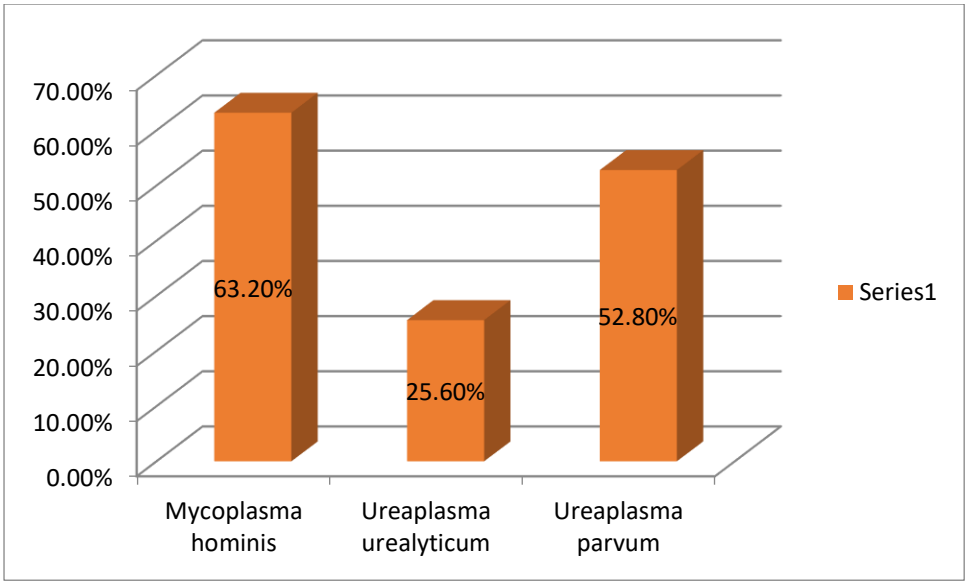

Figure 4: Placental Infection with Mycoplasma hominis and Ureaplasma spp. in preterm births.

NBU admission amongst preterm babies was 29.6%. 64.9% of the admissions were due to respiratory distress with or without low birth weight. Very Low and low birth weight as the only reasons for admission contributed to 16.2%, suspected chorioamnionitis and meconium aspiration with asphyxia accounting for 8.1%, and inability to breastfeed at 2.7%. At 48 hours 51.3% (19/37) of the neonates had shown clinical improvement while 16.2% (6/37) had died. The overall neonatal death rate at day 7 was 24.3% (9/37).

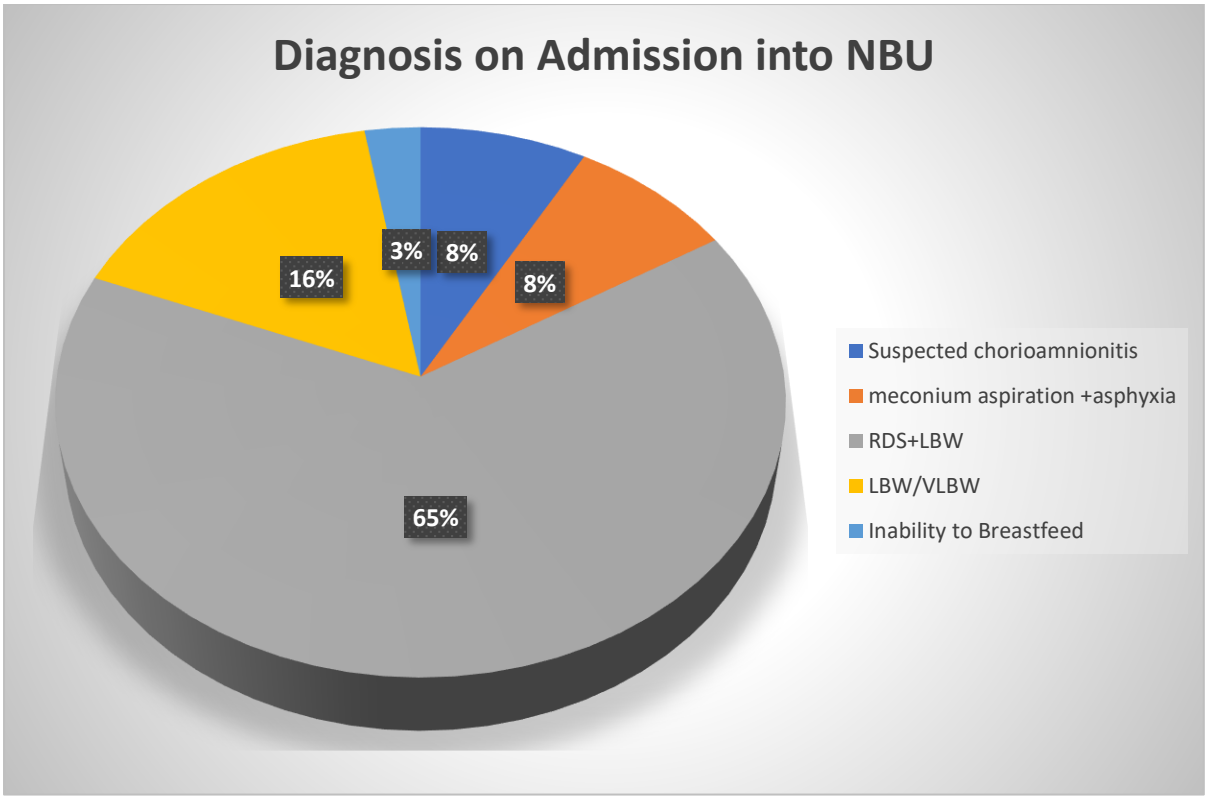

Figure 5: Diagnosis on admission to the newborn unit

All the neonatal deaths were complicated by respiratory distress, with the majority in the very low birth weight category.

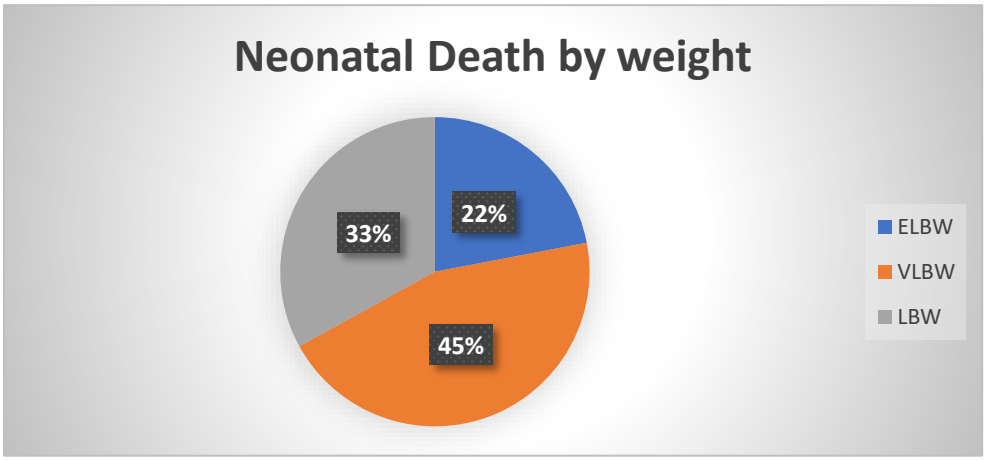

Figure 6: Neonatal death by weight

Table 2: Maternal factors and neonatal outcomes associated with Placental infection with Mycoplasma hominis

Patient Characteristic		Frequency n (%)			
		Positive	**Negative**	**Total**	**p-value**
Maternal Age (Years)	<18	2 (2.5)	0 (0.0)	2 (1.6)	0.277
	18-25	40 (50.6)	22 (47.8)	62 (49.6)	0.762
	26-30	24 (30.4)	12 (26.1)	36 (28.8)	0.609
	31-35	12 (15.2)	5 (10.9)	17 (13.6)	0.497
	>35	1 (1.3)	7 (15.2)	8 (6.4)	0.001
Gravidity	Primigravida	32 (40.5)	17 (37)	49 (39.2)	0.695
	Gravida 2-3	41 (51.9)	22 (47.8)	63 (50.4)	0.661
	Gravida 4-5	5 (6.3)	6 (13)	11 (8.8)	0.201
	Gravida >5	1 (1.3)	1 (2.2)	2 (1.6)	0.696
Gestation Age	<32 weeks	11 (13.9)	7 (15.2)	18 (14.4)	0.843
	>32 to 37	68 (86.1)	39 (84.8)	107 (85.6)	
Neonatal Outcomes					
5th MinAPGAR	<7	5 (6.3)	3 (6.5)	8 (6.4)	0.966
	≥7	74 (93.7)	43 (93.5)	117 (93.6)	
Birth Weight	<1000gm	2 (2.5)	0 (0)	2 (1.6)	0.277
	1000-1499gm	6 (7.6)	5 (10.9)	11 (8.8)	0.533
	1500-2499gm	35 (44.3)	15 (32.6)	50 (40)	0.198
	≥2500gm	36 (45.6)	26 (56.5)	62 (49.6)	0.238
At 48 hrs	Clinically okay	70 (88.6)	37 (80.4)	107 (85.6)	0.209
	Deteriorated	6 (7.6)	6 (13)	12 (9.6)	0.319
	Dead	3 (3.8)	3 (6.5)	6 (4.8)	0.492
At Day 7	Clinically okay	65 (82.3)	37 (80.4)	102 (81.6)	0.798
	Deteriorated	10 (12.7)	4 (8.7)	14 (11.2)	0.550
	Dead	4 (5.1)	5 (10.9)	9 (7.2)	0.687

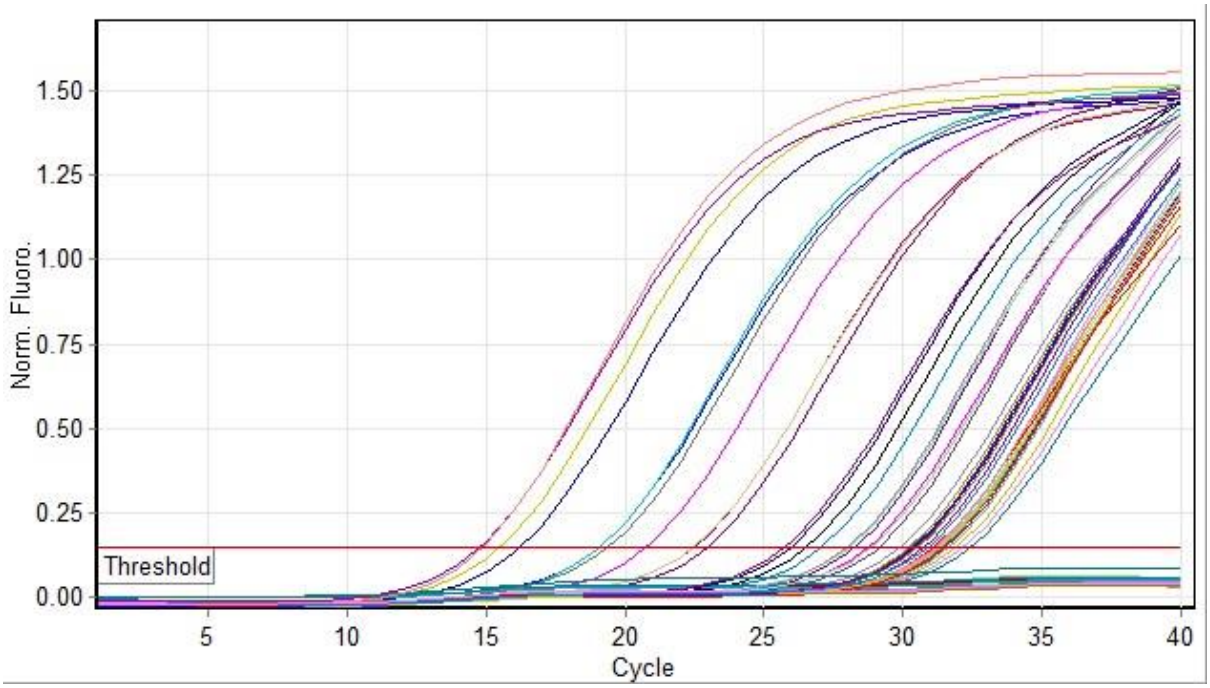

Figure 7: Placental M. hominis PCR curve

Table 3: Maternal factors and neonatal outcomes associated with Placental infection with Ureaplasma urealyticum

Patient Characteristic		Frequency n (%)			
		Positive	**Negative**	**Total**	**p-value**
Maternal Age (Years)	<18	1 (3.1)	1 (1.1)	2 (1.6)	0.425
	18-25	17 (53.1)	45 (48.4)	62 (49.6)	0.644
	26-30	7 (21.9)	29 (31.2)	36 (28.8)	0.316
	31-35	5 (15.6)	12 (12.9)	17 (13.6)	0.698
	>35	2 (6.3)	6 (6.5)	8 (6.4)	0.967
Gravidity	Primigravida	9 (28.1)	40 (43)	49 (39.2)	0.137
	Gravida 2-3	20 (62.5)	43 (46.2)	63 (50.4)	0.112
	Gravida 4-5	2 (6.3)	9 (9.7)	11 (8.8)	0.555
	Gravida >5	1 (3.1)	1 (1.1)	2 (1.6)	0.425
Gestation Age (weeks)	<32	4 (12.5)	14 (15.1)	18 (14.4)	0.723
	>32 to 37	28 (87.5)	79 (84.9)	107 (85.6)	
Neonatal Outcomes					
APGAR IN 5TH Min	<7	2 (6.3)	6 (6.5)	8 (6.4)	0.968
	≥7	30 (93.8)	87 (93.5)	117 (93.6)	
Birth Weight	<1000	1 (3.1)	1 (1.1)	2 (1.6)	0.425
	1000-1499gm	2 (6.3)	9 (9.7)	11 (8.8)	0.555
	1500-2499gm	11 (34.4)	39 (41.9)	50 (40)	0.451
	≥2500gm	18 (56.3)	44 (47.3)	62 (49.6)	0.383
At 48 hours	Clinically okay	27 (84.4)	80 (86)	107 (85.6)	0.819
	Deteriorated	4 (12.5)	8 (8.6)	12 (9.6)	0.519
	Dead	1 (3.1)	5 (5.4)	6 (4.8)	0.607
At Day 7	Clinically okay	26 (81.3)	76 (81.7)	102 (81.6)	0.953
	Deteriorated	5 (15.6)	9 (9.7)	14 (11.2)	0.357
	Dead	1 (3.1)	8 (8.6)	9 (7.2)	0.301

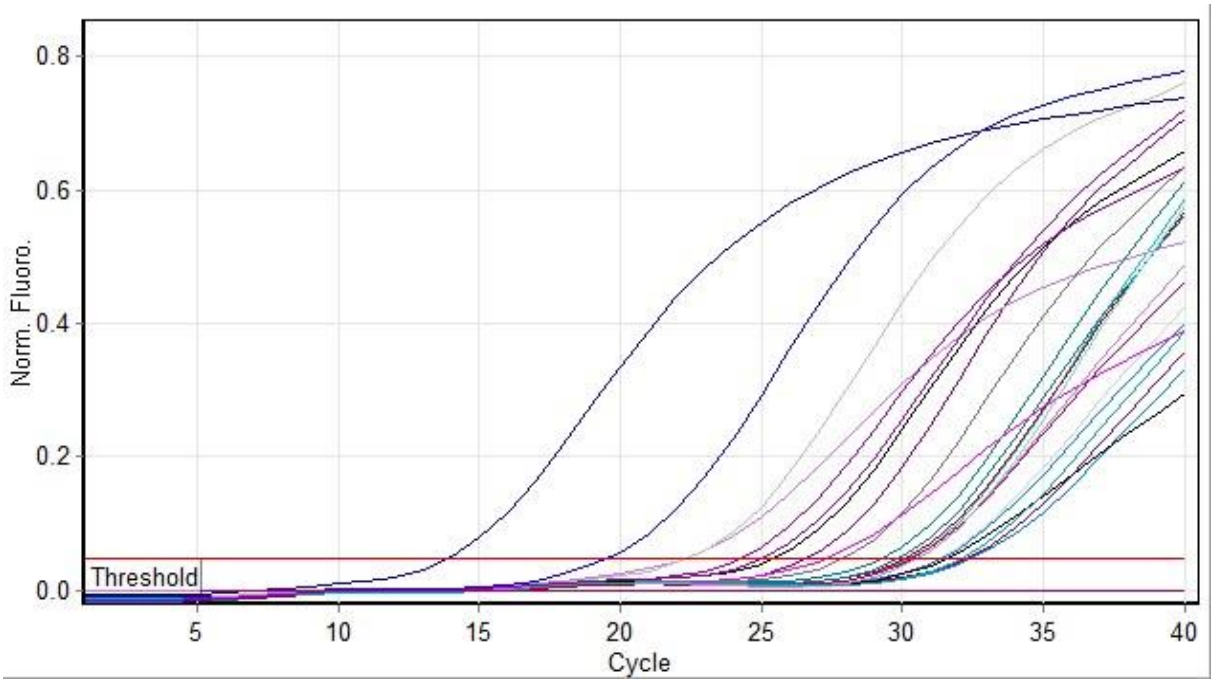

Figure 8: Placental U. urealyticum PCR curve

Table 4: Maternal factors and neonatal outcomes associated with Placental infection with Ureaplasma parvum

Characteristic		Frequency n (%)			
		Positive	**Negative**	**Total**	**p-value**
Maternal Age (Years)	<18	1 (3.1)	1 (1.1)	2 (1.6)	0.425
	18-25	17 (53.1)	45 (48.4)	62 (49.6)	0.644
	26-30	7 (21.9)	29 (31.2)	36 (28.8)	0.316
	31-35	5 (15.6)	12 (12.9)	17 (13.6)	0.698
	>35	2 (6.3)	6 (6.5)	8 (6.4)	0.967
Gravidity	Primigravida	27 (40.9)	21 (36.2)	48 (38.7)	0.592
	Gravida 2-3	34 (51.5)	29 (50)	63 (50.8)	0.866
	Gravida 4-5	4 (6.1)	7 (12.1)	11 (8.9)	0.240
	Gravida >5	1 (1.5)	1 (1.7)	2 (1.6)	0.927
Birth Weight	<1000gm	2 (3)	0 (0)	2 (1.6)	0.181
	1000-1499gm	4 (6.1)	7 (12.1)	11 (8.9)	0.240
	1500-2499gm	28 (42.4)	22 (37.9)	50 (40.3)	0.611
	≥2500gm	32 (48.5)	29 (50)	61 (49.2)	0.866
After 48 hours	Clinically okay	57 (86.4)	49 (84.5)	106 (85.5)	0.767
	Deteriorated	6 (9.1)	6 (10.3)	12 (9.7)	0.814
	Dead	3 (4.5)	3 (5.2)	6 (4.8)	0.871
After 7 days	Clinically okay	54 (81.8)	47 (81)	101 (81.5)	0.911
	Deteriorated	9 (13.6)	5 (8.6)	14 (11.3)	0.379
	Dead	3 (4.5)	6 (10.3)	9 (7.3)	0.214

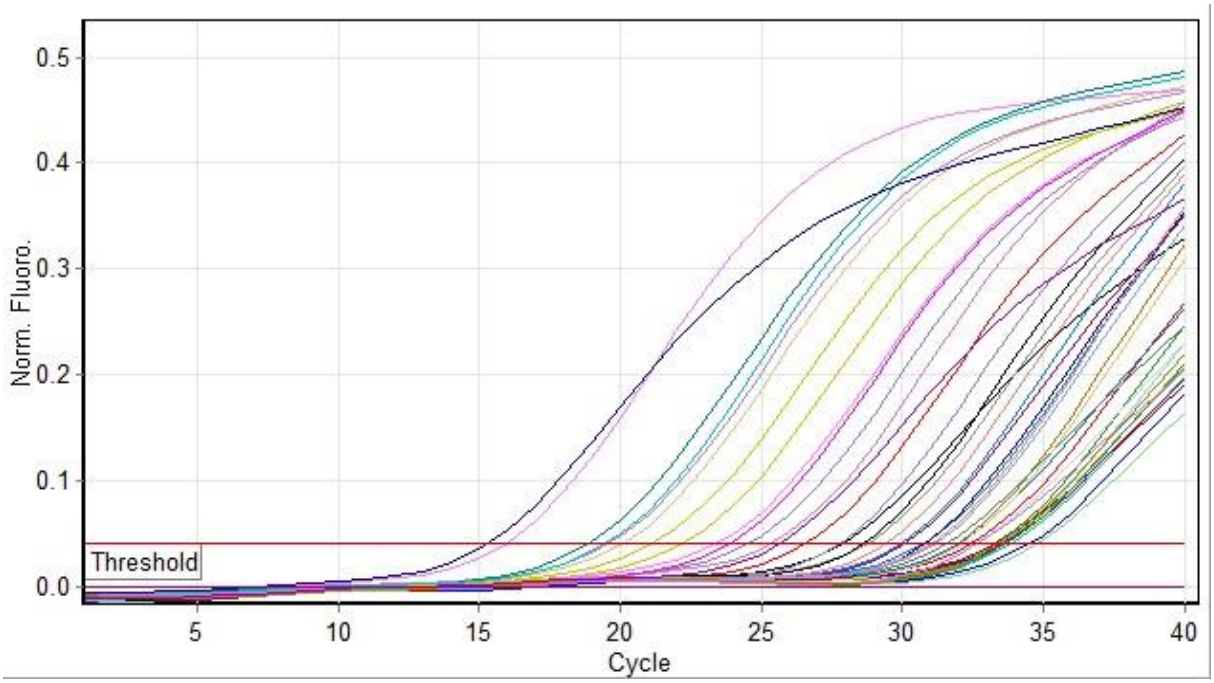

Figure 9: Placental U. parvum PCR curve

CHAPTER 5: DISCUSSION

The prevalence of *Mycoplasma hominis* was found to be 63.3%, that of *Ureaplasma urealyticum* at 25.6% and *Ureaplasma parvum*52.8% in placentae following preterm birth. Infection with the three organisms was found to be present in 12.8% of the placentae. Majority of the placentae had co-infection with *M. hominis* and *U. parvum*. There was no relationship between infection and the mode delivery: vaginal or abdominal delivery.56% (70/125) of the newborns were male while 44% (55/125) female consistent with findings of Post Jamvander (46).

A total of 37 (29.6%) babies delivered preterm were admitted to the newborn unit of Kenyatta National Hospital, with majority (64.9) having respiratory distress syndrome. 24.3% had died at day 7 of life. The main diagnosis associated with death in the first 7 days was very low birth weight in 6 of the 9 deaths as shown in figure6.

There were no significant differences we noted in adverse neonatal outcomes at 48 hours and day 7 of life as a result of placental with the three organisms compared to those without infection.

Clinical improvement within 48 hours was at 51.4% and the mortality rate within 48hours of life.

M. hominis was found to be the most prevalent organism at 63.2% as shown in table 1. This prevalence differs with most other studies which have shown Ureaplasma spp. to be the commonest. This could be possibly due to geographical variations(16)(47). Keskin et al was able to demonstrate the presence of *M. hominis* at 16-21 weeks gestation in amniotic fluid during genetic testing in 72% using culture and 69% real time PCR(48).

Our isolation rate for *U. urealyticum* of 25.6% is comparable with findings of Andrea Stein who found *U. urealyticum* to be the most isolated organism at 24%, and demonstrated chorioamnionitis in 69% of placentae infected with *U. urealyticum*(17).

Dammann et al were able to culture U. urealyticum in 30% (139/464) of placentae of very low birth weight. Histological examinations of the placenta showed significant chorioamnionitis as well as

inflammation of the fetal vessels(47). Kundsin et al in 1996 found 28% placental infection with ureaplasmas and 6% with M. hominis infection(49). These results are partly similar to our findings.

U. parvum as opposed to *U. urealyticum* has been associated with higher incidences of spontaneous preterm labor and delivery, especially if the vaginal colonization is high in the first trimester(40). Fumihiko et al, found the prevalence of *Ureaplasma spp* among preterm deliveries at 42% with higher isolation among lower gestations. Term deliveries had isolation rates of 24%.

Hassanein et al, were able to isolate *U. urealyticum* in 43.3% septic preterm neonates through cord blood PCR. There were also significantly high levels of C-reactive protein and interleukin-6 in the neonates who were positive for *U. urealyticum*. Additionally, the infected neonates were delivered 2 weeks earlier with more males than females as compared to those who were negative for the organism. Overall, there were higher adverse outcomes for those preterm neonates that tested positive(50).

Kacerovsky et al, found no association between an elevated microbial load of *M. hominis* and *Ureaplasma spp.* in the umbilical cord blood and severe early neonatal morbidity in pregnancies that had preterm premature rupture of membranes and subsequent preterm delivery. In the study, there was no association between the microbial load and levels of interleukin 6 production in the neonates. However, there was a notable increase in cases of chorioamnionitis in those with positive *Ureaplasma spp.* and *Mycoplasma hominis* infections(18).

Our findings are also comparable those of Tank et al 2019 at KNH who were auditing antibiotic prescribing practices for neonatal sepsis and measurement of outcome in New Born Unit. They noted that despite use of antibiotic in 97.1% of the neonates, clinical improvement rate at 48 hours was 53.63% while the overall mortality rate was 55% and involving mainly the preterm neonates who contributed 86.4% of all the deaths(42).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion:

Based on the findings of this study, the prevalence of *M. hominis was found to be 63.2%, U. urealyticum 53.2%* and U. parvum 53.2% in placentae from preterm deliveries.

There is no association of placental infection with *M. hominis U. urealyticum* or *U. parvum* on the early neonatal outcomes, instead it is the effect of prematurity that contributes to neonatal morbidity and mortality.

6.2 Recommendations

- Case control or cohort studies are recommended to establish causal relationship of infection with *M. hominis, U. urealyticum* and *U. parvum* and preterm birth
- Studies to screen for these organisms before conception especially in women with a history of preterm delivery and offering targeted treatment.

The studies may inform future practices in an attempt to reduce global preterm deliveries and the attendant neonatal morbidity and mortality.

7.0 Study Timelines

	June 2019	July 2019	Aug 2019	Sept 2019	Oct 2019	Nov 2019	Dec 2019	Jan 2020	Feb 2020	March 2020	April 2020	May 2020
Concept development												
Proposal development												
Proposal Presentation												
Ethical approval												
Data collection												
Data analysis												
Results presentation												

8.0 Budget

Items	**Unit Cost Ksh.**	**Units**	**Total Ksh.**
Research assistant allowances/ administration of questionnaires and consent	30,000	3	90,000
Stationery & Flash drives	1,000	3	3,000
Printing / Photocopy	10	1000	10,000
Binding	500	4	2,000
Communication/ Airtime	5,000	4	20,000
Data analysis/ statistician	30,000	1	30,000
Cool Box	2,000	1	2,000
Cryovials 2.0ml 100/pk	2800	4	11,200
Microtube 2ml (500)2/5	1800	1	1,800
U.parvum/U.urealyticum/M.hominis Real-TM Quant	165,417	2	165,417
Strip tubes & cups, 0.1ml (250)	26,082	1	26,082
Art Barrier Tips 10µl	12,500	1	12,500
KAVI Laboratory Fee	20,000	1	20,000
Miscellaneous	20,000	1	20,000
TOTAL			**413,999**

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ANNEXES

ANNEX 1: LETTER TOERC

Dr Samuel Muriithi Kagema (MBChB)
H58/7122/2017,
P.O.BOX 5486-00200,
NAIROBI.

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. Samuel Muriithi Kagema,
Resident,
Department of Obstetrics and Gynaecology,
College of Health Sciences
University of Nairobi

ANNEX 2: CONSENT EXPLANATION INFORMATION

STUDY TITLE: PREVALENCE OF MYCOPLASMA HOMINIS, UREAPLASMA UREALYTICUM AND UREAPLASMA PARVUM IN PLACENTAE OF MOTHERS WITH PRETERM DELIVERY AND THE ASSOCIATED NEONATAL OUTCOMES AT KENYATTA NATIONAL HOSPITAL.

Investigator

I, DR. SAMUEL M. KAGEMA am a postgraduate student at the University of Nairobi in the department of Obstetrics and Gynaecology. I am conducting the above named study in partial fulfilment for the award of the degree of Master of Medicine in Obstetrics and Gynaecology. My cellphone number is **0732023189, email address sammiekagz@gmail.com, postal address P.O.BOX 5486 – 00200, Nairobi.**

Lead supervisor,

Prof. Eunice Cheserem, Associate professor at the department of Obstetrics and Gynaecology, University of Nairobi. **Contacts: 0722722440.**

Aim of the study

The study aims to determine the prevalence of *Mycoplasma hominis, Ureaplasma urealyticum and Ureaplasma* in placentae following preterm delivery and the associated neonatal outcomes at Kenyatta National Hospital.

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. Following delivery, we shall use the placenta for laboratory tests and examination (PCR for *M. hominis*, *Ureaplasma urealyticum* and Ureaplasma *parvu*m. If your baby is admitted to the new born unit, we will review the file to determine the outcome for the first 7 days of life. If not admitted, we will call you after one week to ask you about the health of your baby.

BENEFITS:

The study participants may not directly benefit from this study, however findings of will be used we hope that the results 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.

COSTS AND PAYMENTS

You will not be charged any money in order to participate in this study. Neither will you be paid. Participation in this study is purely voluntary.

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 will be allowed to participate in the study. In Kenya, Pregnant women below 18 years are legally allowed to give consent. (Emancipated minors)

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.

If you have any questions or would like clarifications, please contact any of the following:

1. **The principal investigator,** Dr. Kagema Samuel Muriithi

Cellphone: **0732023189**/0726294218, P.O.Box5486-00200, Nairobi**.**

Email address**: sammiekagz@gmail.com,**

2. **Lead Supervisor**, Prof. Eunice Cheserem,

University of Nairobi, College of Health Sciences,

Cellphone: **0722722440, P.O.BOX 19676-00202 Nairobi**

Email address**: eunicecheserem@yahoo.com**

3. **The Secretary** KNH-UON Ethics and Review Committee

Tel: 2726300 Ext. 44102

Email: uonknh_erc@uonbi.ac.ke

ANNEX 3: CONSENT FORM.

I ……. do confirm that I have read / been explained to about the study, understood the information presented to me and have had the opportunity to ask questions. I understand that the study is voluntary and that I am free to withdraw from this study at any time without giving reasons. I have not been coerced or enticed in any way in order to participate in this study. I confirm that I have agreed to allow the researchers use the placenta for laboratory tests as well as enquire about the condition of my baby for the first 7 days of life.

Signature of the participant……... Date …….....

Signature of the Investigator / Assistant ……Date…….

1. Dr. Kagema Samuel Muriithi

Department of Obstetrics and Gynaecology, University of Nairobi
Tel. 0732023189/0726294218

2. Professor Eunice Cheserem.

Department of Obstetrics and Gynaecology,
University of Nairobi, P.O. Box 19676 - 00202
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5. Dr. Edwin Walong

Department of Human Pathology,
University of Nairobi, P.O. Box 19676 - 00202
Tel: 0738590623

6. The Secretary KNH-UON Ethics and Review Committee

Tel: 2726300 Ext. 44102
Email: uonknh_erc@uonbi.ac.ke

ANNEX 4: MAELEZO YA IDHINI

STUDY TITLE: PREVALENCE OF MYCOPLASMA HOMINIS, UREAPLASMA UREALYTICUM AND UREAPLASMA PARVUM IN PLACENTAE OF MOTHERS WITH PRETERM DELIVERY AND THE ASSOCIATED NEONATAL OUTCOMES AT KENYATTA NATIONAL HOSPITAL.

Mtafiti,

Mimi DR. SAMUEL M. KAGEMA ni mwanafunzi wa uzamili katika chuo kikuu cha Nairobi kwenye idara ya afya na magonjwa ya wanawake. Ninafanya utafiti uliotajwa hapo juu kama sehemu ya kutimiza matakwa ya kupata shahada kuu ya somo la udaktari la afya ya akina mama na magonjwa ya wanawake. Nambari yangu ya simu ni 0732023189, barua pepe sammiekagz@gmail.com, sanduku la posta P.O.Box 5486 – 00200 Nairobi.

Msimamizi mkuu: Prof. Eunice Cheserem, Associate professor at the department of Obstetrics and Gynaecology, University of Nairobi. **Nambari ya simu: 0722722440.**

LENGO LA UTAFITI:

Lengo letu ni kuweza kueleza mtawanyiko wa bakteria za aina ya *Mycoplasma hominis, Ureaplasma urealyticum na Ureaplasma parvum* katika akina mama ambao hupata uchungu na kujifungua kabla siku za kujifungua kufika na matokeo yanayoambatana na watoto wanaozaliwa.

JINSI YA KUFANYA UTAFITI

Utaulizwa maswali machache kuhusu umri wako, idadi ya watoto na idadi ya mimba ambayo umewahi shika, kiwango cha elimu, kazi yako, kuhusu mimba iliyotangulia na mimba uliyo nayo sasa, na kwenda kwako kwa kliniki ya wajawazito, uvutaji wa sigara, matumizi ya dawa zozote, matibabu uliyopewa kabla ya kujifungua, dalili zinazoambatana kama vile maji ya mtoto kupasuka. Kasha tutaangalia kadi yako ya kliniki kutambua kama matukio ya hapo awali yanakuhatarisha kupata uchungu na kujifunguaa mapema. Bada ya kujifungua, tutatumia kondo la nyuma / plasenta kufanya

vipimo katika maabara. Kisha tutafuatilia mtoto wako kwa wiki moja ili kutambua kama atapata shida yoyote ya kiafya. Kama mtoto atalazwa katika chumba cha watoto wachanga, tutatumia faili kupata maarifa. Kama atapewa ruhusa kwenda nyumbani kabla ya wiki moja kuisha, tutakupigia simu ili utueleze jinsi mtoto atavyokuwa kwa wiki ya kwanza tangu kuzaliwa.

FAIDA

Wanaoshiriki katika utafiti huu wanaweza kosa kupata faida ya moja kwa moja. Hata hivyo, matokeo ya utafiti huu yatasaidia kutoa mwelekeo katika matumizi ya dawa za kuua viini hivi kwa wale kina mama ambao hujifungua kabla ya siku kuwadia, na pia kwa wanao wanapolazwa katika chumba cha watoto wachanga.

KUSHIRIKISHWA KATIKA UTAFITI NA IDHINI

Mwenye kufanya utafiti pamoja na wasaidizi wake watakuelezea jinsi utafiti utafanywa, watakupatia maelezo yaliyoandikwa kwa wakati unaofaa na kupata itikio kutoka kwako kabla ya kuanza utafiti.

HATARI YA UTAFITI

Utafiti huu hautakuweka wewe ama mwanao katika hatari yoyote kwani tutatumia kondo la nyuma / plasenta katika uchunguzi wetu. Utapatiwa uangalizi wa afya yako kikamilifu ambayo mama anayejifungua anastahili.

GHARAMA NA MALIPO

Hakuna malipo yoyote ambayo utaitishwa kwa kuhusika katika utafiti huu. Pia, hakuna malipo yoyote ambayo utapata kwa kushiriki. Kuhusika ni kwa hiari yako.

USIRI

Jina lako, nambari ya hospitali ama alama zozote ambazo zinaweza kukutambulisha hazitayumika kamwe. Badala yake, namba tambulishi ndiyo itakayotumika. Habari ambayo tutapata kutoka kwa orodha ya maswali itatumiwa na mchunguzi na wasaidizi wake pekee.

Wasichana Wadogo

Wale wote ambao watakuwa na mimba wataruhusiwa kujiunga na utafiti huu. Nchini Kenya, wajawazito walio chini ya miaka 18 wameruhusiwa na sheria kupatiana idhini.

KUSHIRIKI NA KUJIONDOA KATIKA UTAFITI

Kushiriki kwako katika utafiti huu ni kwa hiari yako. Hutalazimishwa kujibu maswali ambayo yatakuondolea starehe. Unaweza jiondoa kutoka utafiti huu wakati wowote bila kuathiri jinsi ya kuhudumiwa kiafya unavyostahili. Hakuna haja kufuatilia nasi baada ya kushiriki katika utafiti huu. Lakini utashauriwa kufuatilia kliniki za wale ambao wamejifungua.

SHIDA AU MASWALI:

Ikiwa una swali lolote ama kutaka kufafanuliwa chochote kuhusu huu utafiti, wasiliana na mmoja wa hawa:

1. Mtafiti mkuu **Dr. Samuel Muriithi Kagema**

   Sanduku la posta **5486-00200, Nairobi,** Nambari ya simu **0732023189**/0726294218

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

Au **2**. msimamizi mkuu **Prof. Eunice Cheserem,**

University of Nairobi, College of Health Sciences,

P.O.BOX 19676-00202 Nairobi, Nambari ya simu **0722722440,**

Barua pepe**: eunicecheserem@yahoo.com**

Au3. Karani KNH-UON Ethics and Review Committee

Nambari ya simu: **2726300 Ext. 44102**

Barua pepe: uonknh_erc@uonbi.ac.ke

KAULI YA ITIKIO NA SAHIHI:

Mimi …….. nathibitisha ya kwamba nimesoma / nimeelezwa kuhusu utafiti huu, nimeelewa na nimepata fursa ya kuuliza maswali. Naelewa kuwa kushiriki ni kwa hiari yangu na kwamba niko huru kujiondoa kwenye utafiti wakati wowote bila kutoa sababu yoyote. Sijalazimishwa wala kushawishiwa kwa njia yoyote ile ili kushiriki utafiti huu. Nathibitisha kwamba nimekubali watafiti kuchukua kondo la nyuma (placenta) kufanya vipimo vya kimaabara na pia kuchunguza hali ya mtoto itakavyokuwa siku za kwanza saba za maisha yake.

Sahihi ya mshirika ……..... Tarehe ……...

Sahihi ya mchunguzi …..…….. Tarehe ……

1. Dr. Kagema Samuel Muriithi

Department of Obstetrics and Gynaecology, University of Nairobi

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6. The Secretary KNH-UON Ethics and Review Committee

Tel: **2726300 Ext. 44102**

Email: uonknh_erc@uonbi.ac.ke

ANNEX 5: QUESTIONNAIRE

Serial number …….. Cellphone number ……

1. **Section A: Sociodemographic Data**

Age (Years) …… Parity …… Gravidity…….

Gestation by dates (LNMP) ……

Gestation by 1st ultrasound……. Date u/s done…….

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 ☐ Married ☐ Separated ☐

Divorced ☐ Widowed ☐

d) What is your occupation? ……

e) How many sexual partners have you had to date……

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 haemorrhage Yes ◯ No ◯

Post-partum haemorrhage 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

IV..…… 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) Where were you when membranes ruptured: At home ☐ In hospital ☐

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

Yes ☐ No ☐

i) Have you consumed alcohol in the current pregnancy?

Yes ☐ No ☐

j) If yes, what kind of alcoholic drink do you take?..... How many times in a week…… How many glasses/bottles per drinking session……..

k) 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……..

QUESTIONNAIRE: KISWAHILI VERSION.

NAMBARI MFULULIZO……

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

Umri (Miaka)…… Nambari ya mimba……..

Umri wa mimba kwa wiki kulingana na siku ya kwanza ya damu ya mwezi ya mwisho?.....

Mimba ina wiki ngapi kulingana na picha ya kwanza?..... Siku picha ilifanywa ……..

a) Kiwango cha Elimu

Hakuna ⬭

Shule ya Msingi ⬭

Shule ya Upili ⬭

Chuo Kikuu/Kolejia ⬭

b) Dini

Mkristo ⬭

Muislamu ⬭

Kihindi ⬭

Dini aina nyingine……

c) Hali ya ndoa

Hujaolewa ⬭ Umeolewa ⬭ Umetalakiwa ⬭

Umetengana na Mume wako ⬭ Mjane ⬭

d) Ni kazi gani unayoifanya? ……..

e) Umelala na wanaume wangapi maishani yako? …….

2. Historia ya Mimba

a) Ulijifungua mwisho lini?.....

b) Umewahi pasuliwa ukijifungua? Ndio ⬭ La ⬭

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

c) Ulipata matatizo wakati ulizaa mwisho?

Shinikizo la damu Ndio ⬭ La ⬭

Kisukari Ndio ⬭ ⬭

Kuvuja damu ukiwa mja mzito Ndio ⬭ La ⬭

Kuvuja damu baada ya kujifungua Ndio ⬭ La ⬭

Maambukizi (kuwa maalum) Ndio ⬭ La ⬭

d) Umewahi tibiwa uchungu wa mwana kabla ya siku kufika? Ndio ⬭ La ⬭

Kama ni ndio, matokeo yalikuwa gani?

Alikuwa ameaga ⬭

Kuzaliwa mapema ⬭

Kuzaliwa akiwa amefikisha siku ⬭

3. Mimba unayobeba

a) Umekuwa ukienda kliniki ya wajawazito? Ndio ⬭ La ⬭

Kama ni ndio, kituo ni cha kiwango gani?

Zahanati ⬭

Hospitali Kaunti ndogo ⬭

⬭

Hospitali ya Kaunti

Hospitali ya Rufaa

b) Umeenda kliniki mara ngapi?.....

c) Umepimwa 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……

d. Umeanzishwa dawa aina yoyote katika hii mimba? Ndio La

Kama ni ndio, ni dawa gani ulipewa…….

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?..... Kama ndio, tafadhali fafanua.....

Umedungwa sindano za kufanya mapafu ya mtoto kukomaa?Ndio La

h. Ulikuwa wapi wakati maji ya mtoto yalipopasuka? Hospitalini Nyumbani

i) Umetumia pombe kwa hii mimba? Ndio La

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

Chupa/glasi ngapi kwa kila kikao…….

k) Umevuta sigara ukiwa na hii mimba? Ndio La

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

Kwa wale watoto waliozaliwa mapema na hawakulazwa sehemu ya watoto wachanga

1. Jinsia ya mtoto ni gani? .....
2. Mtoto alikuwa na uzito wa kilo ngapi alipozaliwa? .....
3. Ulipewa ruhusa kwenda nyumbani baada ya siku ngapi? ……
4. Je, mtoto wako amepata shida zifuatazo:
5. Kuendesha ama kutapika Ndio ☐ La ☐
6. Joto jingi kwa mwili? Ndio ☐ La ☐

a. Macho kubadilika rangi kuwa ya manjano? Ndio ☐ La ☐

a. Shida ya kupumua ama kupumua kwa haraka? Ndio ☐ La ☐

b. Kukataa kunyonya? Ndio ☐ La ☐

c. Kuwashwa haraka ama kulia Zaidi? Ndio ☐ La ☐

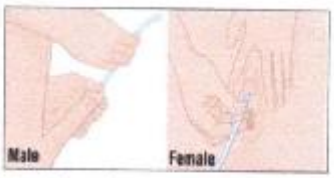
Kama ni ndio kwa swali lolote hapo juu, ulitafuta matibabu yoyote? Ndio…….. La……

Kama ulitafuta matibabu, ulielezwa mtoto alikuwa na ugonjwa gani?

Mtoto alilazwa hospitali? Ndio …… La ……..

ANNEX 6: Champs Protocol for specimen collection

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 7: LABORATORY REQUEST FORM 1: PLACENTA FOR PCR STUDY

STUDY TITLE: PREVALENCE OF MYCOPLASMA HOMINIS, UREAPLASMA UREALYTICUM AND UREAPLASMA PARVUM IN PLACENTAE OF MOTHERS WITH PRETERM DELIVERY AND THE ASSOCIATED NEONATAL OUTCOMES AT KENYATTA NATIONAL HOSPITAL.

Patient identification number:

Age:

LNMP:

Gestational age at delivery:

Parity:

Baby admitted to NBU Yes ◯ No ◯

Clinical summary and diagnosis:

……

……

Mode of delivery: SVD ◯ Cesarean section ◯

Date collected: ……..

Collected by: (Name and signature)……

Investigation required: PCR for *M. hominis* and *U. urealyticum* and *U. parvum*.

Requesting clinician:…….…. Signature…….

ANNEX 7: NEONATAL OUTCOME DATA COLLECTION SHEET

PREVALENCE OF MYCOPLASMA HOMINIS, UREAPLASMA UREALYTICUM AND UREAPLASMA PARVUM IN PLACENTAE OF MOTHERS WITH PRETERM DELIVERY AND THE ASSOCIATED NEONATAL OUTCOMES AT KENYATTA NATIONAL HOSPITAL

Patient identification number:

Date of Delivery ……. Date of admission to NBU …….

APGAR Score ……

Birth Weight ……..

Diagnosis upon admission in the NBU ……

Outcome after 48 hours

Clinically okay ◯

Deteriorated ◯

Dead ◯

Discharged home ◯

Outcome at day 7

Clinically okay ◯

Deteriorated ◯

Dead ◯

Discharged home ◯