Article published online: 2022-07-14

Original Article

Identification of Microorganisms associated with intraamniotic infection among women with preterm birth at Ruhengeri Referral Hospital, Rwanda: A case control study

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Abstract

Background: Preterm birth is a global public health threat for maternal and child health. Each year, 15 million neonates are born preterm worldwide, with 40% resulting from intrauterine infections. **Materials and Methods:** This cross-sectional and case–control study was conducted from October to February 2019 at Ruhengeri Referral Hospital. A total of 120 swab samples were collected from 40 women, of which 20 were full-term delivery, while the other 20 were preterm delivery. The three samples, including the placental membranes, amniotic fluids, and fetal membranes, were collected immediately after birth. A sterile cotton swab was used to collect the samples and put into swab Stuart sterile plastic container to avoid sample contamination. Samples were transported in a tightly covered carrier to the clinical microbiology laboratory at INES Ruhengeri for microbiological investigation. Gram staining, culture, and biochemical tests were performed. The independent *t*-test was used to test for significant differences between the means of the two groups, while the Chi-square test (χ^2) was used to test for significant association with microorganisms and intra-amniotic infections. **Results:** A half of the participants were in the age range of 24–29 years. Non-albicans candida (32.7%) and mold (27.9%) were the predominant microorganisms isolated. Non-albicans candida and mold were common to preterm and full-term samples. *Staphylococcus* species were observed in placental and fetal membrane samples. *Escherichia coli, Klebsiella*

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Submitted: 18-Aug-2021 **Revised:** 11-Sep-2021
Accepted: 11-Sep-2021 **Published:** 16-Dec-2021

How to cite this article: Yadufashije C, Umugwaneza J, Muhimpundu L, Izere C, Munyeshyaka E, Mala AO, *et al*. Identification of Microorganisms associated with intraamniotic infection among women with preterm birth at Ruhengeri Referral Hospital, Rwanda: A case control study. Ibnosina J Med Biomed Sci 2021;13:196-203.

Access this article online

Quick Response Code:

Website: www.ijmbs.org

DOI: 10.4103/ijmbs.ijmbs_64_21

species, *Streptococcus* species, and *Candida albicans* were observed among women with preterm birth samples. There was a statistically significant difference between the two means in the amniotic fluid isolates ($t = 4.023$, $P < 0.007$), placental membrane isolates ($t = 7.17$, $P < 0.0004$), and fetal membrane isolates ($t = 6.7$, $P < 0.0006$). Association with microorganisms and intra-amniotic infection was statistically significant with *E. coli* ($\chi^2 = 3.98$, $P < 0.05$), *Streptococcus* species ($\chi^2 = 5.53$, $P < 0.019$), non-albicans candida ($\chi^2 = 8.37$, $P < 0.004$), and *C. albicans* ($\chi^2 = 3.98$, $P < 0.05$). **Conclusions:** Invasion of the amniotic fluid, placenta, and fetal membranes by pathogenic microorganisms may be associated with the incidence of preterm labor and birth. Early diagnosis is recommended to avoid both maternal and fetal complications.

Keywords: Chorioamnionitis, intra-amniotic infection, microorganisms, uterus

Introduction

Preterm birth is a birth that comes earlier than 37 weeks of gestation. Existing data shows that every year, 15 million babies are born preterm around the world. Preterm delivery is the leading cause of neonatal death in the developed world.[1] However, the burden of preterm birth in sub-Saharan Africa and Asia accounts for half of the world's total birth annually. More than 60% of preterm births are found in developing countries (sub-Saharan Africa and Asia), and above 80% of global neonatal deaths are attributed to the complications of preterm delivery.[2] The health consequences of preterm delivery commonly occur in neonatal periods but can be long-lasting across life. Spontaneous development of the fetus or medical intervention provided for pregnancy could be the leading cause of preterm birth to women.[3] The specific known factors of spontaneous preterm birth include infections, placental abruption, hormonal disorders, and multiple gestations. Intrauterine infection (intra-amniotic infection) accounts for 40% of preterm deliveries globally.[4] It has been challenging to determine whether infections cause preterm birth in women, but evidence shows that infection in the uterus could cause preterm birth. Inflammatory diseases on gestational tissues caused by bacterial, fungal, or viral infections are the major causes of preterm birth.[5] This is evidenced by a relatively higher rate of microbial colonization and inflammatory conditions in the intra-amniotic fluid of preterm labor patients than in full-term births.[6]

Rupture of the membrane (ROM) happens either during or at the beginning of labor. Before the onset of labor begins, it is considered premature rupture of membrane (PROM).[7] On the other hand, when the ROM happens before 37 weeks, it is known as preterm PROM (PPROM), which is a sign of intra-amniotic infections in some cases.[8] Bacterial intrauterine infections are the major cause of preterm birth-related infections. The amniotic cavity is considered to be sterile and free from infections. However, <1% of women in preterm labor have bacteria in their amniotic fluids.[9] It has been challenging to isolate bacteria from amniotic fluids before birth because of the risk posed by secondary complications from pregnancy. Most intra-amniotic microbial colonization is asymptomatic and cannot be diagnosed without a microbiological analysis of amniotic fluid.[10] It has been estimated that 12.8% of positive amniotic fluid culture occurs in women with premature labor with an intact membrane. About 22% of positive amniotic fluid samples were reported in women who have experienced premature labor with the intact membrane and preterm birth.[11] In the same report, the rate of positive amniotic fluids was 32.4% among women experiencing PPROM. During labor, more than 75% of these women suffer from the microbial invasion of the amniotic cavity (MIAC) due to the ruptured membrane, which plays a protective role of cushioning the baby from ascending infections from the cervix.[12] Women with MIAC are at high risk of delivering a preterm baby and developing clinical chorioamnionitis.[13] Recent findings reported that the rate of preterm birth in Rwanda stood at 10%,

despite efforts by the Government of Rwanda to mitigate the same.[14] However, there are limited studies on intra-amniotic-related infections and their influence on preterm birth. This study, therefore, investigated significant microorganisms that contribute to intra-amniotic fluid invasion among women with preterm birth at Ruhengeri Referral Hospital.

Materials and Methods

Study population

A total of forty women were selected for this study. Cases consisted of women with preterm birth, and controls were selected from women with full-term birth. Nonprobability purposive sampling was used to select twenty women from preterm and full-term birth participants to recruit the women with preterm birth, and Simple Random Sampling (SRS) was used for women without preterm birth. All pregnant women suspected to have chorioamnionitis at birth were included. Pregnant women without chorioamnionitis and who did not face any pregnancy-related complications were recruited as a control group. We excluded women with serious obstetrical complications, where they have symptoms of chorioamnionitis or not.

Sample processing and collection

We aimed to compare the presence of microorganisms and intra-amniotic infection between cases and controls. Swab samples were collected from amniotic fluid, placental, and fetal membrane in maternity service. There were three samples collected from each woman. Samples were collected immediately after delivery in the regional referral hospital, Musanze. This was done using a sterile cotton swab and put in sterile specimen bags (Stuart plastic) to avoid sample contamination. Samples were transported to INES Ruhengeri clinical microbiology laboratory for microbiological analysis. Microbiological analysis techniques including Gram staining, culture, and biomedical tests were performed to isolate and identify microorganisms in amniotic fluid, placental membrane, and fetal membrane samples.

Microbiological methods

Gram stains (HiMedia® Ref K001-1KT) were performed to classify and differentiate the bacteria into Gram-positive and Gram-negative microorganisms. After smear preparation and application of different dyes, the slides were examined with the aid of light microscopy. Gram-positive microorganisms retain the primary dye crystal violet, and Gram-negative microorganisms take the color of the counterstain Safranin O. The blood agar (Fluka® Ref 70133-500G) and MacConkey agar (HiMedia® Ref M081-500G) culture media were used to isolate bacteria from the placental, fetal membrane, and amniotic fluid samples. This was performed based on Gram stain results. The samples were inoculated into prepared plates with solidifying culture media, and the inoculated plates were incubated at 37°C for 24 h. Therefore, the plates with growth colonies were morphologically observed, and biochemical tests were performed.

Sabouraud dextrose agar (TM Media Ref TM 387) selective media for yeast and molds was used. Prepared Sabouraud agar plates were inoculated by streaking, as with standard bacteriological media. Molds were typically incubated at room temperature (22°–25°C for 2–5 days), and yeasts were incubated at 37°C for 24 h. The growing yeast colonies (creamy whitish) were confirmed by germ tube to differentiate *Candida albicans* and *Candida* other species, whereas molds grew as filamentous colonies of various colors were identified morphologically and by using lactophenol cotton blue stain.

Biochemical tests

Different biochemical tests were performed to differentiate bacterial species. The catalase (Faholo B/No: 32017 FHP) and coagulase (HiMedia® Ref FD248-5VL) tests were used to differentiate *Staphylococcus* species and *Streptococcus* species Gram-positive bacteria. For the identification of Gram-negative bacteria isolates, different culture media were used to test different biochemical parameters. Simmons' citrate agar (HiMedia® Ref M099-500G) was used to test the microorganism's ability to utilize citrate as a source of energy. Motility was confirmed when a wide filament-like form appeared in the SIM medium (HiMedia® Ref M181-500G). An appearance of a red ring like into its surface after adding two to three drops of Kovac's

reagent (HiMedia® Ref R008-100ML) after 24 h of incubation indicates indole positive. Urease positive was proved by a color change in pink for urea broth (HiMedia® Ref M111-500G). Furthermore, Kligler iron agar (HiMedia® Ref M078-500G) permitted differentiation of Gram-negative bacilli by their ability to ferment glucose or lactose was also used. Red color changes from yellow due to the PH indicator in response to acid production of the fermentation of the sugar.

Statistical analysis

We analyzed the significant difference in the mean between the two groups and the associations with isolated microorganisms and intra-amniotic infection among women with preterm delivery. SPSS version 22 (IBM Company located in New York, USA) was used for data analysis. Both *t*-test and Chi-square tests were carried out to test for statistical associations. The level of significance was $\alpha = 0.05$.

Results

Age distribution of the study participants

Table 1 shows the age distribution of the study population. Fifty percent of the participants in both preterm and full-term women were in the age range of 24–29 years, and 20% were aged 30–35 years. Preterm women were 15%, while full-term women were 10% in the age range of 36–41 years. Fifteen percent of the preterm women compared with 20% full-term women were less likely to be aged 18–23 years.

Microorganisms isolated from amniotic fluid

Table 2 shows microorganisms isolated from amniotic fluids for both women with preterm and full-term birth. The predominantly isolated microorganisms among women with preterm birth were mold (28.8%) and non-albicans candida (28.8%). The non-albicans candida (66.7%) and molds (33.3%) were also more prevalent among women with full-term birth than the preterm births. *Escherichia coli* (9.6%), *Klebsiella* species (3.8%), *Streptococcus* species (13.4%), *Staphylococcus* species (5.7%), and *C. albicans* (9.6%) were only isolated among women with preterm birth. The difference between the means of the two groups was statistically significant ($t = 4.023$, $P < 0.007$).

Microorganisms isolated from placental membrane samples

Table 3 shows isolated microorganisms from the placental membrane samples among women with preterm and full-term birth. Isolated microorganisms from placental membrane samples collected from women with preterm birth were predominated by mold (25.8%) and non-albicans candida (25.8%); the same conditions were observed among women with full-term birth where the predominant microorganisms were non-albicans candida (50%) and mold (30%). *Staphylococcus* species were also isolated from women with full-term birth and stood at 20%. Other microorganisms were isolated from women with preterm delivery including *Staphylococcus* species (13.7%), *Streptococcus* species (12%), *C. albicans* (8.6%), *E. coli* (5.6%), and *Klebsiella* species (5.2%). The mean difference ($t = 7.17$, $P < 0.001$) of the two groups was statistically significant.

Microorganisms isolated from fetal membrane samples

Table 4 reveals microorganisms isolated from fetal membrane samples of women with preterm and full-term delivery. Mold (26.3%) and non-albicans candida (26.3%) were the isolated predominant microorganisms among women with preterm birth. These microorganisms also were predominant among women with term birth and stood at 33.3% and 55.5%, respectively. *Staphylococcus* species were observed among preterm and full-term birth next to mold and non-albicans candida with 14% and 11.1%, respectively. *Streptococcus* species (12%), *C. albicans* (8.7%), *E. coli* (8.7%), and *Klebsiella* species (3.5%) were only observed among women with preterm delivery. The difference between

Table 1: Age distribution of the study participants

Age group	Preterm women (%)	Full-term women (%)	Total (%)
18-23	3 (15)	4 (20)	7 (17.5)
24-29	10 (50)	10 (50)	20 (50)
30-35	4 (20)	4 (20)	8 (20)
36-41	3 (15)	2 (10)	5 (12.5)
Total	20 (100)	20 (100)	40 (100)

Table 2: Comparative profiles of microorganisms isolated from amniotic fluid

Microorganism	Women with PTB (%)	Women without PTB (%)	SD	Independent *t*-test	df	*P*
Escherichia coli	5 (9.6)	0				
Klebsiella species	2 (3.8)	0				
Streptococcus species	7 (13.4)	0				
Staphylococcus species	3 (5.7)	0				
Mold	15 (28.8)	2 (33.3)				
Non-albicans candida	15 (28.8)	4 (66.7)				
Candida albicans	5 (9.6)	0				
Total	52 (100)	6 (100)	4.0773	4.023	6	0.006522

$\sum D=46$, MD=6.5, $\sum(D\text{-}MD)^2=99.75$, SE=1.541. PTB: Preterm birth, SD: Standard deviation, SE: Standard error

Table 3: Comparison of microorganisms isolated from placental membrane samples

Microorganisms	Women with PTB	Women without PTB	SD	Independent *t*-test	df	*P*
Escherichia coli	5 (8.6)	0				
Klebsiella species	3 (5.2)	0				
Streptococcus species	7 (12)	0				
Staphylococcus species	8 (13.7)	4 (20)				
Mold	15 (25.8)	6 (30)				
Non-albicans candida	15 (25.8)	10 (50)				
Candida albicans	5 (8.6)	0				
Total	58 (100)	20 (100)	1.988	7.17	6	0.000372

$\sum D=38$, MD=5.4, $\sum(D\text{-}MD)^2=23.72$, SE=0.753. PTB: Preterm birth, SD: Standard deviation, SE: Standard error

Table 4: Comparison of microorganisms isolated from fetal membrane samples

Microorganisms	Women with PTB (%)	Women without PTB (%)	SD	Independent *t*-test	df	*P*
Escherichia coli	5 (8.7)	0				
Klebsiella species	2 (3.5)	0 (0.0)				
Streptococcus species	7 (12.2)	0 (0.0)				
Staphylococcus species	8 (14)	2 (11.1)				
Mold	15 (26.3)	6 (33.3)				
Non-albicans candida	15 (26.3)	10 (55.5)				
Candida albicans	5 (8.7)	0				
Total	57 (100)	18 (100)	2.15	6.7	6	0.000537

$\sum D=39$, MD=5.5, $\sum(D\text{-}MD)^2=27.75$, SE=0.814. PTB: Preterm birth, SD: Standard deviation, SE: Standard error

the means of the two groups was statistically significant ($t = 6.7$, $P < 0.001$).

Microorganisms associated with intra-amniotic infection

Table 5 shows microorganisms associated with intra-amniotic infection among women with preterm delivery. Both samples from preterm and full-term birth were considered. Seven microorganisms are identified: *E. coli, Klebsiella* species, *Streptococcus* species, *Staphylococcus* species, mold, non-albicans candida, and *C. albicans*. However, *E. coli* ($\chi^2=3.98$, $P<0.05$), *Streptococcus* species ($\chi^2=5.53$, $P<0.03$), non-albicans candida ($\chi^2 = 8.37$, $P < 0.004$), and *C. albicans* ($\chi^2=3.98$, $P<0.05$) were statistically significant. The overall association ($\chi^2=24.084$, $P<0.0006$) with all isolated microorganisms and intra-amniotic infections was statistically significant.

Discussion

The study analyzed the microbial differences in amniotic fluids, placental membranes, and fetal membranes. Bacteria associated with urinary tract infections (UTIs) were observed among women with preterm birth and absented among women without preterm birth. The bacterial differences in each sample site were studied. For the amniotic fluids, *E. coli*, the main contributor to the urinary tract infection, was observed among women with preterm birth but was not isolated among women with full-term birth. *Klebsiella* species

Table 5: Microorganisms associated with intra-amniotic infection

Microorganisms	Women with PTB (%)	Women without PTB (%)	Total	χ^2	df	*P*
Escherichia coli	15 (11.8)	0 (3.12)	15	3.98	1	0.046044
Klebsiella species	7 (5.5)	0 (1.4)	7	1.8	1	0.179712
Streptococcus species	21 (16.6)	0 (4.37)	21	5.53	1	0.018693
Staphylococcus species	19 (19.7)	6 (5.2)	25	0.14	1	0.708281
Mold	45 (46.6)	14 (12.3)	59	0.284	1	0.594091
Non-albicans candida	45 (55)	24 (14.3)	69	8.37	1	0.003815
Candida albicans	15 (11.8)	0 (3.12)	15	3.98	1	0.046044
Total	167	44	211	24.084	6	0.000504

PTB: Preterm birth

and *Staphylococcus* species were also isolated among women preterm birth but not in full-term birth. Other microorganisms were isolated. The invasion of the isolated pathogens in the uterus leads to a safe pregnancy or childbirth. We assume that these women should have been experiencing UTIs or any other vaginal infection throughout pregnancy [Table 2]. This study agrees with the Comparative Microbial Analysis of Paired Amniotic Fluid and Cord Blood from Pregnancies Complicated by Preterm Birth and Early-Onset Neonatal Sepsis. The study identified the microorganisms in both paired amniotic fluid and cord blood. The *E. coli* was isolated in both samples.[15] Other different microorganisms were isolated, but *E. coli* is common between the two studies. The invaders of the amniotic cavity were isolated in the study conducted on short-term neonatal outcomes in women with preterm labor and intact membranes, and the *E. coli* was the third most isolated bacteria apart from *Ureaplasma* spp. and *Fusobacterium* spp.[16] The same situation was observed for microorganisms isolated in placental membranes, despite the sharp difference in percentages. The placenta is connected to the funiculus umbilicalis, which connects the fetus to the mother, and could be the pathway through which bacteria from the mother invade the fetus. The invasion of microorganisms in the placenta is the risk of amniotic fluids' invasion. This justifies the microorganisms' similarities among these both sites [Table 3]. *E. coli, Staphylococcus aureus, and Streptococcus* spp. isolated in the current study in the placental membrane were also observed in the study on microorganisms in the human placenta associated with altered CpG methylation of immune and inflammation-related genes.[17] As the amniotic fluids surround the fetal membranes, the similarities of the microorganisms in the fetal membranes, amniotic fluids, and placental membranes are not critical. Bacteria such as *E. coli, Klebsiella* spp., *Streptococcus* spp., *Staphylococcus* spp., and others were isolated in the fetal membranes as per placental and amniotic fluids [Table 4]. The microbial differences in the amniotic fluids, placental membranes, and fetal membranes were statistically significant among women with preterm and full-term birth. Microorganisms such as *E. coli, Klebsiella* species, *Staphylococcus, Streptococcus*, and *C. albicans* were observed in all samples among women with preterm birth but were not isolated among women with full-term birth. The association between intra-amniotic infection and preterm birth was tested. The *E. coli, Streptococcus* species, *C. albicans*, and non-albicans candida were associated with preterm birth [Table 5]. We better know that *E. coli* contributes to 90% of UTIs, and its ascendancy toward the uterus can lead to devastating birth outcomes, including preterm birth. Some *Streptococcus* species are the leading cause of serious maternal infections. *Candida* species contribute to candidiasis during pregnancy, and their invasion of the uterus may lead to pregnancy and birth complications. *E. coli, Streptococcus* species, non-albicans candida, and *C. albicans* were associated with intra-amniotic infections and were observed in all three types of samples [Tables 2-4]. The study on the intra-amniotic infection reported that isolated microorganisms from amniotic fluid samples were the normal flora of the vagina, including *E. coli* and *Streptococcus* species.[18] The ascendancy of vaginal flora to the uterus can cause intrauterine infection, a precursor to preterm

birth and fetal deaths. A similar finding from a systematic review of *Candida* chorioamnionitis among pregnant women revealed a high prevalence of *C. albicans* among *Candida* chorioamnionitis patients.[19] *C. albicans* is the leading cause of most vaginal yeast infections among women during pregnancy. Ascending infections from the vagina to the uterus could lead to preterm delivery, birth impairment, neonatal infections, and fetal death. This is made possible when preterm rupture of membranes washes away the intracervical mucus plug, giving room for bacterial colonization in the amniotic sac.

Some microorganisms were isolated from women with preterm birth and absent from women that had carried their pregnancy to a full-term birth. Similar findings reported that *E. coli* and *Streptococcus* species were isolated from women with intra-amniotic infections.[20] The current study compared the microbial differences between women with preterm and women with full-term delivery. The mean difference between the two samples was statistically significant [Tables 2-4]. The findings reported the microbial differences between preterm and term birth women; *Fusobacterium*, *Streptococcus*, *Mycoplasma*, *Aerococcus*, *Gardnerella*, *Ureaplasma,* and Enterobacteriaceae were more prevalent among preterm birth women but were absent in samples collected from women who had undergone full-term birth.[21] The findings on preterm, prelabor, amniorrhexis, and intra-amniotic infection reported *Streptococcus agalactiae* and *Streptococcus milleri, Lactobacillus sp., Enterobacter sp., C. albicans, Streptococcus viridans, Streptococcus sanguis, Haemophilus influenzae, and Staphylococcus epidermidis*. These microorganisms were isolated from fetal blood samples while *Enterobacter, C. albicans, H. influenzae*, *S. agalactiae,* and *S. epidermidis* were isolated from amniotic fluid samples.[22] We isolated some similar microorganisms in both fetal membranes and amniotic fluid samples. This study did not consider fetal blood samples.

Tests of associations with isolated microorganisms and intra-amniotic infection identified a statistical association of *E. coli, Streptococcus* species, yeast, and *C. albicans* [Table 5]. A similar finding reported different isolates but showed similarities by having similar isolates. The study isolated S. *agalactiae, E. coli,* and *Staphylococcus coagulase-negative*.[23] Peng *et al*.(1996) indicate the importance of these biomarkers in diagnosing intrauterine infection or chorioamnionitis and instituting antibiotic management early to prevent preterm birth. However, the possibility of an antepartum diagnosis of these infections may be a challenge given the invasiveness of amniocentesis for obtaining an amniotic fluid sample.[24] Molds*, Klebsiella, and Staphylococcus* spp. were not associated with intra-amniotic infection, and past studies did not report any of them contributing to clinical chorioamnionitis.

The study is limited by the accessibility of various samples due to the status of women undergoing birth, which affected our sample size. The conclusion of the study cannot be generalized to other populations.

Conclusions

There is a microbial difference between preterm and full-term birth in all placental membranes, amniotic fluid, and fetal membrane samples. The mean difference between the two groups was statistically significant. *E. coli*, *Streptococcus* species, non-albicans candida, and *C. albicans* are the primary cause of intra-amniotic infection or clinical chorioamnionitis among women with preterm birth. Preterm rupture of membranes may be associated with intra-amniotic bacterial or fungal infections. Ascending microorganisms may be associated with intra-amniotic infection or clinical chorioamnionitis among pregnant women, leading to preterm birth. Although intra-amniotic infections may be subtle, pregnant women should seek immediate medical care when they show signs such as prelabor draining of fluids or are at higher or risk of intra-amniotic infections.

Acknowledgments

The authors would like to express their gratitude to the staff in Ruhengeri Referral Hospital for support in sample collection. We also thank INES Ruhengeri for allowing time and providing the microbiological analysis of samples. We are grateful to Dr. Roger

Harrison from the University of Manchester for help in manuscript revision.

Authors' contribution

All authors contributed to the analysis and interpretation of the results, methodology elaboration, drafting and revision of the manuscript, and approval of its final version.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Compliance with ethical principles

The Research Ethics Committee of INES Ruhengeri Institute of Applied Sciences approved the research to be conducted. Ethical approval was also sought from Ruhengeri Referral Hospital (ref: 1209/HDR/RHH/2018) or swab sample collection. Data were analyzed and reported anonymously.

References

1. Bond DM, Middleton P, Levett KM, van der Ham DP, Crowther CA, Buchanan SL, *et al*. Planned early birth versus expectant management for women with preterm prelabour rupture of membranes prior to 37 weeks gestation for improving pregnancy outcome. Cochrane Database Syst Rev 2017;3:CD004735.
2. Kagura P, Wasunna A, Laving A, Wamalwa D, Ng'ang'a P. Prevalence and factors associated with preterm birth at Kenyatta national hospital. BMC Pregnancy Childbirth 2018;18:107.
3. Astek JA, Srinivas SK, Sammel MD, Elovitz MA. Do neonatal outcomes differ depending on the cause of preterm birth? A comparison between spontaneous birth and iatrogenic delivery for preeclampsia. Am J Perinatol 2010;27:163-9.
4. Lamont RF. Infection in the prediction and antibiotics in the prevention of spontaneous preterm labour and preterm birth. BLOG 2003;110:71-5.
5. Sebire NJ. Choriodecidual inflammatory syndrome (CoDIS) is the leading, and under recognized cause of early preterm delivery and second trimester miscarriage. Med Hypotheses 2001;56:497-500.
6. Kemp MW. Preterm birth, intrauterine infection, and fetal inflammation. Front Immunol 2014;5:1-11.
7. Menon R, Fortunato SJ. Fetal membrane inflammatory cytokines: A switching mechanism between the preterm premature rupture of the membranes and preterm labor pathways. J Perinat Med 2004;32:391-9.
8. Abbasalizadeh S, Bagherifard R, Mahdavi F, Abbasaizadeh F, Raouf S. Comparison of maternal and fetal outcomes in pregnancies with preterm premature rupture of membrane (PPROM) terminating in 34th or 36th gestational weeks: A clinical trial. ABCmed 2017;5:1-12.
9. Agrawala V, Hirscha E. Intrauterine infection and preterm labor. Semin Fetal Neonatal Med 2017;17:12-9.
10. Holst RM, Hagberg H, Wennerholm UB, Skogstrand K, Thorsen P, Jacobsson B. Prediction of microbial invasion of the amniotic cavity in women with preterm labour: Analysis of multiple proteins in amniotic and cervical fluids. BLOG 2010;118:240-9.
11. Ventura W, Nazario C, Ingar J, Huertas E, Limay O, Castillo W. Risk of impending preterm delivery associated with the presence of amniotic fluid sludge in women in preterm labor with intact membranes. Fetal Diagn Ther 2011;30:116-21.
12. Hong JS, Park KH, Noh JH, Suh YH, Yoon BH. Cervical length and the risk of microbial invasion of the amniotic cavity in women with preterm premature rupture of membranes. Am J Obstet Gynecol 2006;195:S232.
13. Seong HS, Lee SE, Kang JH, Romero R, Yoon BH. The frequency of microbial invasion of the amniotic cavity and histologic chorioamnionitis in women at term with intact membranes in the presence or absence of labor. Am J Obstet Gynecol 2008;199:375.e1-5.
14. Ahishakiye A, Abimana MC, Beck K, Miller AC, Betancourt TS, Magge H, *et al*. Developmental outcomes of preterm and low birth weight toddlers and term peers in Rwanda. Ann Glob Health 2019;85:147.
15. Wang X, Buhimschi CS, Temoin S, Bhandari V, Han YW, Buhimschi IA. Comparative microbial analysis of paired amniotic fluid and cord blood from pregnancies complicated by preterm birth and early-onset neonatal sepsis. PLoS One 2013;8:e56131.
16. Cobo T, Vives I, Rodríguez-Trujillo A, Murillo C, Ángeles MA, Bosch J, *et al.* Impact of microbial invasion of amniotic cavity and the type of microorganisms on short-term neonatal outcome in women with preterm labor and intact membranes. Acta Obstet Gynecol Scand 2017;96:570-9.
17. Tomlinson MS, Bommarito PA, Martin EM, Smeester L, Fichorova RN, Onderdonk AB, *et al.* Microorganisms in the human placenta are associated with altered CpG methylation of immune and inflammation-related genes. PLoS One 2017;12:e0188664.
18. Fahey JO. Clinical management of intra-amniotic infection and chorioamnionitis: A review of the literature. J Midwifery Womens Health 2008;53:227-35.
19. Maki Y, Fujisaki M, Sato Y, Sameshima H. *Candida* chorioamnionitis leads to preterm birth and adverse fetal-neonatal outcome. Infect Dis Obstet Gynecol 2017;2017:1-11.
20. Romero R, Gomez-Lopez N, Winters AD, Jung E, Shaman M, Bieda J, *et al*. Evidence that intra-amniotic infections are often the result of an ascending invasion – A molecular microbiological study. J Perinat Med 2019;47:915-31.
21. Doyle RM, Alber DG, Jones HE, Harris K, Fitzgerald F, Peebles D, *et al*. Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery. Placenta 2014;35:1099-101.
22. Carroll SG, Ville Y, Greenough A, Gamsu H, Patel B, Philpott-Howard J, *et al*. Preterm prelabour amniorrhexis: Intrauterine infection and interval between membrane rupture and delivery. Arch Dis Child Fetal Neonatal Ed 1995;72:F43-6.
23. Mazor M, Hershkovitz R, Ghezzi F, Maymon E, Horowitz S, Leiberman JR. Intraamniotic infection in patients with preterm labor and twin pregnancies. Acta Obstet Gynecol Scand 1996;75:624-7.
24. Peng CC, Chnag HJ, Lin YH, Cheng JP, Su HB. Intrauterine inflammation, infection, or both (TripleI): A new concept for chorioamnionitis. Pediatr Neonatol 2018;59:231-7.

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