
**“VAGINAL MICRO BIOME AND SOCIO-
DEMOGRAPHIC DETERMINANTS OF
SPONTANEOUS ABORTION IN A TERTIARY CARE
HOSPITALS OF NORTH KARNATAKA”**

**Thesis Submitted to
KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
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**For the award of the degree of
Doctor of Philosophy
In the Faculty of Nursing**

By

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Registration No: KLEU/Ph.D.-19-20/DO1219030

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2024

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Let's choose today to quench our thirst for the 'good life' we think others lead by acknowledging the good that already exists in our lives. We can then offer the universe the gift of our grateful hearts.

Sarah Ban Breathnach

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ABBREVIATIONS

Sr. No	Abbreviations	Expanded forms
1.	SA	Spontaneous Abortion
2.	DNA	Deoxy Ribose Nucleic Acid
3.	RNA	Ribose Nucleic Acid
4.	WHO	World Health Organization
5.	(ACOG),	American College of Obstetricians and Gynecologists
6.	E coli	Escherichia coli
7.	STDs	Sexually Transmitted Disease
8.	RPL	Recurrent Pregnancy Loss
9.	L. iners	Lactobacillus Iners
10.	BV	Bacterial Vaginosis
11.	PCR	Polymerase chain reaction
12.	HPV	Human Papilloma Virus
13.	M Hominis	Mycoplasma hominis
14.	C. Trachomatis	Chlamydia trachomatis
15.	RM	Recurrent Miscarriage
16.	RR	Risk Ratio
17.	OR	Odds Ratio
18.	ART	Artificial Reproductive Technique

19	PTB	Pre-Term Birth
20	NP	Non Pregnant
21	(URSA)	Unexplained recurrent spontaneous abortion
22	(CSTs)	Community state type
23	(OTU)	Operational taxonomic units
24	(PCoA),	Principal coordinates analysis
25	VMB	Vaginal Micrbiome
26	(sETBs)	Early term births
27	L.	Lactobacillus
28	(PRISMA)	Preferred Reporting Items for Systematic Review and Meta-Analysis Statement
29	(PROM),	Preterm rupture of membranes
30	(SES)	Socio Economic Status
31	LR	Logistic regression
32	ANC	Ante Natal Care
33	MRS	De Man–Rogosa–Sharpe
34	Hb	Haemoglobin

ABSTRACT

Background:. Spontaneous abortion (SA) is the termination of a pregnancy without medical or mechanical intervention before the fetus is sufficiently developed to survive. In other words, miscarriage is early pregnancy loss before 20th week of gestation, or 139 days, counting from the first day of the last normal menses.

At least 15-20% of pregnancies that are clinically detected end in spontaneous abortion in the first trimester. First trimester, or the first three months of pregnancy, accounts for around 80% of all incidences of pregnancy loss

Numerous factors are linked to SA. Age, clotting disorders, immunological factors, infections, and genetic disorders are the significant risk factors associated with recurrent SA in Indian women, additionally, women whose first pregnancy resulted in miscarriage are more likely to experience another miscarriage during their second pregnancy compared to those with term pregnancies. More specifically, infections have been linked to 66% of late losses and 15% of early miscarriages.

Lack of a thorough grasp of risk factors, differential diagnosis, and inadequate management planning can all make accurate diagnosis difficult, especially in the early stages of pregnancy.

Therefore, the aim of this study was to identify and compare the vaginal microorganism of women experiencing SA of those who have continued pregnancy of the same gestational age, and also elucidate if certain sociodemographic and reproductive factors are associated with a higher risk of miscarriage, or if screening pregnant women for modifiable factors might lead to a lower pregnancy loss rate.

Objectives

1. To compare the vaginal microbiome in mothers who have undergone spontaneous abortion within 20 weeks of gestation and those who have continued pregnancy with same gestational age.
2. To determine the Socio demographic and Reproductive determinant of spontaneous abortion and compare it with the socio demographic and Reproductive determinants of mothers those who have continued pregnancy with same gestational age.

Materials and Method: The study design adopted for the present study was: Cross-sectional comparative research design. The setting for the study was tertiary care hospitals of northern Karnataka. Using the Purposive Sampling technique 200 mothers were recruited for the study

Measures: Vaginal swab to detect the vaginal microbiome among the participants. DNA was extracted from the vaginal samples. 16S rRNA sequencing method was used to identify the organisms present. And also participants were interviewed using a structured questionnaire on socio demographic as well as reproductive factors were collected.

Results: A pathogen detection rate of 13.15% (n=200) was found in the vaginal discharge cultures. The percentages of bacterial detection in women who had spontaneous abortions and those who continued their pregnancies were 19.9% and 7.9%, respectively ($p < .05$). Compared to the women who sustained their pregnancy with same gestational weeks, the women who experienced a spontaneous abortion had five common species: *Escherichia coli* (48%), *Enterococcus faecalis* (28 %), *Enterococcus faecium* (12%), *Pseudomonas fluorescens* (7%), and *Pseudomonas cedrina* (5%); the most prevalent species among those who sustained pregnancy were

Lactobacillus inners (58%), Lactobacillus crispatus (32%), and Escherichia coli (10%) ($p < 0.05$). . Risk of abortion with unadjusted odds ratio 1.87 times more in the age group of 19-20years , parity primi mother with unadjusted odds ratio 1.16 times had more risk of abortion, Area of residence the mother who stayed in rural area with unadjusted ratio 1.47 times more risk of abortion, occupation the mothers who were self-workers with unadjusted odds ratio 1.50 times more risk of abortion, when looking into the education of mother illiterate mother with unadjusted odds ratio 0.50 times had risk of abortion, in view with religion muslims with unadjusted odds ratio 0.38 times more risk of abortion considering the type of family the mother who belong to nuclear family with unadjusted odds ratio 1.04 times had more risk of abortion, consanguineous marriage with unadjusted odds ratio 3.95 had times more risk of abortion

Conclusion: Women who had spontaneous abortions had a vaginal microbiome that was more varied and irregularly distributed with bacteria. The bacteria identified could affect the women's pregnancy outcome and perhaps result in spontaneous abortion. It is vital for nurses to provide education to women who are in the fertile period regarding prenatal screening, sexually transmitted infections, and perineal care. And also our study shed light to clarify whether certain socio demographic and reproductive determinants do increase miscarriage risk, or screening of pregnant women for treatable determinants would improve the rate of pregnancy loss

Keywords: Spontaneous abortion, vaginal microbiome, microbiological analysis, DNA sequencing, women's health, pregnancy, Cross sectional, comparative analysis, Sociodemographic and Reproductive, Determinants, Spontaneous abortion.

TABLE OF CONTENT

Sr. No	Particulars	Page No
1	INTRODUCTION	1-13
2	REVIEW OF LITERATURE	14-52
3	MATERIALS AND METHOD	53-61
4	RESULTS	62-103
5	DISCUSSION	104-108
6	CONCLUSION	109-113
7	SUMMARY	114-119
8	RECOMMENDATIONS	120-122
9	BIBLIOGRAPHY	123-140
10	ANNEXURES	141-174

LIST OF TABLES

Sr. No	Particulars	Page No
1	The table representing results of biochemical testing	67
2	The table representing the results of IMVIC test	68
3	The table representing the results of Gram staining for few representative samples	70
4	The table representing the result of Gram Staining	71
5	The table representing distribution of isolated pathogens bacteria among study group	84
6	Association between abortions with Maternal Age	87
7	Association between abortions with Parity	89
8	Association between abortions with Area of Residence	90
9	Association between abortions with Occupations	91
10	Association between abortions with Maternal Education	92
11	Association between abortions with Religion	93
12	Association between abortions with consanguineous marriage	94
13	Association between abortions with socio demographic characteristics by simple logistics regression analysis	95
14	Association between abortions with age at menarche	97
15	Association between abortions with any complication during last pregnancy	98
16	Association between abortions with history of previous Abortion	99

17	Association between abortions with levels of Hb%	100
18	Association between abortions with Maternal infection	101
19	Association between abortions with pregnancy/ reproductive related variables by simple logistic regression analysis	102

LIST OF FIGURES

Sr. No	Particulars	Page No
1	Factors leading to miscarriage	3
2	Dysbiosis of vaginal micro biota reciprocally impacts Reproductive Health	7
3	Conceptual Framework	13
4	Protocol/Methodology- Cross Sectional Comparative Research Design	53
5	Flow chart of microbial processing : Detection of vaginal Micro biome among mothers undergoing spontaneous abortion	56
6	Culture plates	64
7	Showing the shift in color in IMVIC Test	66
8	Depicting the result of gram staining	69
9	DNA and 16S Amplicon data for sample with code no 0N 42	73
10	DNA and 16S Amplicon data for sample with code no 0N 98	73
11	DNA and 16S Amplicon data for sample with code no 0N 62	74
12	DNA and 16S Amplicon data for sample with code no 0A 64 Sample 4 with code no 0A12	74
13	DNA and 16S Amplicon data for sample with code no 0A 12	75
14	DNA and 16S Amplicon data for sample with code no 0A 72	75
15	DNA and 16S Amplicon data for sample with code no 0A 54	76
16	DNA and 16S Amplicon data for sample with code no 0A 55	76

17	DNA and 16S Amplicon data for sample with code no 0A 22	77
18	Phylogenetic tree depicting the species of <i>Pseudomonas cedrina</i>	81
19	Phylogenetic tree depicting the species of <i>Pseudomonas fluorescens</i>	81
20	Phylogenetic tree depicting the species of <i>Enterococcus faecium</i>	82
21	Phylogenetic tree depicting the species of <i>Enterococcus faecalis</i>	82
22	Phylogenetic tree depicting the species of <i>Escherichia coli</i>	83

LIST OF GRAPHS

Sr. No	Particulars	Page No
1	Depicting the ATGC peak for the sample OA64	78
2	Depicting the ATGC peak for the sample OA12	78
3	Depicting the ATGC peak for the sample OA72	78
4	Depicting the ATGC peak for the sample OA54	79
5	Depicting the ATGC peak for the sample OA54	79
6	Pie Graph showing the Types of abortion in abortion group	86
7	Bar Graph showing Comparison of normal group and abortion group with Maternal Age	88
8	Bar Graph Showing the : Comparison of normal group and Abortion group with Parity	89
9	Bar Graph showing the Comparison of normal group and abortion group with Area of Residence	90
10	Bar Graph showing Comparison of normal group and abortion group with Occupation	91
11	Bar Graph showing Comparison of normal group and abortion group with Maternal Education	92
12	Bar Graph showing the Comparison of normal group and abortion group with Religion	93
13	Bar Graph showing the Comparison of normal group and abortion group with consanguineous marriage	94

14	Bar Graph showing the Comparison of normal group and abortion group with age at menarche	97
15	Bar Graph showing the Comparison of normal group and abortion group with any complication during last pregnancy	98
16	Bar Graph showing the Comparison of normal group and abortion group with history of previous abortion	99
17	Bar Graph showing the Comparison of normal group and abortion group with levels of hb%	100
18	Bar Graph showing the Comparisons of normal group and abortion group with maternal infection	101

CHAPTER I

INTRODUCTION

1.1) Background

The World Health Organization (WHO) defines abortion as "the expulsion or extraction from its mother of a fetus or embryo weighing less than 500 grams."¹

Spontaneous abortion (SA) is the termination of a pregnancy without medical or mechanical intervention before the fetus is sufficiently developed to survive. In other words, miscarriage is early pregnancy loss before 20th week of gestation, or 139 days, counting from the first day of the last normal menses.²

According to estimations from the American College of Obstetricians and Gynecologists (ACOG), SA is the most prevalent form of pregnancy loss.³ Approximately 26% of pregnancies worldwide terminate in miscarriage, while the first trimester accounts for 80% of early pregnancy losses.⁴ Significant rates of SA have been reported in India. A household study carried out in Bihar, India; found that miscarriages usually happened later in pregnancy, with a prevalence of 46 per 1,000 pregnancies (4.6%).⁵ On the other hand, an epidemiological study conducted in the largest Indian cities found that up to 32% of Indian women had recurrent (SAs), with the majority of SAs (80%) happening in the first 3 months of pregnancy.⁶

When compared to data from the western countries, the study showed that Indian women had a greater rate of recurrent SA²

At least 15-20% of pregnancies that are clinically detected end in spontaneous abortion in the first trimester. First trimester, or the first three months of pregnancy, accounts for around 80% of all incidences of pregnancy loss.⁷

The March of Dimes reports that spontaneous abortions occur in about 50% of pregnancies, usually before a woman misses her monthly cycle or even realizes she is pregnant.

According to the study conducted in Belagavi city, Karnataka it is estimated that of 1000 ongoing pregnancies at 6 weeks' gestation, nearly 40% will be lost prior to 20 weeks' gestation, two thirds to miscarriage.⁸

A spontaneous abortion may occur in several phases, which might include:

- **Threatened abortion** (pregnancy may continue): (Pregnancy may continue): Abortion may or may not occur, and there is vaginal bleeding without cervical dilatation.
- **Inevitable abortion:** This is a condition when the cervix has dilated and there is excessive vaginal bleeding. The pregnancy will end, and either a partial or full abortion will take place.
- **Incomplete abortion:** Products of conception are partly expelled
- **Complete abortion:** Products of conception are completely eliminated.⁹

Numerous factors are linked to SA. Age, clotting disorders, immunological factors, infections, and genetic disorders are the significant risk factors associated with recurrent SA in Indian women.² additionally, women whose first pregnancy resulted in miscarriage are more likely to experience another miscarriage during their second pregnancy compared to those with term pregnancies. More specifically, infections have been linked to 66% of late losses and 15% of early miscarriages.¹⁰

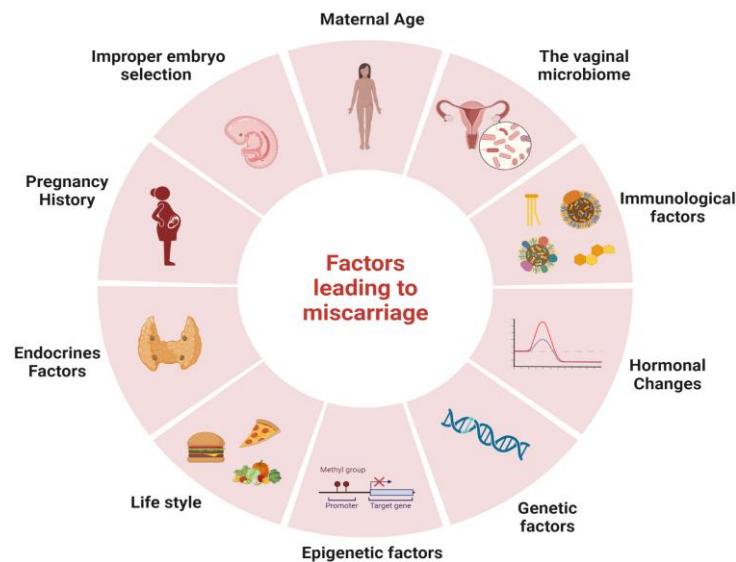


Fig. 1 Factors leading to miscarriage

The health of the mother and fetus throughout pregnancy is significantly influenced by the diversity of the vaginal micro-biota. Pregnant women's micro biomes are more stable and less diverse than those of non-pregnant women.¹¹ vaginal microbiota can be impacted by urogenital infections, diet, sexual behavior, medicinal treatments, and hormonal fluctuations. Possible causes of SA include toxoplasmosis, bacterial vaginosis, rubella, Cytomegalo virus herpes simplex, Lactobacillus, and chlamydia.¹⁰ Consequently, it is imperative that the specific organism be identified. In a prospective study Nasioudis et al., 2017 of 155 women who were seen at an outpatient obstetrical service, the vaginal microbiome differed between women with a first or subsequent pregnancy. Lactobacillus crispatus was prevalent in 76.4% of primigravid women, 50% of those with a history of prior abortion, and 22.2% of individuals with a prior birth ($p \leq 0.01$). Conversely, Lactobacillus iners prevalence was observed in 3.8% of primigravid women, compared to 19.2% ($p = 0.03$) and 20.8% ($p = 0.03$) in those with a history of prior SA or birth, respectively. The prevalence of Gardnerella as the predominant bacterial genus increased from 3.8% in primigravid women to 15.4% and 14.3% in individuals with a history of prior SA or birth,

respectively ($p>0.05$). Moreover, prevalence of *Lactobacillus iners* was significantly associated with a history of SA ($p\leq 0.02$). Researchers in Iraq (Sherzad Hussien., 2019) analyzed 80 deep vaginal swabs from 40 women with recurrent SA and 40 women who maintained their pregnancies to evaluate the prevalence of vaginitis due to bacterial causes and its relationship with SA. Findings revealed that the highest rate of isolated bacteria from the high vaginal swab culture of women who experienced SA was *Gardenella vaginalis* (36.36%), *Escherichia coli* (27.27%), and *Staphylococcus aureus* (22.73%); the highest rate of isolated bacteria from women who maintained their pregnancies was *E coli* (50%).¹²

The composition of the vaginal micro biota and its influence on pregnancy outcomes, specifically SA, varies with pregnancy history. Worldwide there is evidence that preventable infections may account for up to 15% of early (within 13 weeks of gestation) SAs and up to 66% of late SAs (13-22weeks of gestation).¹⁰ If researchers can identify the microbes that are correlated with SA, routine screening and treatment for these organisms could decrease the incidence of SA and can be made mandatory for pregnant women. This will allow for early detection and management of the infection in the genital tract and hence for a successful pregnancy outcome. Therefore, the aim of this study was to identify and compare the vaginal microorganism of women experiencing SA of those who have continued pregnancy of the same gestational age.

On the other arm numerous risk factors, including chromosomal abnormalities and fetal deformities, chronic illnesses in mothers, uterine disorders, immunological factors, and infections, are associated with spontaneous abortion in many cases. Yet, a number of modifiable risk factors for spontaneous abortion were also found to exist: increased smoking, alcohol and caffeine consumption, obesity, and older parenteral

age.¹³ Nevertheless, the therapeutic care of these individuals faces significant challenges because the etiology of around 50% of recurrent pregnancy loss cases is still unclear¹⁴ Because of its identified etiology, recurrent pregnancy loss is generally considered to be a complicated illness.

Lack of a thorough grasp of risk factors, differential diagnosis, and inadequate management planning can all make accurate diagnosis difficult, especially in the early stages of pregnancy.

There hasn't been any agreement reached yet, despite the fact that few studies have particularly looked into the connection between socioeconomic and reproductive factor and the occurrence of spontaneous abortion.

Hence our research also aims to elucidate if certain sociodemographic and reproductive factors are associated with a higher risk of miscarriage, or if screening pregnant women for modifiable factors might lead to a lower pregnancy loss rate.

1.1) Need for the study

The spontaneous loss of a pregnancy before 12 weeks (early miscarriage) or between 12 and 20 weeks (late miscarriage) of gestation is known as a spontaneous abortion.

In obstetrics, miscarriage is a prevalent issue that affects around 25% of pregnancies globally. Based on when they happen, miscarriages can be classified as early or late: early miscarriages happen before 12 weeks of gestation, while late losses happen between 12 and 20 weeks of pregnancy.¹⁵

One in five pregnancies results in a spontaneous abortion, which can have serious physiological and psychological effects on the mother. Significant medical expenses are also linked to it.¹⁶ Research suggests that infections that might have been prevented could be the cause of up to 15% of early spontaneous abortions and up to 66% of late spontaneous abortions.¹⁹

When cardiac activity stops and embryonic or fetal development stagnates, but there is no spontaneous expulsion from the uterus cavity for several days or weeks, this is known as a missed abortion. It is described as a non-developing pregnancy in which the cervix stays closed. Women who are of childbearing age may experience major consequences to their reproductive health.¹⁷ Genetic, endocrine, anatomical anomalies of the reproductive system, infection, and variables connected to the environment, and drugs are among the recognized causes.¹⁸

Pregnant women's vaginal micro biome is more stable and less diverse than that of non-pregnant women, which has a significant impact on the health of the mother and the fetus during pregnancy. Hormonal fluctuations, diet, sexual behavior, medical conditions, and urogenital infections are some of the variables that might affect the vaginal microbiota.²⁰ Women's vaginal microbiome has a major impact on reproductive health. Imbalance in this microbiota has been connected to bacterial vaginosis, increased susceptibility to STDs, and poor reproductive outcomes. The *Lactobacillus* species are the predominant inhabitants of the human vagina in a healthy condition, and they are essential to preserving the health of the vagina. In addition to producing lactic acid to keep the vagina's pH between 3.6 and 4.5 normal, these helpful bacteria shield the vagina from antimicrobial agents. However the composition of the vaginal microbiome affects conception and pregnancy in significant ways.^{21, 22}

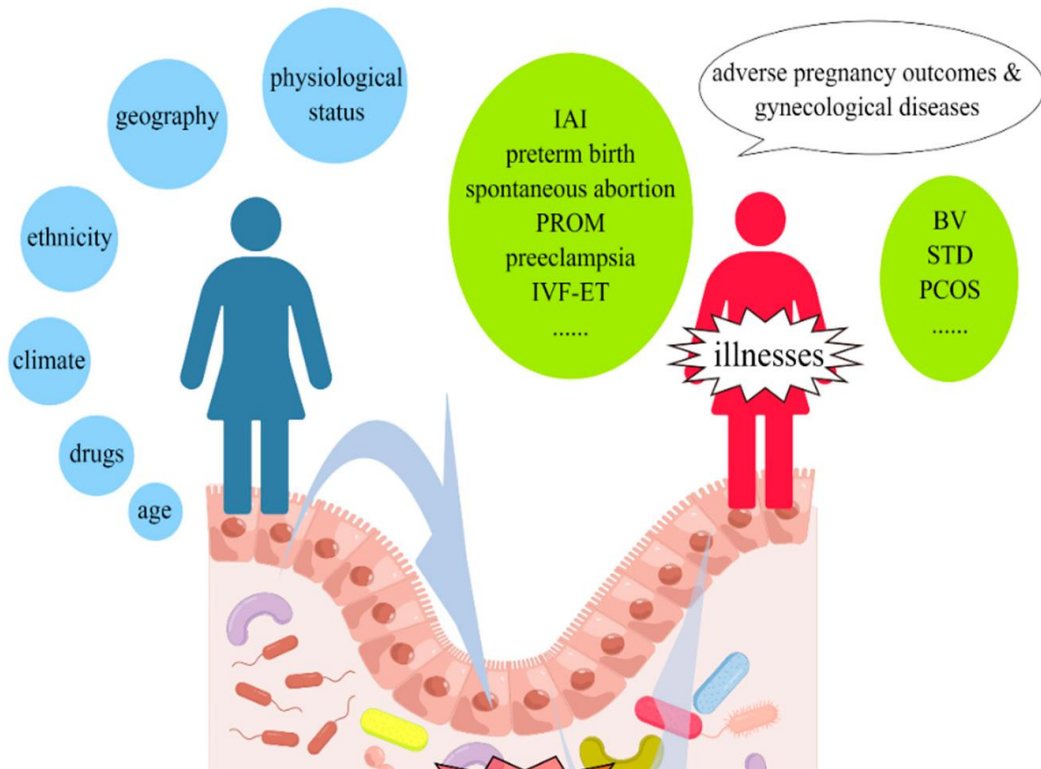


Fig.2 Dysbiosis of vaginal micro biota reciprocally impacts Reproductive Health

In fact, the high diversity raised the possibility of pathogen colonization and ascending infection, which can trigger the immune system and inflammatory response. As a result, the possibility of miscarriage was enhanced by uterine contractions and fetal membrane modification.²³

Contrarily, the prevalence of *Lactobacillus* species inhibits the colonization of pathogens, resulting in a decreased incidence of pregnancy complications including miscarriage.²²

However, the causes of these variances remain unknown. *Lactobacillus* dominates a healthy vaginal microbiota, and an overabundance of other species is frequently linked to harmful conditions one of the main defenses against genital infections, which are thought to be a prevalent cause of miscarriage, is a healthy vaginal flora, which is dominated by *Lactobacillus* species. A reduction in

Lactobacillus and an increase in a number of facultative or anaerobic bacteria indicate a shift in the vaginal bacterial flora.²⁴

Numerous factors can impact the composition of the vaginal microbiome, including lifestyle choices (like hygiene and sexual habits), environmental circumstances (like antibiotics, contraception, and pre- and probiotics), individual characteristics (like age, genetic and immunological factors, hormonal status, socioeconomic status, and ethnicity), and overall health.²⁵

A Nested Case Control research was done to determine the composition of the vaginal or endometrial microbiome associated with recurrent miscarriages. Between March 2018 and December 2020 at Helsinki University Hospital, Helsinki, Finland, endometrial and vaginal samples were taken from 39 healthy control women without a history of pregnancy loss and 47 women who had lost consecutively two or more pregnancies. Using 16S rRNA gene amplicon sequencing, the endometrial and vaginal microbiota compositions were compared between individual vaginal and endometrial samples and the RPL and control women. Internal transcribed spacer 1 amplicon sequencing was used to analyze the microbiota composition and provide a descriptive overview. The models were modified to account for age, parity, and body mass index. The endometrial samples of women with RPL had lower levels of *Lactobacillus crispatus* than those of controls (mean relative abundance 17.2% versus 45.6%, $q = 0.04$). *Gardnerella vaginalis* was found in higher concentrations in the RPL group compared to the controls in both vaginal (8.7% vs 5.7%, $q = 0.002$) and endometrial (12.4% against 5.8%, $q < 0.001$). There was a significant correlation between the individual endometrial and vaginal microbial compositions ($R = 0.85$, $P < 0.001$). In 22% of the endometrial and 36% of the vaginal samples, fungi were

found. Study concluded that Reproductive tract microbiome dysbiosis is linked to RPL and might be a new risk factor for miscarriages.²⁶

As per previous studies, genetic abnormalities, diseases in mothers and cardiovascular issues are among the other recognized causes of SA. Concurrently, lifestyle decisions such as excessive work, stress, nutrition, smoking, heavy alcohol use, and coffee consumption are also probable risk factors for SA.²⁷

Socioeconomic factors also affect expectations and health throughout pregnancy, in addition to the results of pregnancies. The risk of SA is increased by low socioeconomic status, which is demonstrated by low levels of education, income, and occupation that are associated with poor housing, insufficient nutrition, and restricted access to healthcare.²⁸

It has long been understood that a person's health is influenced by their socioeconomic standing, which is established by their level of income, education, and employment. Because those with low socioeconomic level are more likely to engage in hazardous behavior and have less access to healthcare, these socioeconomic variables have an impact on pregnancy outcomes and health. Numerous studies—possibly even as many as abundant—have conclusively shown a link between socioeconomic class and the onset of non-communicable illnesses including cancer. Although few research have specifically examined the relationship between socioeconomic position and the prevalence of SA, no consensus has yet been achieved.⁷⁶

Despite the fact that SA is not often linked to maternal death it can. Indeed, it can. On the other hand, morbidities such as recurrent SA, hemorrhage, sepsis,

psychological stress, and secondary infertility might occur. It is estimated that 25% of SA may be prevented if the risk factors were eliminated.²³

Since women are responsible for the entire population's physical, mental, and social well-being, they are a nation's greatest asset. The welfare and progress of a nation are shaped by the health of its mothers and children.

One of the main goals of the Reproductive and Child Health approach is to emphasize that women can give birth and go through pregnancy safely, and that pregnancy results in favourable outcomes in terms of both mother and fetal survival.

Globally, researchers face significant challenges in managing and treating numerous fatal pregnancy problems, which places a significant burden on society.

Only 30% of human conceptions result in a live birth with the other conceptions leading to pregnancy failure; this not only inflicts medical suffering but also psychological stress on a family. Therefore, in order to effectively manage them, it is imperative that we comprehend the intricate pathophysiology and genetics of inexplicable pregnancy loss.²⁹

In light of this, the current study is being conducted to investigate the vaginal microbiome and the sociodemographic factors that influence spontaneous abortion. The results of this study will assist medical professionals in treating infections at an early stage and in developing screening protocols for women in the preconception and early stages of pregnancy.

Title of the study

Vaginal Microbiome and Socio-Demographic Determinants of Spontaneous Abortion in a Tertiary Care Hospitals Belagavi, Karnataka

1.3) Objectives

1. To compare the vaginal microbiome in mothers who have undergone spontaneous abortion within 20 weeks of gestation and those who have continued pregnancy with same gestational age.
2. To determine the socio demographic and Reproductive determinant of spontaneous abortion and compare it with the socio demographic and Reproductive determinants of mothers those who have continued pregnancy with same gestational age.

1.4) Operational Definitions

Spontaneous abortion: In the present study spontaneous abortion is defined as a clinically recognized pregnancy loss before the 20th week of gestation.

Vaginal Microbiome: In the present study vaginal microbiome refers to the microorganisms that colonize the vagina.

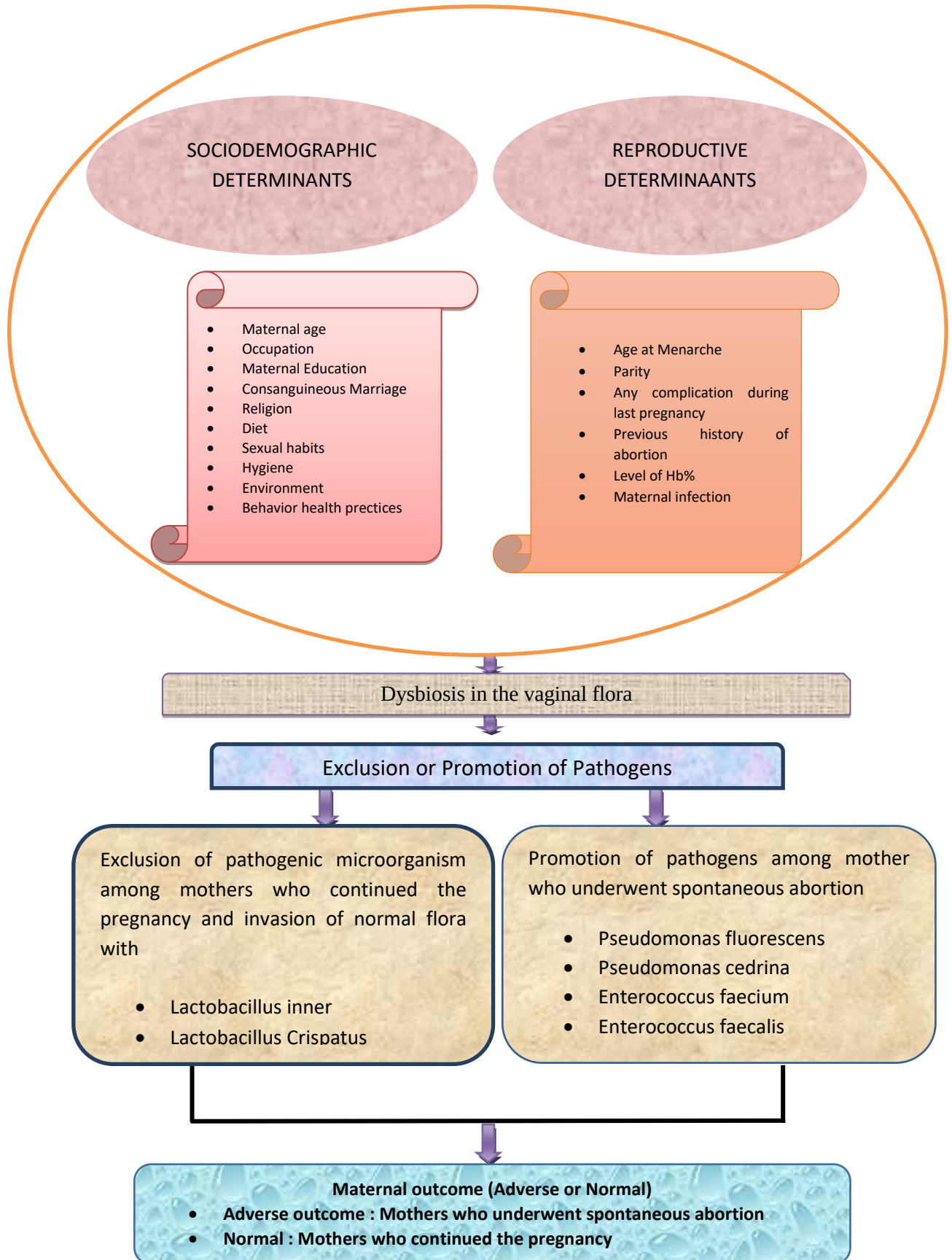
Determinants: In the present study determinants refers to the factors or variables which determines the spontaneous abortion.

1.5) Conceptual Framework

An event of interest, such as spontaneous abortion, can be identified by the proximate determinants framework, which pinpoints the precise process by which components interact. The causes of spontaneous abortion have been conceptually outlined in a framework provided by Odogwu, N. M., Onebunne, C. A. C et al.³⁰ According to this theory, the vaginal microbiome is directly impacted by lifestyle choices (like hygiene and sexual habits), environmental circumstances (like antibiotics, contraception, and pre- and probiotics) and also the socio demographic

and reproductive determinants as maternal age, parity, consanguineous marriage etc. and reproductive factors like Hb% of the mothers, age at menarche, previous complication during pregnancy etc.

The term "dysbiosis" refers to a change in the micro biome that can arise from a variety of physiological and pathological states. It has long been thought that the female genital tract is primarily inhabited by Lactobacilli, which prevent infections. Pathologic changes in their profile may make the vagina susceptible to infections, which could result in spontaneous abortion. The micro biome can also change with environment, weight, diet pattern, and hormonal milieu. Therefore, immunological, endocrinological, and metabolic changes during pregnancy can bring about major changes in the micro biome. . According to this conceptual framework, we would anticipate that the vaginal microbiome would subsequently influence maternal outcomes by either promoting or excluding infections.

CONCEPTUAL FRAMEWORK**Fig.3: Representing the Conceptual framework**

CHAPTER II

REVIEW OF LITERATURE

A literature review can serve as an inspiration for novel research concepts in addition to helping to set the groundwork for the study. The report starts by providing a review of the literature, which gives readers a context for understanding the state of the field and highlights the importance of the new study.

The following literature has been explored by the investigator and is organized under the following topics in the course of conducting the current investigation.

Literature pertaining to:

- i. Vaginal micro biome determinant of spontaneous abortion.**
- ii. Socio demographic determinant of spontaneous abortion.**
- i. Vaginal micro biome determinant of spontaneous abortion**

A Nested case-control study was carried out at Queen Charlotte's and Chelsea Hospital, Imperial College Healthcare London, to characterize the vaginal bacterial composition in early pregnancy and explore its relationship with first and second trimester miscarriages. Of the 161 pregnancies, 64 resulted in a first trimester miscarriage, 14 in a second trimester miscarriage, and 83 were term pregnancies. Using 16S rRNA gene-based metataxonomics, prospective characterization and analysis of the composition of vaginal bacteria from 5 weeks of gestation in miscarriages or simple term births were matched for age, gestational age, and body mass index data. The study found that, when first trimester miscarriages were compared to viable pregnancies, the vaginal microbiota dominated by *Lactobacillus* spp. was less prevalent, the alpha diversity was higher (mean Inverse Simpson Index

2.5 [95% confidence interval 1.8-3.0] versus 1.5 [1.3-1.7], $P = 0.003$), and the richness was higher (25.1 (18.5-31.7) versus 16.7 (13.4-20), $P = 0.017$). This was independent of vaginal bleeding and observable before first trimester miscarriage diagnosis ($P = 0.015$). Incomplete/complete miscarriage associated with higher proportions of *Lactobacillus* spp.-depleted communities compared with missed miscarriage. Early pregnancy vaginal bacterial stability was similar between miscarriage and term pregnancies. These results show that early pregnancy establishes an unsatisfactory community composition and link the bacterial component of vaginal microbiome to miscarriages in the first trimester. Although further research is needed to fully understand the process, vaginal bacterial makeup could be a modifiable risk factor for miscarriage in the first trimester.³¹

A prospective study was carried out to see whether the vaginal microbiota of women who had their first or second conception varied. By categorizing DNA sequences, the relative abundance of bacteria in the vaginal microbiome was found in first trimester pregnant women: 52 with their first known conception, 26 with a prior spontaneous or induced abortion but no delivery, and 77 with at least one previous birth. . In 76.4% of women who conceived for the first time, 50.0% who had previously had a planned or spontaneous abortion, and 22.2% who had previously given birth, *Lactobacillus crispatus* was the bacterium with the highest numerical abundance ($p \leq 0.01$). The most prevalent bacteria in 3.8% of women having their first conception was *L. iners*, whereas it was in women who had previously had an abortion or given birth, respectively. In women who had their first conception, the most common bacterial genus was 3.8%; however, in those who had previously had an abortion or given birth, it was 15.4% and 14.3%, respectively ($p > 0.05$). The

presence of *L. iners* was similarly linked to a history of spontaneous abortion ($p \leq 0.02$).³²

A retrospective case–control longitudinal study was order taken to define the vaginal microbiota during a typical human pregnancy, sequence-based approaches were employed in a research including 32 non-pregnant women and 22 pregnant women who delivered their babies at term (38 to 42 weeks) without any difficulties. Women who were not pregnant as well as those who were pregnant provided serial vaginal fluid samples. Pyro sequencing was used in a study based on the 16S r RNA gene sequence to investigate the stability and structure of the vaginal micro biota The vaginal microbiome of healthy pregnant women differed from that of non-pregnant women (pregnant women had lower abundances of 22 other phylotypes and greater abundances of *Lactobacillus vaginalis*, *L. crispatus*, *L. gasseri*, and *L. jensenii*). High relative abundance of *Atopobium* species and the absence of *Prevotella*, *Sneathia*, *Gardnerella*, *Ruminococcaceae*, *Parvimonas*, *Mobiluncus*, and other taxa previously linked to bacterial vaginosis were characteristics of either Bacterial Community State Type, which is associated with bacterial vaginosis.³³

The aim of this study was to assess the relationship between BV and previous instances of spontaneous abortion and repeated miscarriages there were two hundred women in the trial who were fertile. Out of 200 women, sixty one (30.5%) had experienced at least three recurrent pregnancy losses ($N = 31$) and one spontaneous abortion in the previous six months ($N = 30$). Papanicolaou staining, Gram staining, or culture with *Gardnerella vaginalis*, the BV-associated bacterium, were the three methods used to diagnose BV. There was no significant correlation found between BV and recurrent pregnancy losses, although there was a statistically significant association between the presence of BV and the history of a spontaneous miscarriage

during the past six months ($P < 0.05$). The gestational durations of these women were also taken into consideration throughout their evaluation. Of the 61 women, forty seven experienced a miscarriage within the first trimester (≤ 12 weeks), while fourteen experienced one within the second trimester (> 12 weeks). A significantly significant correlation ($P < 0.05$) was seen between BV and second trimester miscarriages. Discharge, itching, and discomfort were not related to positive BV results ($P > 0.05$).³⁴

The study's objective was to assess the vaginal microbiota in first-trimester pregnant women who had previously experienced miscarriages. Ten pregnant women with a straightforward obstetric history and sixty pregnant women with a history of pregnancy loss were examined using real-time polymerase chain reaction, microscopic, and cultural techniques. Vaginal microbiota disorders were exclusively seen in women who had previously experienced miscarriages. Three *Lactobacillus* species—*Lactobacillus crispatus*, *Lactobacillus jensenii*, and *Lactobacillus iners*—were found to make up the majority of the lactoflora in both groups. Nevertheless, the species *L. iners* was only found in the women who had already experienced miscarriage. The prevalence of non-*Lactobacillus* microbiota was much higher in expectant mothers with a history of miscarriage. In both groups, facultative and obligate anaerobes were found. There were no appreciable variations in the levels of *Mycoplasma hominis* and *Ureaplasma* spp. in the females in either group. According to the study's findings, women who have already had miscarriage are more likely to have aberrant microbiota.³⁵

A Nested case-control research was carried out at Queen Charlotte's and Chelsea Hospital, Imperial College Healthcare London, to examine the link between first and second trimester miscarriages and the characterization of vaginal bacterial composition in the early stages of pregnancy one hundred sixty one pregnancies were

reported, of which sixty four resulted in a miscarriage in the first trimester, fourteen in the second trimester, and eighty three ended in term. Using 16S rRNA gene-based metataxonomics, prospective profiling and analysis of the composition of vaginal bacteria from 5 weeks of gestation in miscarriages or simple term births matched for age, gestational age, and body mass index principal outcome indicators. First trimester miscarriage linked to decreased frequency of vaginal microbiota dominated by *Lactobacillus* species, which was characterized by hierarchical clustering analysis. Compared with viable pregnancies. Before the diagnosis of a first-trimester loss, this was noticeable and unrelated to vaginal bleeding ($P = 0.015$). Comparing incomplete or full miscarriage to missed miscarriage, the former is linked to greater percentages of communities with reduced levels of *Lactobacillus* species. Vaginal bacterial stability throughout the first trimester of pregnancy was comparable in term pregnancies and miscarriages. Final Thoughts These results suggest that inadequate community composition is formed in the early stages of pregnancy and link the bacterial component of vaginal microbiome with miscarriages in the first trimester. Vaginal bacterial makeup may be a modifiable risk factor for first trimester miscarriage, while further research is needed to fully understand the mechanism.³⁶

To ascertain the part infections play in miscarriages, a retrospective investigation was carried out. The chorionic villi derived from aborted material underwent cytogenetic assessment and were examined for the presence of human papillomaviruses, adeno-associated virus, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and human cytomegalovirus. PCR is used in placenta tissues for karyotyping and the identification of viral and bacterial DNA. The karyotype was found to be normal in fifty four (50%) of the one hundred and eight samples, aberrant in thirty eight (35%) of the samples, and unknown in sixteen (15%) of the samples. Of

the one hundred and eight samples, the karyotype was found to be normal in fifty four (or 50%), aberrant in thirty eight (or 35%), and unknown in sixteen (15%) of the samples. AAV-2, M. hominis, HCMV, U. urealyticum, or M. hominis DNA were not found; (8%) samples had HPV DNA and one (1%) sample had C. trachomatis DNA. There was no discernible link found between the karyotype status and HPV-positive results. This study investigated into the possible impact of abnormalities in the vaginal flora on recurrent miscarriages that go unexplained. It is yet unclear how a balanced vaginal microbiome protects women from gynaecological illnesses and promotes their general health.³⁷

A case control study was carried out to investigate the potential effect of aberrations in the vaginal flora on unexplained recurrent miscarriage (RM). Ten patients with unexplained RM and ten healthy volunteers had their vaginal bacterial communities extracted, and the Illumina MiSeq platform was used to perform sequencing studies of the V3 and V4 sections of the bacterial 16S ribosomal RNA gene. The bacterial community architectures in the RM and control groups differed, according to beta diversity and main component analyses. Alpha diversity estimation showed lower microbial diversity in RM patient samples. Taxonomic study showed a substantial difference in the abundance of three phyla (Actinobacteria, Bacteroidetes, and Firmicutes) between the RM and the normal control group. Moreover, Lactobacillus was the most prevalent genus in both groups at the genus level. Five genera showed statistically significant variations between the two groups. Only two bacterial taxa (Lactobacillus and Gardnerella) were overrepresented in the control group, compared to three bacterial taxa (Atopobium, Prevotella, and Streptococcus) that were considerably more common in the RM group. To sum up, the current

findings offer experimental evidence in favor of vaginal flora dysbiosis in RM women.³⁸

To determine the effect of human papillomavirus (HPV) infection on spontaneous abortion, spontaneous preterm birth, the pregnancy rate of women using assisted reproductive technologies and spontaneous abortion of an pregnancy, a systematic review was conducted. Researcher searched the Cochrane Library, PubMed, Medline, and Embase up until December 16, 2016. An effect size was chosen based on the relative risk (RR) or OR and its associated 95% confidence interval. Both indiscriminate and high-risk HPV genotype infections were subjected to subgroup analysis. STATA 12.0 was used for statistical analysis. There were eighteen studies total. There was no significant correlation found in eight studies between HPV infection and spontaneous abortion. Subgroup analysis, however, revealed that although HRHPV infection had no discernible impact indiscriminate genotype HPV infection raised the probability of spontaneous abortion. The findings showed that sPTB was at risk for HR-HPV infection. It was discovered that HPV infection was unrelated to both the spontaneous abortion of an ART pregnancy and the ART-based clinical pregnancy rate. The incidence of spontaneous abortion can be elevated by indiscriminate HPV genotype infection, and sPTB was associated with a risk factor for HR-HPV infection. Nevertheless, there was insufficient data to support a link between HPV infection and the rate of ART pregnancy as well as spontaneous ART pregnancy termination. Various HPV infection genotypes may contribute differently to unfavourable pregnancy outcomes.³⁹

A comparative study was carried out to evaluate the distribution of flora in women who had spontaneous abortions (SA) and those who were in good health, in the Gynaecological and Public Health Centre of hospital; samples of vaginal

discharge were taken from 3,233 women. Hospital's Microbiological Diagnostic Centre tested for pathogens through culture, testing non-pregnant women (control group, n = 812) and the SA group (n = 1,513) as well as normal first-trimester groups (NP group, n = 908). There were four hundred twenty five samples (13.15%) of vaginal discharge out of 3,233 that tested positive for a pathogen.. *Enterococcus faecalis*, *Candida albicans*, *Escherichia coli*, group B *Streptococcus* (GBS), and *Ureaplasma urealyticum* (Uu) were the five most common bacteria in the SA group; Uu, *C. albicans*, *Candida tropicalis*, GBS, and *E. faecalis* were in the NP group; and in the control group, it was *Gardnerella vaginalis*, Uu, *E. coli*, and *C. albicans*. The pathogen detection rate was significantly higher in the SA group. It was relatively common for pregnant women to have a *Candida* sp. infection. Overtreating asymptomatic candidiasis is not advised in standard prenatal care. The fact that there was no discernible variation in the Uu detection rate between the SA group and the other two groups indicates that SA is not caused by it.⁴⁰

This study set out to assess the relationship between BV and previous instances of spontaneous abortion and repeated miscarriages. There were 200 women in the trial who were fertile. Out of two hundred women, 61 (30.5%) had experienced at least three recurrent pregnancy losses (N = 31) and one spontaneous abortion in the previous six months (N = 30). BV was identified by growing *Gardnerella vaginalis*, a bacterium linked to BV, or by utilizing Papanicolaou staining or Gram staining. The history of a spontaneous abortion within the last six months was statistically linked to the presence of BV ($P < 0.05$), although there was no significant correlation found between BV and repeated miscarriages ($P > 0.05$). The women were also assessed considering their gestational durations. Out of sixty one women, forty seven or (77%) experienced a miscarriage within the first trimester (≤ 12 weeks), while fourteen (or

23%) experienced a miscarriage within the second trimester (>12 weeks). A statistically significant correlation was seen between BV and miscarriage during the second trimester ($P < 0.05$). Discharge, itching, and pain were not linked to positive BV results ($P > 0.05$). BV may be a factor in second trimester miscarriages and spontaneous abortion.⁴¹

In the realm of obstetrics and gynaecology, the diagnosis and treatment of unexplained recurrent spontaneous abortion (URSA) are significant and often discussed. Researcher discovered via clinical inquiry (observation) that vaginal dysbacteriosis or recurrent vaginitis is typically experienced by URSA patients during non-pregnancy, pregnancy, and the post-abortion period. On the other hand, the impact of vaginal dysbacteriosis on URSA has not been studied. This study explores the relationship between difference bacteria at the genus level and the incidence of URSA utilizing women who had normal induced abortions as a control group and 16S rRNA sequencing, which aids in screening differently expressed flora. Determining if specific pathogenic genera can induce an imbalance in immunological tolerance of the maternal and fetal interface through regulating chemokines, which results in recurrent spontaneous abortion, was another goal of this research. The pathogenesis of URSA has been examined in this paper from the standpoint of differently expressed vaginal flora, which has important theoretical implications for the early detection and management of URSA.⁴²

In a longitudinal study spanning two prenatal visits and one postpartum visit, researcher examined the vaginal microbiota in a sample of women aged 18 and over who were both HIV-positive and negative. HIV testing self-collected vaginal swabs for microbiome sequencing and point-of-care STI testing was carried out. The investigator classified microbiological communities and assessed alterations during

gestation as well as correlations with HIV status and STI diagnosis. Researcher found four main community state types (CSTs) among 242 women (mean age 29, 44% living with HIV, 33% diagnosed with STIs): two CSTs that are dominant in lactobacillus (dominated by *Lactobacillus crispatus* and *Lactobacillus iners*, respectively) and two CSTs that are diverse and non-dominant (dominated by *Gardnerella vaginalis* and another by other facultative anaerobes). 60% of women in the *Gardnerella*-dominant CST switched to *Lactobacillus*-dominant CSTs from the first prenatal visit to the third trimester (24–36 weeks gestation). 80% of the women in *Lactobacillus*-dominant CSTs switched to non-*Lactobacillus*-dominant CSTs from the third trimester to postpartum, including a significant percentage in the facultative anaerobe-dominant CST. The microbial composition varied according to the diagnosis of STIs, and women who had a STI were more likely to have CSTs that were dominant in *Gardnerella* or *L. iners*. Overall, researchers discovered that during pregnancy, there was a change toward *Lactobacillus* dominance and that in the postpartum phase; a unique, extremely diversified anaerobe-dominant microbiome population emerged.⁴³

This study evaluates the state of knowledge on the composition of the vaginal microbiota, with a particular emphasis on the microbiome during pregnancy. The background information on the contribution to world health and the significance of these microbial communities to pregnancy are provided with this knowledge. Included is a quick overview of the techniques used to date to look into the composition of these microbial populations. The vaginal microbiota has been studied using two different approaches: cross-sectional and longitudinal. Both approaches are examined since the data they offer can be utilized to piece together a more comprehensive picture of the vaginal microflora and its changes throughout

pregnancy. The identity of the microorganisms found in the vagina is investigated in relation to health and illness, and in particular, in relation to the consequences of pregnancy. Analyses of the distinct functions of different *Lactobacillus* species are conducted in order to assess the protective function of lactobacilli in preserving a healthy vaginal environment. Study discussed the differences between vagitypes of the vaginal microbiota in pregnant and non-pregnant women. A thorough analysis is conducted on the relationships between certain taxa and the three unfavourable outcomes of pregnancy: miscarriage, stillbirth, and premature birth. This microbiota loses diversity and richness in comparison to the microbiomes seen in non-pregnant women, according to longitudinal studies examining variations in the abundance of different taxa and the composition of the bacterial community. Even with all of the work that has gone into characterizing the vaginal bacterial microbiota, there are still a lot of unanswered questions.⁴⁴

A metagenomic approach for identifying the vaginal microbiota signature during pregnancy allow a metagenomic characterization of the adult human microbiota; nevertheless, population-level research is needed to determine the precise timing and location of these diverse microbial communities' insertion into the host and their reproductive cycles. Because microbial diversity and abundance may vary during pregnancy, the goal of the study was to produce comparative metagenomic signatures for different gestational age groups. Introitus, posterior fornix, and midvaginal DNA were separated, and the V5V3 region of bacterial 16S rRNA genes was sequenced using the 454FLX Titanium platform. 301 non-pregnant controls (60 participants) and 68 samples from 24 healthy gravidae (18 to 40 confirmed weeks) were compared. The sequence data that were generated underwent quality filtering, taxonomy binning, normalization, and organization into operational taxonomic units

(OTU) based on phylogeny. The resulting beta diversity measures were then subjected to principal coordinates analysis (PCoA), which was utilized for visualization and analysis in conjunction with sample clinical metadata. For computational analysis, 1.4 gigabytes of data totaling over 2.5 million reads (averaging 6,837 sequences/sample of 493 nt in length) were produced. A distinct vaginal microbiome signature encompassing several specific OTUs and higher-level clades was nevertheless observed and confirmed using a combination of phylogenetic, non-phylogenetic, supervised, and unsupervised approaches, even though gravidae were not excluded due to a posterior fornix pH >4.5 at the time of screening. Pregnancy decreased both total diversity and richness, with *Lactobacillus* species (*Lactobacillus iners crispatus*, *jensenii*, and *johnsonii*) dominating, as well as the orders Lactobacillales (including the Lactobacillaceae family), Clostridiales, Bacteroidales, and Actinomycetales. With variation of taxa across vaginal subsite and gestational age, study intergroup comparison employing strict standardized collection techniques and analytical methodologies offers strong preliminary evidence that the vaginal microbial 16S rRNA gene catalogue uniquely differs in pregnancy.⁴⁵

A prospective cohort research study was carried out to determine potential vaginal microbiological reasons of missed abortions by investigating the relationship between disturbed vaginal microbiota and missed abortions. Involving a comparative group. Early-stage pregnant women's vaginal secretions from the posterior fornix were collected. Analysis was done on 50 cases of normal pregnancies needing an induced abortion (reference group) and 54 cases of missed abortions (case group). A bacterial chip was used to detect the bacteria after the DNA was extracted and hybridized using primers unique to each gene. α - and β -diversity analyses, as well as the linear discriminant analysis effect size approach, were used to compare the

makeup and relative abundance of vaginal microbiota in the two groups. The case group's Shannon index was higher and its Simpson index was lower than the reference group's, according to the α -diversity analysis. Both the relative abundances of Firmicutes (42.52%) and Lactobacillus (16.51%, reference group 23.00%, $p < 0.05$) were lower in the case group than in the reference group (51.03%, $p < 0.05$). It's interesting to see that the case group had reduced levels of Ureaplasma and Mycoplasma genitalium ($p < 0.05$). Additionally, there was a substantial decrease ($p < 0.05$) in the case group's relative abundance of Lactobacillus crispatus, Lactobacillus jensenii, and Lactobacillus gasseri compared to the reference group. Whereas the reference group's enriched pathways were mostly linked to genetics, the case group's enriched pathways were primarily related to metabolism.⁴⁶

A case-control study was conducted, wherein non-pregnant patients with RSA were chosen and split into groups that received no treatment at all and treatment with medication. Patients on medication were split into three groups: those on metformin alone, those on metformin plus aspirin, and those on other medicines. The control group consisted of well-born women who never had an abortion on their own initiative. Using the Illumina MiSeq platform, 16S rRNA gene sequencing of the V3-V4 region was used to assess the characteristics of the vaginal microbiomes of patients with RSA and healthy women, as well as the effects of medication treatment on the microbiome. The microbial richness was lower in RSA-affected women than in healthy women. Seven taxa in the patient's vaginal microbiota showed substantial changes in relative abundance as compared to controls (Megasphaera, Sneathia sanguinegens, Pseudomonas, Sphingomonas, Rhodococcus, Burkholderia-Caballeronia-Paraburkholderia, and Corynebacterium_1). These changes may be directly linked to RSA. Drug therapy changed the makeup of the vaginal microbial

population in RSA patients. The relative abundance of vaginal *Lactobacillus* spp. in individuals was considerably raised when metformin and aspirin were used together.⁴⁷

A prospective cohort study was carried out to investigate the relationship between the vaginal microbiota and SA before to twelve weeks of gestation, and described the variety and makeup of the vaginal microbiota in pregnant women during their first trimester. At 5–8 weeks of gestation, 16S rRNA gene sequencing in the V3–V4 regions was used to evaluate the bacterial profiles of the participants. The study group consisted of forty eight patients who had SA at 12 weeks of gestation, whereas the control group consisted of one hundred and sixteen women who had normal pregnancies (NPs). Results showed that compared to women with NPs, the vaginal microbiome in SA patients was richer and had a distinct composition. Additionally, there was a notable enrichment of the genus *Apotobium* in patients with SA. *Atopobium*-induced SA could be more accurately classified using an extreme gradient-boosting. Furthermore, the results demonstrated a strong correlation between *Apotobium* and SA after controlling for relevant confounders. In conclusion, SA in the first trimester was linked to greater vaginal *Apotobium* levels.⁴⁸

A study examined whether there were any differences in the vaginal microbiome between women who had their first or second child. Classifying DNA sequences from the V1-V3 region of bacterial 16S rRNA genes allowed researchers to determine the relative abundance of bacteria in the vaginal microbiome in first trimester pregnant women: fifty two with their first known conception, twenty six with a prior spontaneous or induced abortion but no deliveries, and seventy seven with at least one prior birth. In 76.4% of women who conceived for the first time, 50.0% who had previously had a planned or spontaneous abortion, and 22.2% who had previously given birth, *Lactobacillus crispatus* was the bacterium with the highest

numerical abundance ($p \leq 0.01$). 3.8% of women who became pregnant for the first time had the highest concentration of *L. iners*, compared to 19.2% ($p = 0.03$) and 20.8%. The most common bacterial genus, *Gardnerella*, rose from 3.8% in women who conceived for the first time to 15.4% and 14.3% in women who had previously given birth or had an abortion, respectively ($p > 0.05$). A history of spontaneous abortion was similarly linked to *L. iners* dominance ($p \leq 0.02$). Pregnancy history affects the makeup of the vaginal microbiome and how it affects the course of a pregnancy.⁴⁹

A prospective observational cohort study was carried out to compare the local immune response and the makeup of vaginal microbes in chromosomally normal and atypical miscarriages to those of simple pregnancies that end at term. The study examined the vaginal microbiota in a cohort of one hundred and sixty seven women, ninety three miscarriages (54 euploid and thirty nine aneuploid using molecular cytogenetics), and seventy four women who delivered their babies at term using 16S rRNA gene-based metataxonomics. The results were correlated with the aneuploidy status of the miscarriages. The amounts of cervical vaginal fluid were also examined. The findings indicate that, in comparison to aneuploid miscarriage, euploid miscarriage is linked to a much higher incidence of *Lactobacillus* spp. depleting vaginal microbial populations ($P = 0.01$). Combining matching immunological profiles from cervicovaginal fluid revealed that *Lactobacillus* species weakened the vaginal microbiota, which was most substantially linked to pro-inflammatory cytokine levels in euploid miscarriages as opposed to viable term pregnancies.

The study's objective was to reveal that there were no noteworthy variations in alpha diversity between the microbiota of the upper and lower female reproductive tracts from RSA patients and the control group. The vaginal microbiota ($P = 0.036$),

cervical microbiota ($P = 0.010$), and microbiota from uterine lavage fluid ($P = 0.001$) of the RSA group showed considerably higher beta diversity than the control group. Furthermore, the RSA group exhibited significant reductions in interleukin-6 and gamma interferon levels of cytokines. Based on results, the RSA group's vagina, cervix, and uterine lavage fluid had altered microbial biodiversity. A potential risk factor for the development of RSA is changed cytokine levels, which may be linked to changes in the microbiota of the uterine cavity. Additionally, the uterine cavity's microbiota composition varied significantly between endometrial tissue and uterine lavage fluid in the RSA group, as did the microbiome composition in the lower genital tract. Therefore, sampling simultaneously using these two techniques provided for a more thorough understanding of microbial colonization in the uterus.⁵⁰

Study evaluates the state of knowledge on the composition of the vaginal microbiota, with a particular emphasis on the microbiome during pregnancy. The context of the contribution to world health and the significance of these microbial communities for pregnancy are provided with this knowledge. Included is a quick overview of the techniques used to date to look into the composition of these microbial populations. Included is a brief review of the techniques used to date to look into the composition of these microbial populations. The vaginal microbiota has been studied using two different approaches: cross-sectional and longitudinal. Both approaches are examined since the data offered can be utilized to piece together a more comprehensive picture of the vaginal microflora and its changes throughout pregnancy. The identification of the microorganisms in the vagina are investigated in relation to health and illness, and in particular, in relation to the consequences of pregnancy. An assessment is made of the protective function of lactobacilli in preserving a healthy vaginal environment, along with an examination of the distinct

functions of different *Lactobacillus* species. A thorough analysis is conducted on the relationships between certain taxa and the three unfavorable outcomes of pregnancy: miscarriage, stillbirth, and premature birth. Studies conducted over an extended period of time examining alterations in the makeup of the bacterial community and the quantity of different taxa show that the microbiota in this context is less diverse and rich than that seen in microbiomes that are not pregnant.⁵¹

A nested case control study was conducted to determine the composition of the vaginal or endometrial microbiome linked to repeated miscarriages. Vaginal samples were taken from thirty nine healthy control women without a history of pregnancy loss and forty seven women who had lost two or more pregnancies in a row. Using 16S rRNA gene amplicon sequencing, the endometrial and vaginal microbiota compositions were examined between individual vaginal and endometrial samples as well as between the RPL and control women. Internal transcribed spacer 1 amplicon sequencing was used to analyze the makeup of the mycobiota in order to provide a descriptive overview. Age, parity, and body mass index adjustments were made to the models. To define nominal statistical significance at $q < 0.05$, false discovery rate-corrected P-values, or q-values, were employed. Using internal transcribed spacer 1 amplicon sequencing, the makeup of the mycobiota was examined for a descriptive overview. Age, parity, and BMI were taken into account while adjusting the models. At $q < 0.05$, nominal statistical significance was defined using False Discovery Rate-Corrected P-values, or q-values.⁵²

A randomized, double-blind, placebo-controlled intervention study was carried out to examine the microbiota and bacterial vaginosis (BV) settings in pregnant women with normal microbiota and note how oral probiotics affect these parameters and the women's BV status. The 16S rRNA gene amplicons were analyzed

using a high-throughput Illumina-MiSeq sequencing approach, and the pre- and post-probiotic (*Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14) intervention for a month was characterized, as well as the vaginal and gut microbiota of pregnant women with and without BV. The study found that pregnant women with BV and healthy women had different gut and vaginal microbiome compositions. *Lactobacillus helveticus* dominated the vaginal microbiota of healthy women, with *Lactobacillus iners* and *Lactobacillus gasseri* following closely behind. On the other hand, women who tested positive for BV showed higher levels of α -diversity and lower levels of *L. helveticus* abundance. Similar to this, all pregnant women without BV had vaginal samples containing *Prevotella copri*, a gut microbe linked to a normal environment; in contrast, all BV women had *Gardnerella vaginalis*, which dramatically decreased with probiotic treatment. In addition, gut microbiome showed that *P. copri* was more prevalent in healthy women than it was in BV patients. The abundance of the bacterial clade *P. copri* decreased in the placebo group from 7.75 to 4.84 percent, while it grew in the probiotic group from 9.17 to 16.49 percent.⁵³

A prospective cohort study was conducted to evaluate the changes in composition and diversity can impact pregnancy outcomes. The French ethics committee, the "Comité de Protection des Personnes Nord-Ouest III" (Caen, France), assessed the reported study. A signed informed permission form was supplied by each participant in the In SPIRe cohort, authorizing the collection of biological samples and medical data. Only the non-human portion of the DNA taken from vaginal swabs of expecting moms is included in this paper. Patient clinical data was gathered and stored in a secure computerized database. Anonymized exports were made from the analysis database. Vaginal swabs that had been processed were marked with matching anonymous inclusion numbers. in the last trimester of their pregnancy. Study

demonstrates the significance of lactobacilli, particularly *L. crispatus*, in preserving low diversity and the connection between premature delivery and their disappearance. We also show that the strongest risk indicator is not species-specific but rather the overall diversity of the vaginal microbiota.

This study evaluates the state of knowledge on the composition of the vaginal microbiota, with particular attention to the microbiome during pregnancy. The context of the contribution to world health and the significance of these microbial communities for pregnancy are provided with this knowledge. Included is a quick overview of the techniques used to date to look into the composition of these microbial populations. The vaginal microbiota has been studied using two different approaches: cross-sectional and longitudinal. Both approaches are examined since the data they offer can be utilized to piece together a more comprehensive picture of the vaginal microflora and its changes throughout pregnancy. The identity of the microorganisms found in the vagina is investigated in relation to health and illness, and in particular, in relation to the consequences of pregnancy. An assessment is made of the protective function of lactobacilli in preserving a healthy vaginal environment, along with an examination of the distinct functions of different *Lactobacillus* species. Vaginal microbiota classifications into vagitypes in women who are not pregnant and those who are pregnant are discussed. A thorough analysis is conducted on the relationships between certain taxa and the three unfavorable outcomes of pregnancy: miscarriage, stillbirth, and premature birth. This microbiota loses diversity and richness in comparison to the microbiomes seen in non-pregnant women, according to longitudinal studies examining variations in the abundance of different taxa and the composition of the bacterial community. Even with all of the work that has gone into characterizing the vaginal bacterial microbiota, there are still a lot of unanswered questions.⁵⁴

A study conducted to evaluate the vaginal bacterial composition under a microscope and correlated the results with the vaginal metabolic profiles during pregnancy at two distinct gestational ages. A vaginal swab was taken at gestational ages of 9–13 weeks (first trimester) and 20–24 weeks (second trimester) from 67 pregnant Caucasian women who presented to the Family Advisory Health Centres in Ravenna, Italy. Women were grouped into three categories based on the Nugent score: "H" (normal lactobacilli-dominated microbiota), "I" (intermediate microbiota), and "BV" (bacterial vaginosis). Using a ¹H-NMR spectroscopy, a metabolomic study was carried out starting with the cell-free supernatants of the vaginal swabs. As the first trimester progressed, fewer incidences of dysbiosis were seen and more women displayed a typical lactobacilli-dominated microbiome. The vaginal metabolic profiles saw significant alterations in correlation with these microbial modifications. The amounts of metabolites linked to BV, such as acetate, propionate, tyramine, methylamine, and putrescine, were shown to have significantly decreased over the course of the weeks. Higher quantities of lactate and a number of amino acids, such as tryptophan, threonine, isoleucine, and leucine, which are normally present in healthy vaginal settings, were also seen in the vaginal metabolome. The vaginal metabolome grew more homogenous and less varied with time. In the second trimester, women with BV had metabolic profiles that were closer to those of the healthy/intermediate groups than they were in the first. The pathophysiology of pregnancy may benefit from better understanding of the function that vaginal metabolites play.⁵⁵

A complaint review was carried out to literature on VMB composition in pregnant women from sub-Saharan Africa. The PubMed and Embase databases were searched using PRISMA standards. Included were observational and interventional studies that used genetic methods to analyze vaginal microbiome populations from

pregnant mothers in sub-Saharan Africa. There were ten studies that were conducted in seven sub-Saharan African nations. In the groups they analyzed, they separately demonstrated that *Lactobacillus*-dominant VMB (especially *L. iners* or *L. crispatus*) or VMB including *Lactobacilli* are the most common, followed by a more diversified anaerobe-dominant VMB. While *Lactobacillus* dominated the VMB of most pregnant women with a sexually transmitted illness, anaerobic species were much more prevalent. *Lactobacillus* species are the most common VMB species during pregnancy in sub-Saharan African women, according to research done in other cultures. In these groups, diversified anaerobe-dominant VMB is highly frequent. There are few, inconsistently conducted research on VMB during pregnancy in Africa, and the body of information is very small. Further understanding of the makeup of VMBs and potential differences between these groups is required.⁵⁶

A cohort study was carried out to determine whether the rates of conception and miscarriage during the first trimester are impacted by bacterial vaginosis. Research was conducted in the assisted conception section of a teaching hospital in Leeds, and eight hundred sixty seven consecutive women undergoing in vitro fertilization were enrolled in the research. By using a Gram-stained vaginal swab to screen for bacterial vaginosis to assess the rate of conception and miscarriage in the first trimester, as well as the existence of bacterial vaginosis or normal vaginal flora. The findings suggested that one hundred ninety women out of seven hundred seventy one (24.6%) had bacterial vaginosis. There was no discernible difference in the rate of conception between the 61 women (32.1%) and the 146 of 493 women (29.6%) who had normal vaginal flora. Nonetheless, compared to 27 women (18.5%) with normal vaginal flora, 22 women (31.6%) with bacterial vaginosis who became pregnant had a noticeably higher risk of miscarriage in the first trimester. Even after

accounting for variables known to raise the chance of miscarriage, such as smoking, growing older, having had three or more miscarriages in the past, not having had a live delivery in the past, and having polycystic ovaries, the higher risk persisted.⁵⁷

A Nested case-control study was carried out to examine the association between the composition of vaginal bacteria in the first and second trimesters of pregnancy and miscarriage. There were one hundred sixty one pregnancies total: sixty four miscarriages in the first trimester, fourteen miscarriages in the second trimester, and eighty three term pregnancies. from Chelsea and Queen Charlotte's Hospital, London's Imperial College Healthcare were enrolled in the study , Using 16S rRNA gene-based metataxonomics, prospective profiling and analysis of the composition of vaginal bacteria from 5 weeks of gestation in miscarriages or simple term births matched for age, gestational age, and body mass index. When comparing first trimester miscarriages to viable pregnancies, there was a lower prevalence of *Lactobacillus* spp.-dominated vaginal microbiota this was noticeable prior to the diagnosis of a first-trimester miscarriage ($P = 0.015$) and unrelated to vaginal bleeding. When compared to missed miscarriages, incomplete or complete miscarriages were linked to larger percentages of communities with reduced levels of *Lactobacillus* spp. The vaginal bacterial stability during the early stages of pregnancy was comparable in miscarriages and term pregnancies.⁵⁸

A review was done to examine to investigate the areas of current scientific interest on the contribution of infection to miscarriage. PubMed yielded two hundred and ninety two and three hundred and seventy two papers, respectively, including the keywords "miscarriage" and "infection" or "infections," pertaining to papers. Included were pertinent human research (meta-analyses, case-control studies, cohort studies, or case series). Reports with only one case were not included. Based on the Newcastle –

Ottawa Quality Assessment Scale, the studies were graded. It has been established that there is a higher chance of miscarriage when systemic illnesses such as bacterial vaginosis, dengue fever, cytomegalovirus, human immunodeficiency virus, malaria, and influenza virus are present in the vagina. Pregnancy outcomes do not seem to be impacted by Q fever, adeno-associated virus, Bocavirus, Hepatitis C, or *Mycoplasma genitalium* infections. There is ongoing debate on the effects of infections with *Chlamydia trachomatis*, *Toxoplasma gondii*, human papillomavirus, herpes simplex virus, parvovirus B19, hepatitis B, and polyomavirus BK. Some studies suggest an increased chance of miscarriage, while others show no higher risk. The most recent information on syphilis and rubella shows a decline in the frequency of their reported correlations with miscarriage and an increase in prenatal screening globally. The mechanism(s) underlying infection-induced miscarriage remain unclear despite the fact that a number of pathogens have been linked to the condition.⁵⁹

A study was conducted to study the relationship between bacterial vaginosis and miscarriages in the first trimester by screening pregnant women at seven weeks for the disease using the Nugent gram stain score method. Study used the Nugent gram stain scoring system to screen two hundred nineteen pregnant women for bacterial vaginosis at 7 weeks of gestation and track them until the end of the first trimester in order to evaluate the relationship between bacterial vaginosis and first trimester miscarriage. Pregnant women were split into two groups based on the results of the culture and the Nugent Gram stain score system: Group I: one hundred sixty seven women, or 76.3% of the total, are BV-negative. Fifty two women, or 23.7%, tested positive for bacterial vaginosis in Group II, then kept an eye on them till the first trimester's conclusion. Since the purpose of our study was to look at first trimester miscarriage, we decided to use 13 weeks as the cutoff point. Compared the

first trimester miscarriage rates of the two groups. The present investigation found that the incidence of bacterial vaginosis among the percentage of pregnant women was 23.7%. Of the 219 women who took part in the study, thirty eight (17.4%) lost their babies in the first trimester, and one hundred and eighty one women 82% of women carried on with their pregnancies past thirteen week. When comparing pregnant women with bacterial vaginosis to women who are negative for the disease, there is no correlation between bacterial vaginosis and first trimester Miscarriage⁶⁰.

A study was conducted to identify specific BV-associated bacteria associated with miscarriage, researchers at Temple University Hospital in Philadelphia, Pennsylvania, examined the prevalence and concentration of seven BV-associated bacteria in urban pregnant patients receiving regular prenatal care at five urban obstetric practices before 14 weeks of gestation. Of the four hundred and eighteen pregnant women who participated in this examination, seventy four of them had miscarriages. Mean log concentration of BVAB3 was significantly higher among women experiencing a miscarriage (4.27 vs. 3.71, p value = 0.012). Younger women with high levels of BVAB3 had the greatest risk of miscarriage. In addition, we found a significant decreased risk of miscarriage among women with higher log concentrations of *Leptotrichia/Sneathia* species or *Megasphaera* phylotype 1-like species early in pregnancy.⁶¹

The study aim to examine the early pregnancy cervical and vaginal microbiomes in three groups of women: those who miscarried, those receiving treatment for estrogen deficiency, and those who were in good ongoing pregnancies. Fifty one women between the ages of eight and eleven weeks were included in the study. Using the Ion Torrent PGM technology and 16S rRNA sequencing, the microbiome was investigated. *Lactobacillus iners* predominated in all groups,

indicating that most participants share the vaginal community type CST III. Notably, our findings demonstrated the important roles that *Mycoplasma girerdii* and *Gardnerella vaginalis* play in the pathophysiology of early miscarriage. On the other hand, during the early stages of pregnancy, *L. iners* and *Bifidobacterium longum* provide protection. Furthermore, there seemed to be a significant correlation between the vaginal and cervical microbiomes and testosterone intake. In summary, our research has improved comprehension of the composition of the cervical and vaginal microbiomes in early pregnancy among people from Eastern Europe.⁶¹

A study was undertaken to e to assess the relationship between African American women's spontaneous preterm births (sPTBs) and early term births (sETBs) and the vaginal microbiota during the early stages of pregnancy. Four hundred thirty six women who were part of the Emory University African American, vaginal, Oral, and Gut Microbiome in pregnancy had their vaginal samples taken in the early stages of pregnancy (8–14 weeks' gestation). These samples were subjected to 16S rRNA gene sequencing of the V3–V4 region, taxonomic classification, and community state type (CST) assignment. Researcher contrasted the abundance of taxa and vaginal CST between women whose pregnancies ended in sPTB (N = 44) or sETB (N = 84) with those who gave birth at term (N = 231). Just 16% of the women had CST I, II, or V (non-iners *Lactobacillus* dominated), one-third had CST III (*L. iners* dominated), and nearly half had CST IV (Diverse CST) vaginal microbiomes. In multivariate logistic regression, both CST III (*L. iners* dominated) and CST IV (Diverse) were linked to sPTB with adjusted odds ratios (95% confidence interval) of 4.1 and 7.7 respectively, in comparison to vaginal CST I, II, or V (non-iners *Lactobacillus* dominated). On the other hand, sETB was not linked to any vaginal CST. The vaginal microbiota had a significant overall effect on sPTB (p=0.034) but not sETB (p=0.320) according to the

linear decomposition model (LDM) based on amplicon sequence variant (ASV) relative abundance, while the LDM based on presence/absence of ASV revealed a significant influence on sETB ($p=0.030$) but no overall effect on sPTB ($p=0.328$).⁶²

A longitudinal, retrospective case-control study was undertaken using sequence-based methods to describe the vaginal microbiota during a typical human pregnancy comprised thirty two women who were not pregnant and twenty two pregnant women who delivered their babies at term (38 to 42 weeks) without any difficulties. Patients who were not pregnant as well as those who were pregnant provided serial vaginal fluid samples. Pyrosequencing was used to do a survey based on the 16S rRNA gene sequence in order to evaluate the stability and structure of the vaginal microbiota. Generalized estimating equations and linear mixed effects models were utilized to determine which phylotypes had a difference relative abundance between the two research groups. Normal pregnant women had a distinct vaginal microbiota than non-pregnant women; the former had a lower abundance of twenty two other phylotypes and the latter of *Lactobacillus vaginalis*, *L. crispatus*, *L. gasseri*, and *L. jensenii*. In a typical pregnancy, the presence of *Prevotella*, *Sneathia*, *Gardnerella*, *Ruminococcaceae*, *Parvimonas*, *Mobiluncus*, and other taxa previously linked to bacterial vaginosis was less common than in bacterial community state type (CST), which is characterized by a high relative abundance of *Atopobium* species.

The vaginal microbiota of pregnant women was found to be more stable than that of non-pregnant women. Nevertheless, in a typical pregnancy, bacterial populations mostly transition from one CST dominated by *Lactobacillus* spp. to another CST dominated by *Lactobacillus* spp.⁶³

A study was conducted to examine the distribution of common *Lactobacillus* species among pregnant women's. In the study, sixty seven expectant mothers were enrolled, and vaginal samples were taken for Gram staining. Based on Nugent's score, women were categorized as having normal vaginal flora, intermediate flora, or bacterial vaginosis (BV). In order to identify *Lactobacillus* species using the multiplex polymerase chain reaction (PCR) profiling of 16S rDNA amplification method, vaginal samples were also obtained. In pregnant women with normal flora, *Lactobacillus crispatus* (100%) was the most common *Lactobacillus* species. It was followed by *Lactobacillus iners* (77%), *Lactobacillus jensenii* (74%), and *Lactobacillus helveticus* (60%). While *L. crispatus*, *L. jensenii*, and *L. helveticus* dramatically decreased when the vaginal flora shifted to intermediate and BV, *L. iners* was often prevalent across groups in women with normal, intermediate, or BV flora. With the exception of *L. iners*, the prevalence of other lactobacilli was lower in women with BV. No vaginal sample had species like *Lactobacillus rhamnosus*, *Lactoferrulentum*, *Lactocasei paracasei*, or *Lactobacillus casei*. The species that predominated in women with normal flora were *L. crispatus*, *L. jensenii*, and *L. helveticus*. Probiotic qualities of *L. crispatus* alone or in combination with *L. jensenii* and *L. helveticus* may be assessed for BV prevention and treatment.⁶⁴

A retrospective study was carried out to the impact of vaginal *Lactobacillus* species on obstetrical outcomes in women with moderate vaginal flora. The data from all singleton pregnant women who had routinely undergone screening for asymptomatic vaginal infections at our tertiary referral center was retrospectively analyzed by the researcher. Gram-stained vaginal smears were categorized as having normal vaginal flora (score 0-3), intermediate vaginal flora (score 4-6), or bacterial vaginosis (score 7–10) using the Nugent grading system. The study only included

females with moderate vaginal flora. Women with and without lactobacilli were classified based on their Nugent score of 4. A follow-up smear was taken 4-6 weeks following the original smear. Welch's t-test, Fisher's exact test, multiple regression analysis with confounding adjustment, and descriptive data analysis were all carried out. The outcome measures were infant weight and gestational age at delivery. At antenatal screening, 529/8421 women presented with intermediate vaginal flora. Amongst these, 349/529 (66%) had a Nugent score of 4, 94/529 (17.8%) a Nugent score of 5, and 86/529 (16.2%) a Nugent score of 6. Amongst those with a Nugent score of 4, 232/349 (66.5%) women were in the Lactobacilli group and 117/349 (33.5%) in the Non-Lactobacilli group. The preterm delivery rate was significantly lower in the Lactobacilli than in the Non-Lactobacilli group. Mean birth weight was 2979 ± 842 g and 2388 ± 1155 g in the study groups, respectively. On follow-up smears, bacterial vaginosis rates were 9% in the Lactobacilli and 7.8% in the Non-Lactobacilli group.⁶⁵

A Prospective observational study conducted In order to investigate potential variations in the vaginal microbiota between miscarrying and normally pregnant women included twenty four participant women who were pregnant in their first trimester. Using an Illumina MiSeq instrument and QIAGEN reagents, a sequencing analysis of the V4 region of the 16S rRNA gene was performed to characterize the vaginal microbiomes of 24 women. Clinical information relating to pregnancy and vaginal microbiome were correlated. Plotting differentiation revealed variations in the microbiota makeup between controls and miscarried mothers. 50% of nulliparous women had *Lactobacillus crispatus* as their predominant bacteria. In comparison to 15% ($p = 0.011$) of women with no history of miscarriage and no miscarriage in the study, *Lactobacillus iners* was the predominant bacterium in 50% of women with a

history of previous miscarriages and miscarriages during the trial. The quantity of operational taxonomic units and the microbiome richness of miscarried mothers differed significantly from those of term deliveries. Different relative abundances of eight species were discovered in the two groups of women. The results of the study showed that a woman's past pregnancies influence the makeup of her vaginal microbiome. Additionally, there was a notable distinction in the vaginal microbiomes of miscarried and full-term delivery mothers, both in terms of the general microbiome populations and the abundances of specific taxa.⁶⁶

A study was undertaken to determine whether a diagnosis of bacterial vaginosis in the first trimester and termination of pregnancy at ≤ 20 weeks are related. Gestation Microbiologic flora of the vagina was assessed in two hundred and twenty eight women who had a live singleton fetus at 14 weeks gestation in the first trimester and had regular prenatal care in Flanders, Belgium. Microbiologic flora of the vagina was assessed in 228 women who had a live singleton fetus at 14 weeks gestation in the first trimester and had regular prenatal care in Flanders, Belgium. Microbiologic flora of the vagina was assessed in 228 women who had a live singleton fetus at 14 weeks gestation in the first trimester and had regular prenatal care in Flanders, Belgium.

The three methods used to evaluate bacterial vaginosis were microscopically (using clue cells), clinically (using the Amsel et al criteria), or by cultivating bacteria linked to bacterial vaginosis. Both univariate (relative risk) and multivariate analysis of the data were done. A subsequent early pregnancy loss was significantly correlated with the presence of bacterial vaginosis at the first prenatal visit (. Following multivariate analysis, the probability of miscarriage was still linked with bacterial vaginosis, *Mycoplasma hominis*, and *Ureaplasma urealyticum*, but not with any other

microbes. Early pregnancy loss and spontaneous abortion may be caused by mycoplasmas and bacterial vaginosis.⁶⁷

A systematic review was carried out to evaluate the data about the relationship between VMB dysbiosis, BV, and AV, and unfavorable late pregnancy outcomes in women residing in South Africa. Following the recommendations of the Preferred Reporting Items for Systematic Review and Meta-Analysis Statement (PRISMA) was done. The following search terms were used to locate observational and intervention studies carried out in South Africa VMB dysbiosis, BV, or AV and preterm birth/labor/delivery, preterm rupture of membranes (PROM), low birth weight, small for gestational age, intrauterine growth restriction, intrauterine infection, intrauterine (fetal) death, stillbirth, perinatal death, or perinatal mortality. The databases searched were PubMed, Embase (Ovid), and Cochrane. Sixty-three search records from five SSA nations were included in the study. According to one study, low birthweight and VMB dysbiosis are positively correlated. Studies found a correlation between BV and preterm birth (7/9) low birth weight (2/6), PROM (2/4), intrauterine infections (1/1), and small for gestational age (1/1) despite significant variations in study design and outcome reporting. Neither intrauterine growth retardation (1/1) nor pregnancy loss (5/5) were linked to any of the studies that could be retrieved. The correlation between BV and low birthweight, preterm delivery, and PROM in pregnant Nigerian women is supported by at least two studies. There were no publications that looked into the connection between AV and unfavorable late pregnancy outcomes in South Africa There is evidence to suggest the correlation between BV and PROM in two of the South Africa included studies. There are differences in the evidence establishing a connection between BV and low birthweight or preterm birth in the remaining investigations. There was no correlation discovered in any of the investigations

between BV and miscarriage. More investigation is required to fully understand the impact that BV, AV, and VMB dysbiosis play in pregnancy in SSA women. These findings offer valuable support for efforts to prevent vaginal dysbiosis and its link to unfavorable pregnancy outcomes in South.⁶⁸

Section II: Sociodemographic determinant of spontaneous abortion.

A cross sectional study was carried out to investigate the relationship between SA and socioeconomic status (SES) and offers resources to help policymakers enhance their maternal health promotion initiatives. 84,531 women between the ages of 35 and 45 who participated in China Kadoorie Biobank's baseline survey provided data for the study. A standardized questionnaire was used for the participant interviews, and data on the participants' demographic, socioeconomic, and reproductive health conditions was gathered. The relationships between the occurrence of SA and SES variables were approximated using odds ratios (OR) with 95% confidence intervals, which were calculated by multistep logistic regression. In order to determine how SES varied in rural and urban settings for women's reproductive health outcomes, a stratification analysis was also used. Age at study date, tea and alcohol consumption, cigarette smoking, and the number of induced abortions were all taken into account while adjusting the model. SA risk was 1.68 times higher in rural areas compared in urban areas. When comparing women with low incomes to those with high incomes, the former group had a lower risk of SA. those with higher educational achievement showed a lower frequency of SA than those with lower educational attainment. Compared to workers in agricultural and associated industries, the risk of SA was shown to be lower only among factory and professional workers. Following stratification based on rural/urban characteristics, there was a greater correlation between income and SA in urban areas compared to

rural areas Urban areas had a higher association between education and SA than rural areas and there was no difference in the impact of occupation on SA among women in either grouping.⁶⁹

A case control study was designed to o identify the cultural elements that influence spontaneous abortion. The research was planned as a case-control study. Two hundred forty seven women whose pregnancies resulted in spontaneous abortions make up the case group. During the trial, five hundred and fourteen women who had been pregnant for at least 22 weeks made up the control group. In the analyses, chi-square and backward LR logistic regression were used. Four factors—a woman's employment situation, her level of education, her exposure to physical assault, and her refusal to receive an ANC—were found to independently increase the likelihood of spontaneous abortions in multivariate analyses. Primary, secondary, and tertiary healthcare facilities should follow up with pregnant women who have these risk factors more frequently and with greater expertise.⁷⁰

A case-control study was carried out in a hospital. In a 1:2 ratio, the cases (84) were chosen using the sequential sampling technique, and the controls (168) were chosen using the purposive sampling technique. Approved by ethics was acquired. Face-to-face interviews with a structured questionnaire were used to gather data. The data were analyzed using descriptive and inferential statistics (Chi-square and Fisher exact test). A 0.05 P-value was chosen. Study discovered that there was a strong correlation between spontaneous abortion and the kind of family, folic acid intake prior to conception, and prenatal care. Similarly, there was an inverse relationship (p-value < 0.05) between monthly income and the SA. Study's results support the notion that reproductive history, sociodemographic factors (such as living in a joint or

nuclear family, taking folic acid, earning a monthly income, and having prenatal checkups) and lifestyle choices can have a major impact on spontaneous abortions.⁷¹

A cross sectional retrospective study into how sociodemographic factors affect abortion rates and examined the relationships between abortion and factors like age, education, community, and religion, 205 abortion cases were registered in the RMCH, Bareilly Department of Obstetrics and Gynecology. A variety of questionnaires, including ones about education, community, religion, and gravida in specified Performa, were used to gather data. Maximum abortion rates were 42.44%, 42.95% for Hindus, and 37.04% for Muslims in the 26–28 age range. Urban communities accounted for the majority of instances (62.93%), as did women with up to a high school education (32.19%), gravida 3 (46.34%), and women with two live children (46.34%).⁷²

A case control research was carried out to assess what factors put women at risk of spontaneous abortions. Ninety four women were classified as cases when they had two or more unexplained miscarriages and no term pregnancy. The controls were one hundred seventy six women who were admitted to the same clinic for a normal delivery. Personal traits, routines, and gynecological history were all questioned about. Women who experienced spontaneous miscarriages were more likely than the controls to have a family history of repeated. The RRs for women whose menarche happened between the ages of 12 and 13 and 14 or older were 0.8 and 0.5, respectively, compared to women whose menarche happened at age 11 or younger: this trend in risk was statistically significant. Current smokers had a roughly 40% higher risk of miscarriage compared to never-smokers, and the risk rose with daily cigarette consumption. There was no correlation found with general lifestyle behaviors (like drinking coffee or alcohol) or with sociodemographic traits (like

education, marital status, age of the partner), reproductive history (age at first pregnancy), or kind of contraception used.⁷³

A study was conducted in a view to examine the how sociodemographic variables in Ghana and Mozambique affect the decision of reproductive-age women to terminate their pregnancies.. In both nations, women with only a primary education, older age groups, Christians, and working women had higher likelihood of terminating their pregnancy. Comparably, women who had never married, had given birth to four or less children, and had access to social media (radio and television) had greater chances of terminating their pregnancy. Regular integrated community-based outreach initiatives aimed at increasing community responsiveness to effective contraception and prevention of unwanted pregnancy are necessary to reduce unexpected pregnancies that may result in pregnancy termination.⁷⁴

A hospital based cross-sectional study was carried out in a hospital with the participation of rural women as to determine the possible risk factors linked to SA and acknowledged the necessity of offering home-based counseling for post-abortion care in order to reduce difficulties following the procedure. the rural women, between the ages of 18 and 45, who had lost a child spontaneously at a gestational age of less than 20 weeks. A standardized questionnaire with questions on socioeconomic status, demographics, and reproductive health was used to interview participants. In order to reduce problems following an abortion, a home-based protocol for post-abortion care was introduced to the target groups. After one week of implementation, the level of maternal satisfaction was evaluated. 24.12% of the 485 patients who were evaluated for eligibility had a history of one or more SAs. The results of the multivariate analysis showed a strong correlation between the occurrence of SA in our study and maternal comorbidities, gravidity, multiparity, and past pregnancy problems with a

history of unfavorable fetal fate. Lower post-abortion infection rates and greater maternal satisfaction were the outcomes of a home-based post-abortion care protocol⁷⁵.

A case control study was carried out to analyze the relationship between pregnant women's risk of spontaneous abortion and their age study contrasted 1,352 controls that resulted in healthy live births with 338 cases that ended in spontaneous abortions. Maternal age and spontaneous abortion were the primary exposure and outcome indicators, respectively. To ascertain the relationship, we employed a two-piece-wise linear model in addition to a generalized additive model. In order to assess the strength of the correlation between maternal age and spontaneous abortion in various subgroups, we also conducted stratified analyses. After controlling for a number of factors, the study concluded that there is a J-shaped link between maternal age and the chance of spontaneous abortion. Additionally, researchers discovered that 29.68 years old was the ideal threshold age. On the left side of the turning point, the adjusted odds ratio (95% confidence interval) of spontaneous abortion per year rise in maternal age was 0.97 (0.90–1.06), while on the right side, it was 1.25. Furthermore, the relationship between maternal age and spontaneous abortion was unaffected by any of the factors examined ($P > 0.05$).⁷⁶

A cohort study was undertaken to look at the relationship between the Jinchang Cohort's female workers' reproductive condition and their chance of having a spontaneous abortion. The largest nickel production business in China, Jinchang Industry, employed a baseline survey of its female workers, which provided the data for this study. A comprehensive and standardized questionnaire was utilized to gather information on the health status of 18,834 female employees of the company. To assess the relationship between a woman's reproductive status and her chance of

having a spontaneous abortion, logistic regression models and pearman grade correlation analysis were employed. There was a 6.89% incidence rate of spontaneous abortions, and the risk of spontaneous abortions was linked to weariness. An elevated risk of spontaneous abortion was linked to the number of pregnancies, age at main birth, and age at the previous pregnancy. In addition, there was a negative correlation between the number of artificial abortions and spontaneous abortions ($r_s = -0.129$, $p = 0.000$). Female reproductive status was associated with an increased risk of spontaneous abortion in this cohort. More studies are needed to confirm this observed association.⁷⁷

A case control research was proposed to determine the incidence of spontaneous abortions as well as the relationship between the mother's age and these incidences by controlling for other risk factors such parity, pregnancy interval, and contraceptive use. This research used a case-control design, with one hundred and seventy four pregnant women serving as the case group and those without spontaneous abortions serving as the controlled group. The frequency distribution was employed for the univariable analysis, the chi-square test was used for the bivariable analysis, and multiple logistic regression analysis was used for the multivariable analysis. The age range of 20–35 years old (48.3%), pregnancy interval of 2 years (56.9%), parity giving birth more than four times (62.1%), and use of contraception (56.9%) were the biggest sample proportions in the case group for the age variable. In the meantime, the control group's pregnancy interval was 2 and its age range was 78.4% to 35 years old parity (57.8%) between two and three births, and 73.6% use contraception. Age of the mother, age of the mother pregnancy interval, parity, and use of contraception were all related to the incidence of spontaneous abortion. Mother

age under 20 years old was the most significant factor Abortions that occur spontaneously was most common in mothers under 20 years old.⁷⁸

A case-control study was carried out to identify the sociocultural elements influencing spontaneous abortion in Burdur, Turkey. There are 257 women in the case group whose pregnancies ended in spontaneous abortions. 514 women who had been pregnant for at least 22 weeks at the time of the study made up the control group. Chi-square and logistic regression with backward LR were applied to the analyses. Four factors were found to create independent risk on spontaneous abortions in multifactorial analyses: women's employment status, education level, exposure to physical violence, and non-receipt of ANC. Conclusions: In primary, secondary, and tertiary healthcare facilities, pregnant women with these risk factors ought to receive more frequent and knowledgeable follow-up.⁷⁹

The study's objectives were to compare the miscarriage rates of the two groups, describe the miscarriage trend among Italian and immigrant women in Italy over the past few years, and assess the impact of age, nationality, and educational attainment on the likelihood of miscarrying. The remaining miscarriage risk factors were then briefly and critically reviewed. The database used for this investigation is ISTAT. The statistical program SPSS was utilized to examine all of the data, and multivariate logistic regression, factorial analysis of variance, and Chi square test were the analytical methods applied. Compared to Italian women, immigrant miscarriage rates were higher Italian ones, that didn't change. Depending on the nationality, the impact of maternal age on the incidence of miscarriages varied; for Italian women, the trend was an increase in miscarriages with age, whereas for immigrant women it was the opposite. Furthermore, the prevalence of miscarriages varied greatly based on the mother's educational attainment. Even though the

immigrant women under consideration did not have the same ethnic background, biological or genetic variables were ruled out as the cause of these disparities, and a socioeconomic explanation was offered instead. Lastly, a quick study of the literature was conducted to address additional miscarriage risk factors in order to provide more comprehensive information.⁸⁰

A cross-sectional was carried out using baseline data from a large-scale population-based cohort research that included 5 million participants from ten different regions of China. 84,531 women between the ages of 35 and 45 who participated in China Kadoorie Biobank's baseline survey provided data for the study. A standardized questionnaire was used for the participant interviews, and data on the participants' demographic, socioeconomic, and reproductive health conditions was gathered. The relationships between the occurrence of SA and SES variables were approximated using odds ratios (OR) with 95% confidence intervals, which were calculated by multistep logistic regression. In order to determine how SES varied in rural and urban settings for women's reproductive health outcomes, a stratification analysis was also used. Age at study date, tea and alcohol consumption, cigarette smoking, and the number of induced abortions were all taken into account while adjusting the model. SA risk was 1.68 times higher in rural areas than in urban areas. Compared to women with low incomes, women with high incomes had a lower risk of SA. Those with higher educational achievement showed a lower frequency of SA than those with lower educational attainment. Compared to workers in agricultural and associated industries, the risk of SA was shown to be lower only among factory and professional workers. The relationship between income and SA in urban areas was shown to be stronger than in rural areas after stratifying by rural/urban. In both urban and rural areas, there was a correlation between education and SA, but not in the

latter, and there was no difference in the impact of occupation on SA among women in either cohort. In conclusion: Women with lower socioeconomic position were generally more susceptible to SA. There was an inverse relationship found between the risk of SA and lower educational attainment and income. SA was far more common in women who worked in agriculture and similar fields. Interventions should focus more on women from low-socioeconomic backgrounds in order to improve health outcomes and generate economic benefits for health program⁸¹

A cohort study was carried to evaluate the lifetime frequency of spontaneous abortions and the variables influencing spontaneous abortion in women aged 35 to 65. This study included all 4831 married women, aged 35 to 65, who had previously given birth. The number of abortions divided by the number of live births yielded the abortion ratio. Associated factors influencing abortion were identified by multivariate logistic regression analysis. In the past, spontaneous abortions occurred in about 25.7% of women. The female abortion rate was 0.10. Women with a secondary education level, who was married before the age of 26, who were in a high socioeconomic status, who had hyperthyroidism or diabetes, and who were residents of rural areas had a low abortion rate. Women with high blood pressure have 63% lower odds of having a non-spontaneous abortion, which is statistically significant, according to an assessment of the effective variables. In conclusion. Given that a person's education level, age at first marriage, and age at first pregnancy all have an impact on the likelihood of a spontaneous abortion, more care should be given to these individuals.⁸²

CHAPTER III

MATERIALS AND METHODS

Research materials and methods refer to the strategies employed by investigators to organize a study and collect and examine data pertinent to research inquiries. It is a method for methodically resolving the research challenge. There are several steps involved, starting with the problem's original identification and ending with its resolution by the researcher. The materials and technique of the research show how the process of obtaining accurate and trustworthy data for the issue being studied should be generally organized.⁸³

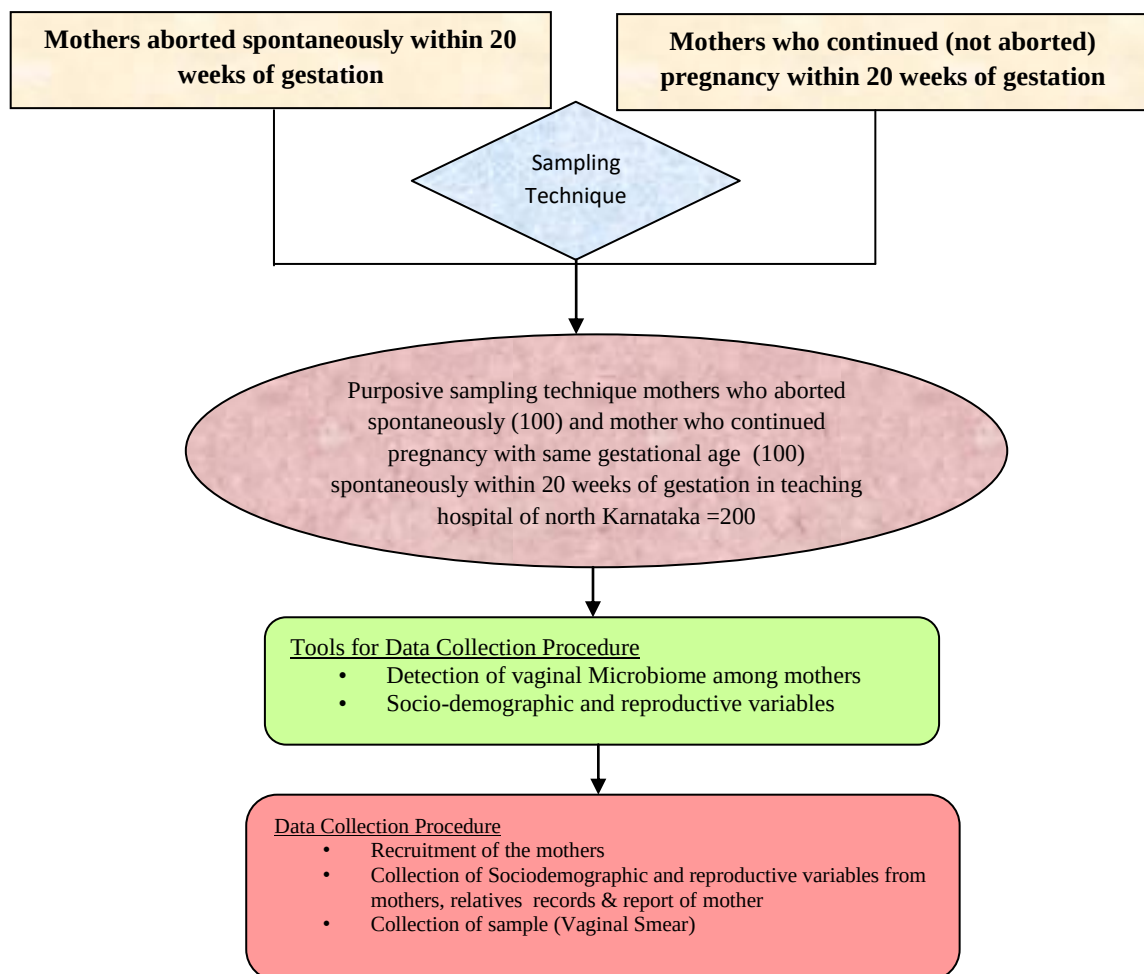


Fig 4: Protocol/Methodology- Cross Sectional Comparative Research Design

3.1 Research approach

An **evaluative approach** was considered appropriate for the present study.

3.2 Study design

The research design used for the present study was a **cross sectional comparative** research design.

3.3 Study Setting

The setting planned for the present study was **tertiary care hospitals** of Belagavi city.

3.4 Study Population

The sample chosen for the present study were mothers undergoing spontaneous abortion within 20weeks of gestation and mothers who continued their pregnancy with same gestational age selected tertiary care hospital of Belagavi city.

3.5 Sampling technique: Purposive Sampling technique

3.6 Sample size

$$n = \frac{Z_{1-\alpha/2}^2}{d^2} SD^2,$$

Where

- The sample size has been computed using 95%CI 15% error in presence of microbiome load and 10% as attrition
- n - Sample size
- $Z_{1-\alpha/2}$ - is one tail Standard Normal Variate value;

Usually taken at 95% Confidence Interval (CI) as 1.96

- SD - is a Standard Deviation of the Variate is 15% of mea
- 'd' -is tolerable error(in present study d is 28%)Considering the above formula the sample size is calculated as **200**
- Among the total (200) sample size 100 were the mother who underwent spontaneous abortion within 20weeks of gestation and remaining 100 were the mother who continued their pregnancy with same gestational week

3.7 Criteria for selecting the sample

The sample selection was based on the following inclusion and exclusion criteria.

Inclusion criteria

1. All mothers undergoing spontaneous abortion within 20 gestational weeks in tertiary care Hospital of North Karnataka.
2. Mothers who have continued their pregnancy with same gestational week

Exclusion criteria

1. Mothers with associated medical condition
2. Mothers who were on antimicrobial therapy or antibiotics

3.8 Study Variables

Socio-demographic variables: Demographic characteristics included in the study were Maternal Age, Maternal Education Occupation, Parity, Gestational age, area of Residence, religion, Type of family, consanguineous marriage and monthly family income

Reproductive determinant: Reproductive determinants included in the study were age at menarche, any complication during last pregnancy, history of previous abortion, level of Hb%

Tools used

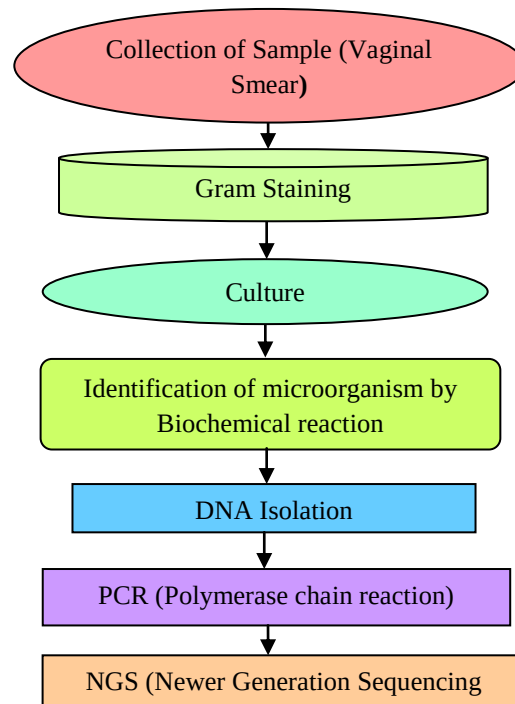


Fig: 5: Detection of vaginal Microbiome among mothers undergoing spontaneous abortion

3.9 Ethical and administrative Deliberation

Approval from the Institutional Ethical Committee (IEC): Ethical clearance was obtained from the Institutional Ethical Committee of KLE University, Belagavi

Hospital Authority Data collection procedure

Permission was obtained from the following authorities:

- Medical Superintendent KLE's Dr. Prabhakar Kore Charitable Hospital, Belagavi, HOD OBG Department, JNMC and BSRC Belagavi.

Patient's consent: Written informed consent was obtained from the mothers after explaining the procedure and their role in the study.

3.10 Data collection instrument and Procedure

3.10.1: Detection of vaginal Microbiome among mothers

3.10.2: Socio-demographic and Reproductive variable

Data collection

- With purposive sampling technique mothers undergoing spontaneous abortion within gestation 20weeks and mothers who have continued pregnancy with same gestational age were enrolled in the study
- The purpose of the study was explained to mothers and relatives during data collection and consent was taken from every subjects
- Detail procedure was explained to the mother after admission with consent and there after keeping her privacy into consideration and maintaining strict aseptic technique vaginal swab was taken from posterior vaginal fornix and then processed for further analysis
- Staff Nurses and Nursing students working in Labor room, Maternity ward were trained for data collection (Socio Demographic and Reproductive data) by using the Performa sheet given by the researcher.
- The mother who undergone spontaneous abortion for them swab was collected on the day of admission

- On the other arm the swab for mother who continued the pregnancy was taken in the OPD when mother comes for her routine check-up considering the same week of the aborted mother

Collection of vaginal swab

The collection of vaginal swab was done considering the below points

Preparation before the Procedure

1. **Gather Supplies:** The articles were gathered the required articles were
 - Sterile vaginal swab
 - Specimen collection tube/container
 - Sterile gloves
 - Disposable speculum (if needed)
 - Antiseptic wipes (for external cleansing)
 - Patient drape or disposable sheet
 - Proper labeling materials
 - Biohazard bag for waste disposal
2. **Prepared the Patient:** After gathering articles the patient was prepared to collect the sample
 - The procedure was explained to the mother to ensure cooperation and comfort.
 - Informed consent was obtained from the mother
 - Asked the mother to empty her bladder to reduce discomfort.
 - Ensured privacy by using a private examination room and draping the mother appropriately.

- Positioned the mother in a lithotomy position (lying on her back with legs bent and feet supported).

3. Hand Hygiene and Personal Protection:

- After preparation of patient, hand washing was performed thoroughly with soap and water.
- Worn sterile gloves to prevent contamination.

4. Procedure for Taking a Vaginal Swab

- Examined for any signs of infection (redness, discharge, swelling, or lesions).
- Cleaned the external genital area with sterile wipes before inserting the swab
- Speculum was used and inserted gently to visualize the vaginal walls and cervix.
- The swab was cautiously inserted into the vagina about 2 inches (5 cm) past the introitus, it was gently spun for 10 to 30 seconds. After the swab came into contact with the vaginal walls and fluids and absorbed the moisture, it was removed without coming into contact with the skin.
- Immediately the swab was placed into the sterile transport medium (Peptone medium) specimen was secured and labeled with patient details and taken to lab within an hour for further analysis.
- After the procedure mother was made comfortable and articles were replaced back.

3.11 Operational definitions:

Spontaneous abortion: In the present study spontaneous abortion is defined as a clinically recognized pregnancy loss before the 20th week of gestation;

Vaginal Microbiome: In the present study vaginal microbiome refers to the microorganisms that colonize the vagina.

Determinants: In the present study determinants refers to the factors or variables which determines the spontaneous abortion

3.12 Data Analysis:

Collected data was coded, entered; in a password protected computer. Data was analyzed by using software SPSS version 22 for following statistical methods:

- Continuous variables was calculated by using measures of central tendency and dispersion
- The sample size was computed using 95% confidence interval 5% error in presence of microbiome load and 10% as attrition. Two hundred women provided enough data to allow for a comparison of the microbiome characteristics between groups of women who sustained their pregnancies and those who had SAs. Subject with respect to microbiome community found in the vaginal niche was employed in metagenomics research to characterize species diversity by using the Shannon diversity index. The significant difference was calculated by the Shannon diversity index.
- Pearson Chi – square test was conducted to examine the relationship between sociodemographic, reproductive determinants and pregnancy termination.
- Descriptive analysis was carried out by frequency and proportion for categorical variables. Binary logistic regression analysis was performed to test the association between the explanatory variables and outcome variables. The odds ratio along with 95% CI is presented. P-value < 0.05 is considered an

association between an outcome variable and an explanatory variable. IBM SPSS version 22 was used for statistical analysis.

- Regression models specifically logistic regression models was used ⁸⁴

Dissemination of the study

The subject related areas were identified and it was disseminated by publishing in National and International Journals and was presented in conferences.

Part A of the study was presented in the international conference on exploring the bondless horizons of mixed method research integrating insights and advancing health care conducted by Yenepoya University Mangalore. With paper published in journal Nursing for Women's Health

Part B of the study was presented in the national conference in association with SOMI on fostering innovation in Midwifery: Moving forward in maternal and newborn care organized by KAHER institute Of Nursing Sciences Belagavi with paper published in journal Scientific Temper.

CHAPTER IV

DATA ANALYSIS AND RESULT INTERPRETATION

In this chapter, data was collected from total of 200 mothers out of which 100 mothers belong to Group 1(mothers those who have undergone spontaneous) and 100 mothers belong to Group 2(those who continued pregnancy with same gestational week

Descriptive analysis was carried out by frequency and proportion for categorical variables. Binary logistic regression analysis was performed to test the association between the explanatory variables and outcome variables. The odds ratio along with 95% CI is presented. P-value < 0.05 is considered an association between an outcome variable and an explanatory variable. IBM SPSS version 22 was used for statistical analysis.⁸⁴

The objectives of the study were

- To compare the vaginal microbiome in mothers who have undergone spontaneous abortion and those who have continued pregnancy with same gestational age
- To determine the socio demographic and reproductive determinant of spontaneous abortion and compare it with the socio demographic determinants of mothers those who have continued pregnancy with same gestational age.

The findings obtained were presented in the form of tables and figures which represented as follow:

SECTION I: Detection of vaginal Microbiome among mothers

SECTION II: Socio-demographic and Reproductive variable

4.1 SECTION I: VAGINAL MICROBIOME AND ITS RELATION WITH MOTHER THOSE WHO HAVE UNDERWENT SPONTANEOUS ABORTION AND THOSE WHO CONTINUE PREGNANCY WITH SAME GESTATIONAL AGE

MICROBIOLOGICAL ANALYSIS

Sample collection

After gaining consent and outlining the procedure in detail. The swab was cautiously inserted into the vagina about 2 inches (5 cm) past the introitus, it was gently spun for 10 to 30 seconds. After the swab came into contact with the vaginal walls and fluids and absorbed the moisture, it was removed without coming into contact with the skin. A swab was obtained and taken to the lab within an hour.⁸⁵

The swab samples were cultivated on MRS agar for a 24 hours the media were prepared and sterilized in accordance with the manufacturer's instructions. The generated medium was used for susceptibility testing, identification, calculating the viable count, and isolation after it had solidified. The swabs were inoculated using MRS agars. The inoculated plates were then incubated at 37°C for 24 hours.⁸⁶

Each inoculated plates had around 10-15 colonies from that a single colony was picked and processed for further analysis.

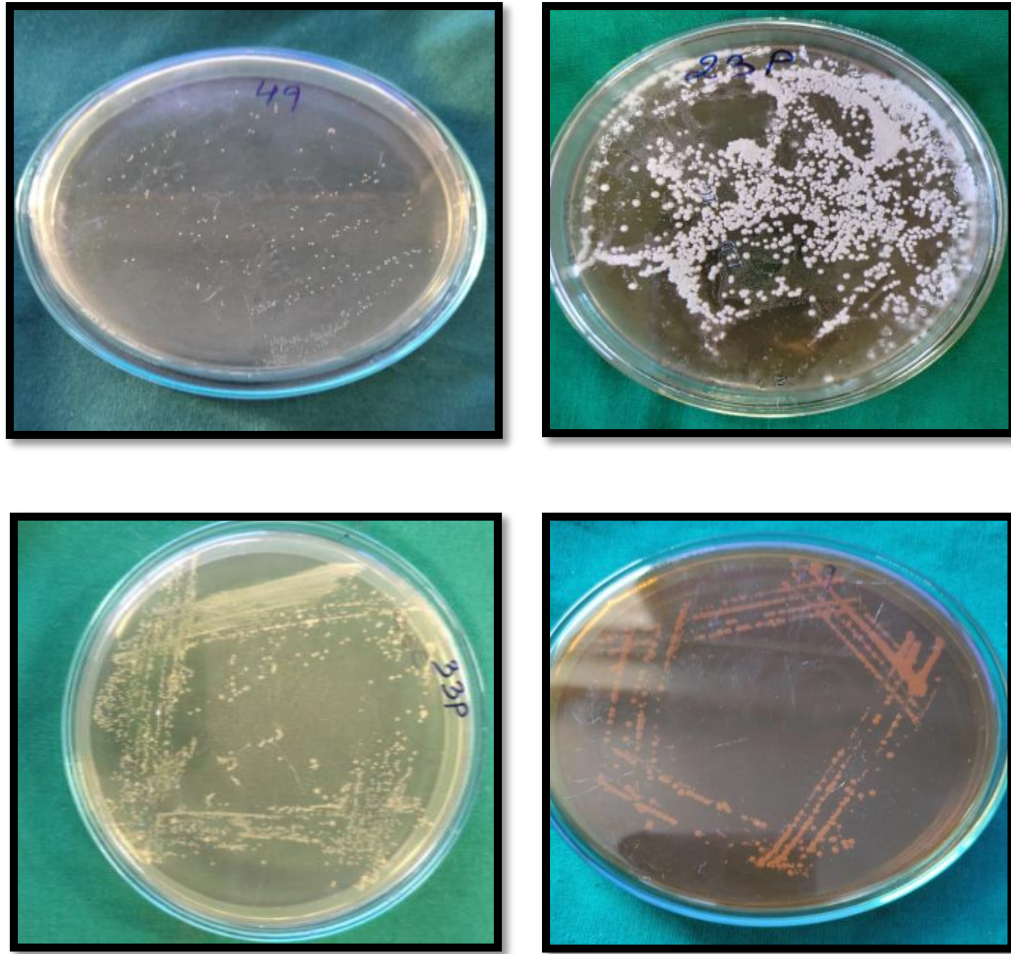


Fig 6: Culture plates

BIOCHEMICAL TESTING

Biochemical tests were performed by distinguishing bacterial species based on their metabolic activities, to identify distinct species. Bacterial identification is aided by several factors such as chemical consumption abilities, enzyme production, carbon metabolism, protein and fat metabolism differences, and more.⁸⁷

The IMViC test is a set of four distinct biochemical assays used to distinguish and identify bacteria, particularly those belonging to the Enterobacteriaceae family. It is mostly used to identify Gram-negative bacteria, however it can (and is) used to identify any sort of bacteria. It is essential for recognizing and separating Enterobacteriaceae family members.⁸⁸

“IMViC” stands for one of these tests. “I” is for indole; “M” is for methyl red; “V” is for Voges- Proskauer, and “C” is for citrate.

Indole test

- The biochemical indole test, part of the IMViC test series, measures an organism's (bacteria's) capacity to use tryptophan as a metabolic product to create indole. It is denoted by the IMViC's letter "I."
- Indole reacts with the aldehyde in the indole reagent to produce a unique color when it was given to a medium containing a bacterial culture that has produced indole.
- To read the result, an indole reagent is added after the test bacteria have been cultivated in the tryptophan-containing medium for 24 to 48 hours. The development of a pink to violet-red or green to blue color ring, , indicates a positive result. The emergence of either a tiny yellowish color ring at the top or no color change at all indicates a negative result.⁸⁷

Methyl red

- This test measures an organism's (bacteria's) capacity to create stable mixed acids as metabolic byproducts of glucose metabolism. It is a biochemical test. It is denoted by the IMViC's letter "M."
- After the MR-VP soup was incubated for 24 hours, methyl red indicator was added to the broth. Red color development suggested a positive outcome, while yellowish color development indicated a negative outcome.⁸⁸

Voges-Proskauer(VP)

- The IMViC test series' (VP) test is a biochemical test that measures an organism's (bacteria's) capacity to convert pyruvate into a neutral intermediate product known as "acetylmethylcarbinol" or "acetoin." It is denoted by the IMViC's letter "V."

- After VP reagents I and II was added, the color shift was visible within 30 minutes of the 48-hour aerobic incubation on MR-VP broth. When the top of the broth turned pink or crimson either right away or after 30 minutes, but not after an hour, it was a sign of a positive result. A negative VP was shown by no color shift.

Citrate Utilization Test

- The biochemical Citrate Utilization Test, part of the IMViC test series, measures an organism's (bacteria's) capacity to use citrate as its exclusive energy source. It is represented by the IMViC's letter "C."
- Bacterial growth and a color shift in the slant section were noted after the 24- to 48-hour incubation period. Growth and the slant's color changing from green to a deep blue hue suggested a positive outcome. There was no change in the slant's hue, indicating a negative outcome.⁸⁸

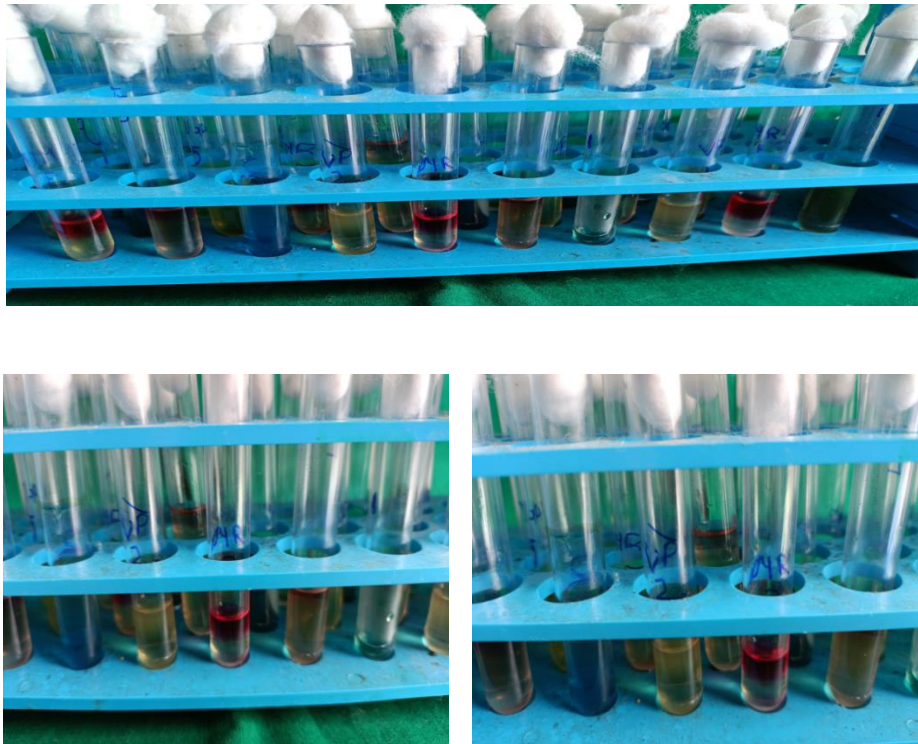


Fig 7: Showing the shift in color

Table 1: Results of biochemical testing are as follows

(n=200)

IMViC”TEST IMVIC/BIOCHEMICAL TEST									
Spontaneous abortion (%) (n=100)					Sustained pregnancy (%) (n=100)				
Code. No	Indole	Methyl red MR	Voges Proskauer VP	Citrate	Code. no	Indole	Methyl Red MR	Voges proskauer VP	Citrate
OA1	-	+	-	+	ON1	+	-	-	+
OA2	+	+	-	+	ON4	+	+	+	+
OA3	+	+	-	+	ON6	-	+	+	+
OA4	+	+	-	+	ON7	+	-	+	+
OA5	+	+	-	+	ON8	-	-	+	-
OA6	-	+	-	+	ON9	-	+	-	-
OA7	+	+	-	+	ON10	+	-	+	+
OA8	+	+	-	+	ON11	+	-	+	+
OA9	+	+	-	+	ON12	+	+	+	+
OA10	-	+	-	+	ON13	+	+	+	+
OA11	-	-	-	+	ON14	-	+	+	-
OA12	+	+	+	-	ON15	+	-	+	+
OA13	-	+	+	-	ON16	-	+	-	-
OA14	+	+	+	-	ON17	+	-	+	+
OA15	-	-	+	+	ON18	-	-	-	-
OA16	+	+	+	+	ON15	+	+	-	+
OA17	+	-	-	-	ON16	+	+	+	+
OA18	+	-	-	-	ON17	+	+	-	+
OA19	-	+	-	-	ON18	-	+	+	+
OA20	+	-	+	+	ON19	-	-	-	-
OA21	+	-	+	-	ON20	+	+	+	+
OA22	+	+	+	-	ON21	-	+	+	+
OA23	+	-	-	-	ON22	-	+	-	-
OA24	-	+	+	+	ON23	+	+	-	+
OA25	+	-	-	-	ON24	+	+	-	+

Table 2: The table representing the results of IMVIC test

S.No	Name of the test	Sustained pregnancy (%) (n=100)		Spontaneous abortion (%) (n=100)	
		+	-	+	-
1	Indole	40	60	48	52
2	Methyl red (MR)	45	55	52	48
3	Voges proskauer (VP)	58	42	35	65
4	Citrate	68	32	54	46

Of the vaginal swabs from women who experienced SA (N=100), 52% were indole negative, 52% were methyl positive, 65% were Voges proskauer positive, and 54% were citrate positive. Similarly, of swabs from women who had continued pregnancy (N=100), 60% were indole negative, 55% were methyl red negative, 58% were Voges proskauer positive and 68% were citrate positive.

GRAM STAINING

The samples were further processed for gram staining

Smears were prepared on glass microscope slide then a series of stains were applied to perform a gram stain

The staining process included four steps

1. Applying a primary stain (crystal violet).
2. Adding a mordant (Gram's iodine).
3. Rapid decolonization with ethanol, acetone or a mixture of both.
4. Counterstaining with safranin.

Then categorization of bacteria is done by the presence of color and shape during the microscopic evaluation

Color: Gram-positive bacteria usually have a purple or blue color, while Gram-negative bacteria have a pink or red color

Shape: The most prevalent forms are rod-shaped (bacilli) or round (cocci).

The researcher also looked for the additional characteristics of the sample by observing the groupings of the bacteria on the slide

- Cocci found alone, in pairs, in clusters, in chains, or in groups of four.
- Bacilli have expanded spores on one end or that were thick, thin, short, or long.⁸⁹

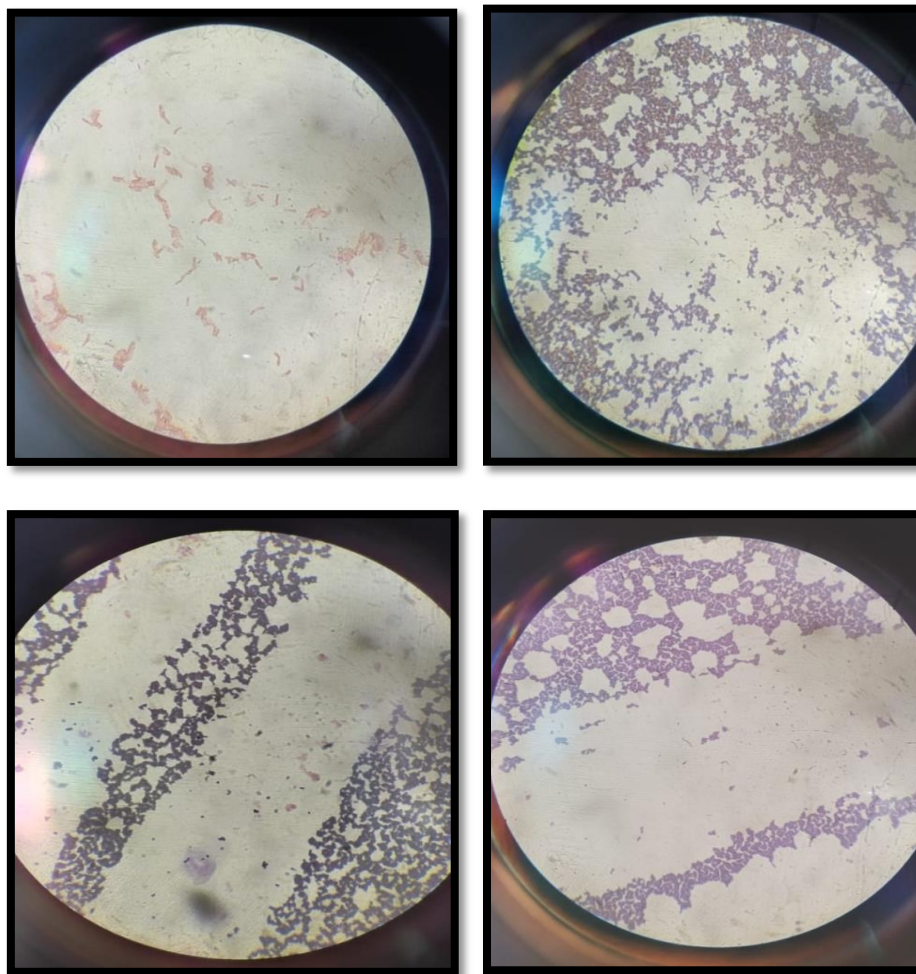


Fig 8: Depicting the result of gram staining

Table 3: The test was carried out for all the samples the results of few representative samples are as follows (n=200)

Code No	Sustained pregnancy (%) (n=100)				Spontaneous abortion (%) (n=100)			
	Gram Positive	Gram Negative	Cocci	Bacilli	Gram Positive	Gram Negative	Cocci	Bacilli
OA1	√		√		√		√	
OA2	√			√		√	√	
OA3		√	√		√		√	
OA4	√			√	√			√
OA5		√	√		√		√	
OA6	√		√			√	√	
OA7		√		√	√			√
OA8		√		√		√	√	
OA9		√	√			√		√
OA10	√		√			√		√
OA11	√		√		√			√
OA12		√	√		√			√
OA13	√			√	√	√	√	
OA14	√		√		√		√	
OA15	√			√	√		√	
OA16		√	√			√		√
OA17	√			√	√			√
OA18	√		√		√			√
OA19		√		√		√	√	
OA20	√		√		√		√	
OA21	√			√		√		√
OA22		√		√	√		√	
OA23		√	√			√	√	
OA24	√		√		√			√
OA25		√	√				√	

Table 4: Representing the result of Gram Staining

S.No		Sustained pregnancy (%) (n=100)	Spontaneous abortion (%) (n=100)
1	Gram Positive	62	56
2	Gram Negative	38	44
3	Cocci	72	45
4	Bacilli	28	28

Gram staining analysis inferred that in the women who had SA (N=100) 56% were gram positive. 44% were gram negative, 45% appeared to be Cocci, and 28% appeared to be Bacilli. Among the samples from women who maintained their pregnancies (N=100), 62% of the samples were gram positive, 38% were Gram Negative, 72% appeared to have cocci, and 28% seemed bacilli Further the sample were processed for DNA Isolation

DNA Isolation

The sample was treated further to extract DNA.

Bacterial stocks were cultured overnight using cells harvested from the culture vessel. Centrifuging cells at 1000 x g for 1 minute pelleted the cells, and the supernatant was discarded. The cell pellet was reconstituted in 100 litres of cold PBS and mixed repeatedly using a pipette.⁹¹

The sample was then processed according to the QIAamp DNA Mini Kit methodology, and DNA extraction was carried out.

After extraction, the sample was kept at 4° Celsius for further examination.

PCR Analysis

The chosen samples were prepared for PCR analysis; the specifics are described below.

PCR GROUP 1(NORMAL)

Experimental method

1. DNA isolation was obtained from the culture. A single band of high-molecular weight DNA was seen when its quality was assessed on a 1.0% agarose gel.
2. **16SrRNA-F and 16SrRNA-R primers** were used to amplify a fragment of the 16S rRNA gene. On an agarose gel, a single distinct 1500 bp PCR amplicon band was seen.
3. To get rid of impurities, the PCR amplicon was filtered.
4. Using the BDT v3.1 Cycle sequencing kit on an ABI 3730xl Genetic Analyzer, the forward and reverse DNA sequencing reaction of the PCR amplicon was performed using 16SrRNA-F and 16SrRNA-R primers.
5. Using aligner software, the consensus sequence for the 16S rRNA gene was produced from forward and reverse sequence data.
6. Using the NCBI GenBank database's "nr" database and the 16S rRNA gene sequence, BLAST was performed. The first ten sequences were chosen based on the maximum identity score, and Clustal W, a multiple alignment software package, was used to align them. Using MEGA 10, a distance matrix and phylogenetic tree were created.⁹²

Sample 1 with code no 0N 42

Analysis Sample which was labeled as **0N42** was found to be **Lactobacillus. crispatus** showed high similarity based on nucleotide homology and phylogenetic analysis.

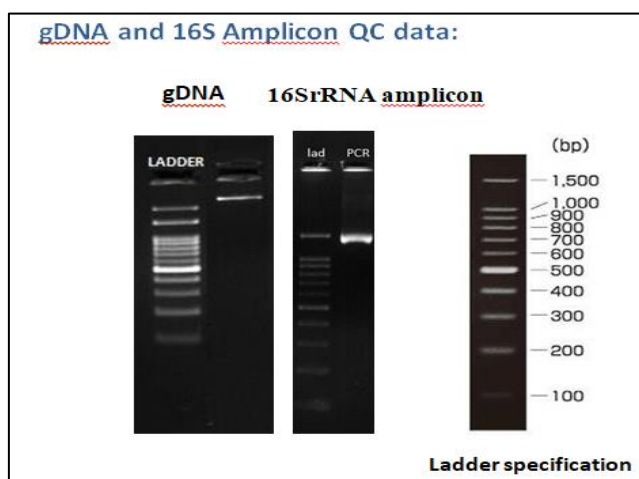


Fig 9 DNA and 16S Amplicon data for sample with code no 0N 42

Sample 2 with code no 0N 98

Analysis: Labeled as 0N98, the sample was discovered to be **Lactobacillus. iners**. Nucleotide homology and phylogeny research revealed a significant degree of commonality amongst **iners**.

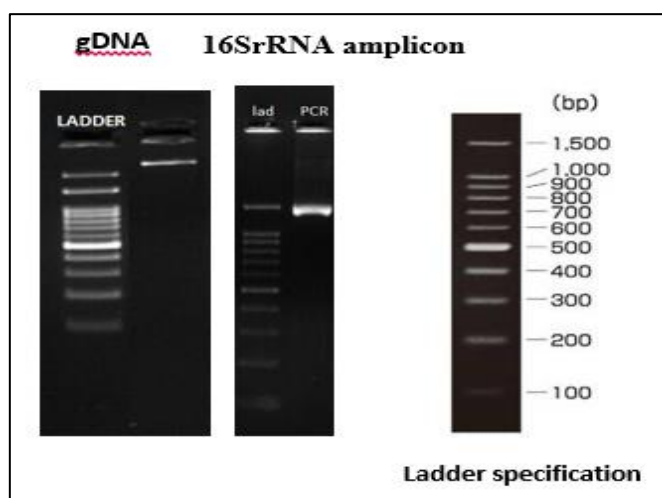


Fig 10 DNA and 16S Amplicon data for sample with code no 0N 98

Sample 3 with code no 0N 62

Analysis *Escherichia coli* were identified in the sample with the label 0N62, which displayed strong similarities based on phylogenetic analysis and nucleotide homology.

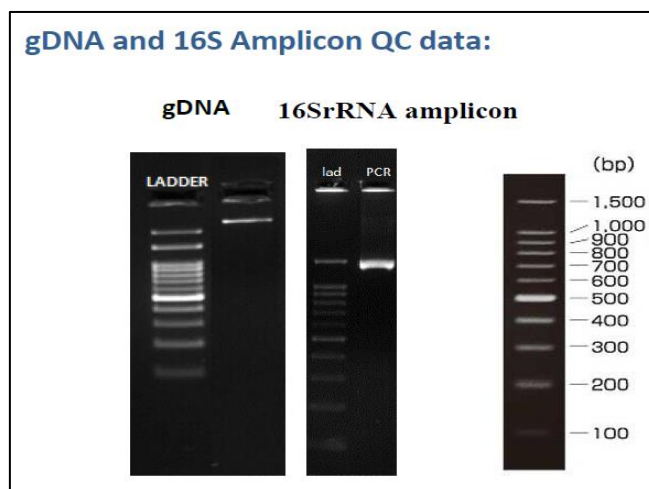


Fig 11 DNA and 16S Amplicon data for sample with code no 0N 62

GROUP 2(ABORTION)

-Sample 1 with code no 0A64

Analysis : Based on nucleotide homology and phylogenetic analysis, the sample with the label 0A64 was determined to be the genome of *Enterococcus faecalis* and shown high similarity.

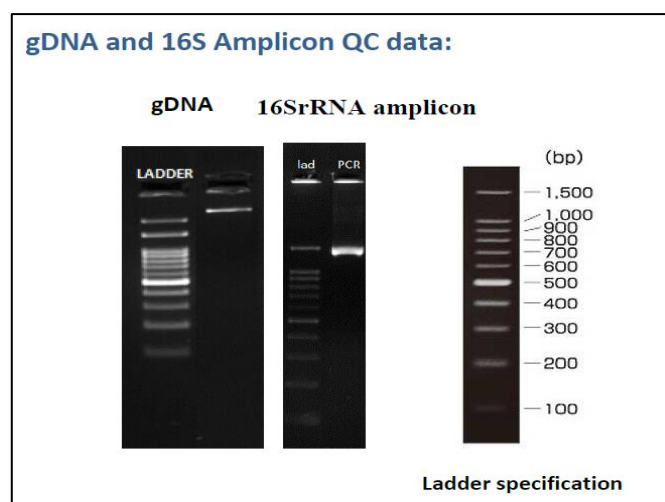


Fig 12 DNA and 16S Amplicon data for sample with code no 0A 64

Sample 4 with code no 0A12

Analysis: Nucleotide homology and phylogenetic analysis revealed that the sample, designated as 0A12, was indeed *Enterococcus faecium*, exhibiting a high degree of similarity.

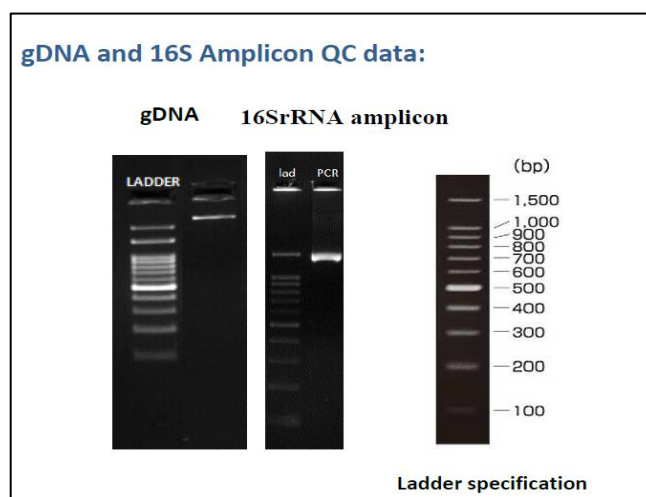


Fig 13 DNA and 16S Amplicon data for sample with code no 0A 12

Sample 6 with code no 0A72

Analysis Nucleotide homology and phylogeny analysis revealed that the sample, designated 0A72, was *Escherichia coli* and that it shared a high degree of similarity.

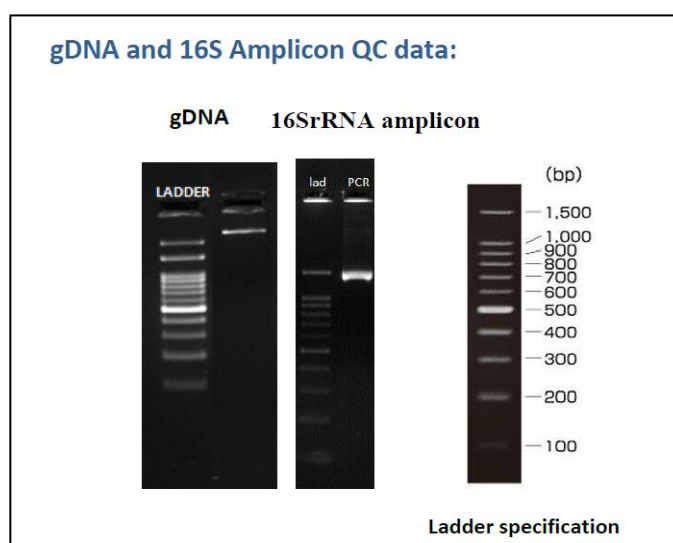


Fig 14 DNA and 16S Amplicon data for sample with code no 0A 72

Sample 7 with code no 0A54

Analysis: *Pseudomonas cedrina* was identified as the sample with the label 0A54; phylogenetic analysis and nucleotide homology revealed a significant degree of similarity.

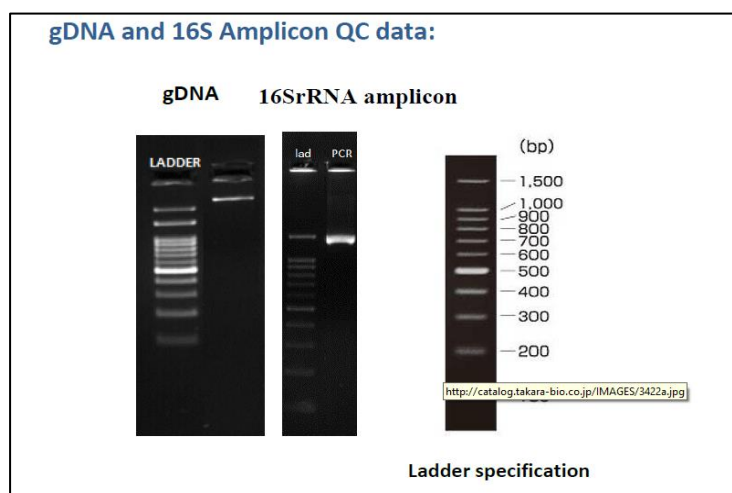


Fig 15 DNA and 16S Amplicon data for sample with code no 0A 54

Sample 7 with code no 0A55

Analysis Using phylogenetic analysis and nucleotide homology, the sample with the label 0A55 was determined to be *Pseudomonas fluorescens*, exhibiting high similarity.

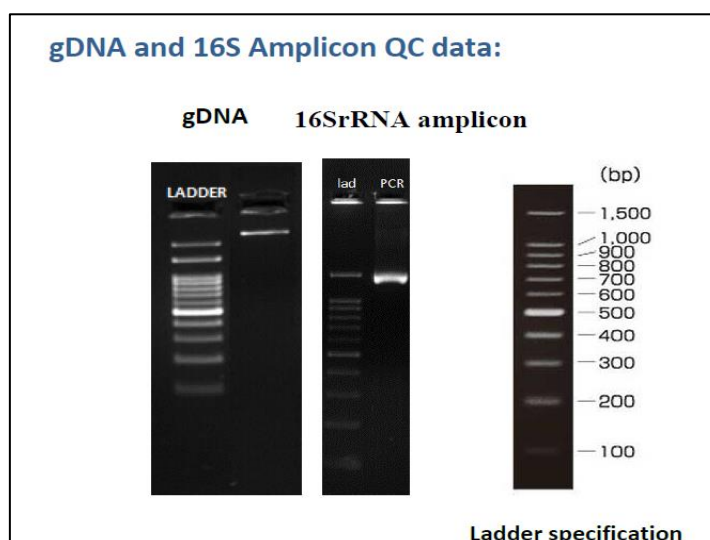


Fig 16 DNA and 16S Amplicon data for sample with code no 0A 55

Sample 2 with code no 0A22

Analysis Based on nucleotide homology and phylogenetic analysis, the sample, designated 0A22 since it was determined to be *Escherichia coli*, exhibited strong similarities.

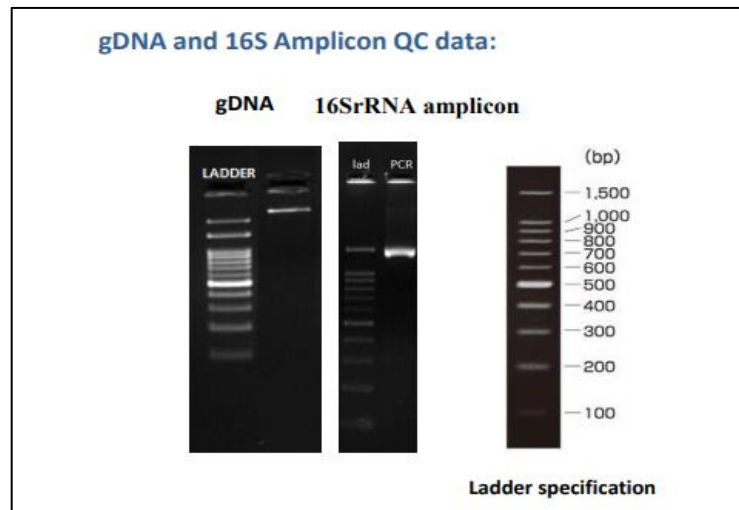
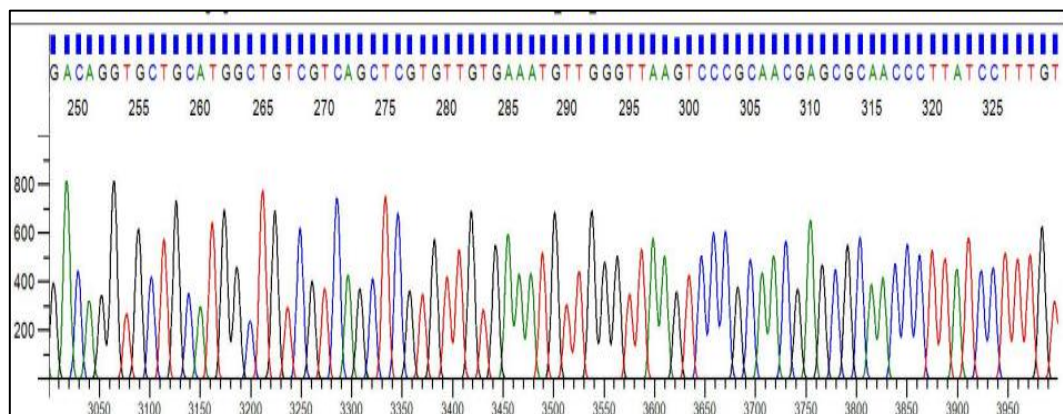


Fig 17 DNA and 16S Amplicon data for sample with code no 0A 22

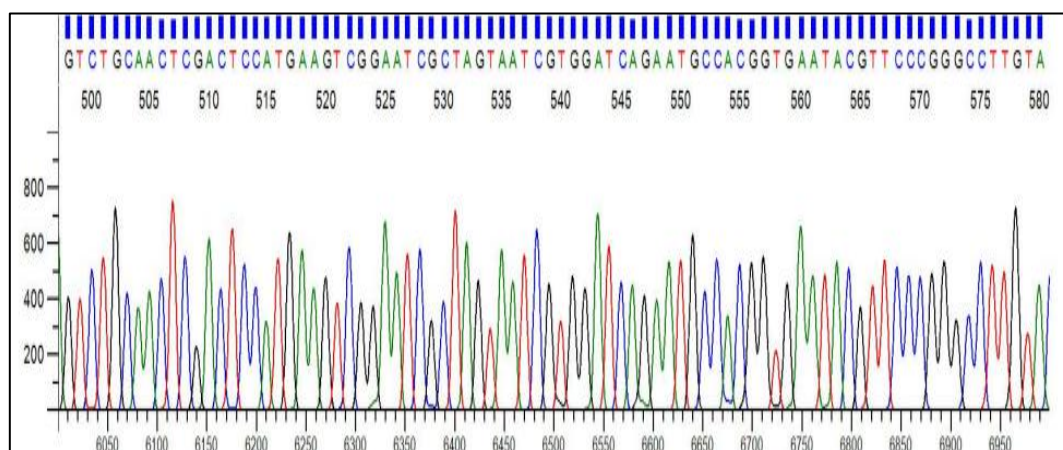
Graph Analysis of Sanger Sequencing

The four bases that make up a DNA molecule are adenine (A), guanine (G), cytosine (C), and thymine (T). Through hydrogen bonds, these bases interact with one another in very particular ways, allowing G to engage with C and A to interact with T. Base pairings are the specific ways that the bases interact with one another. DNA molecules consist of two strands that are oriented in an antiparallel fashion. One strand is oriented from 5' to 3', while the other strand is oriented from 3' to 5'⁹²

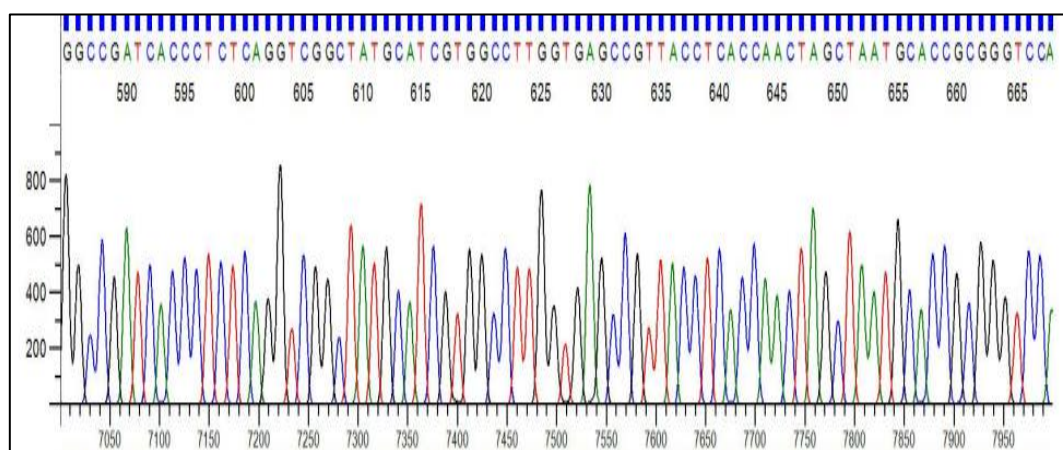
When discussing the base order, the phrases 5' and 3' are essential since they pertain to the directionality of the DNA backbone. A DNA sequence's base order is often described using the 5' to 3' direction and is written from left to right. Therefore, the complementary sequence can be inferred from the sequence of one DNA strand.



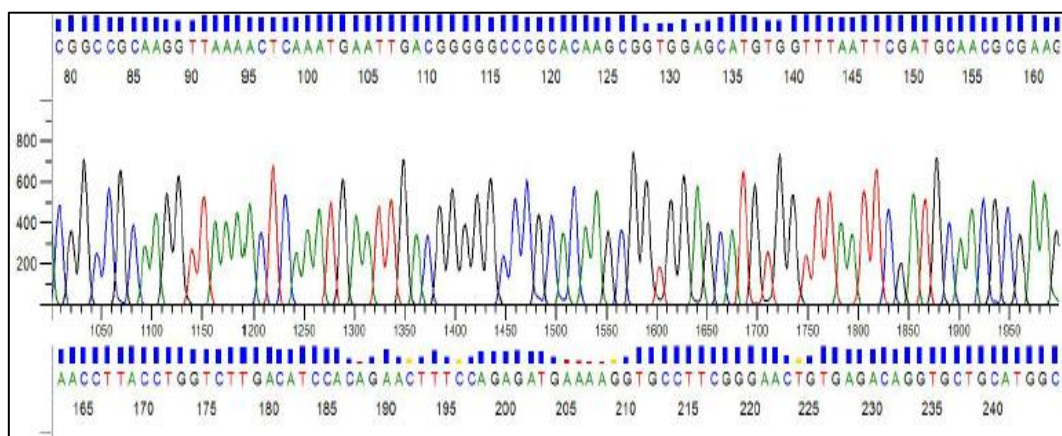
Graph 1: Depicting the ATGC peak for the sample OA64



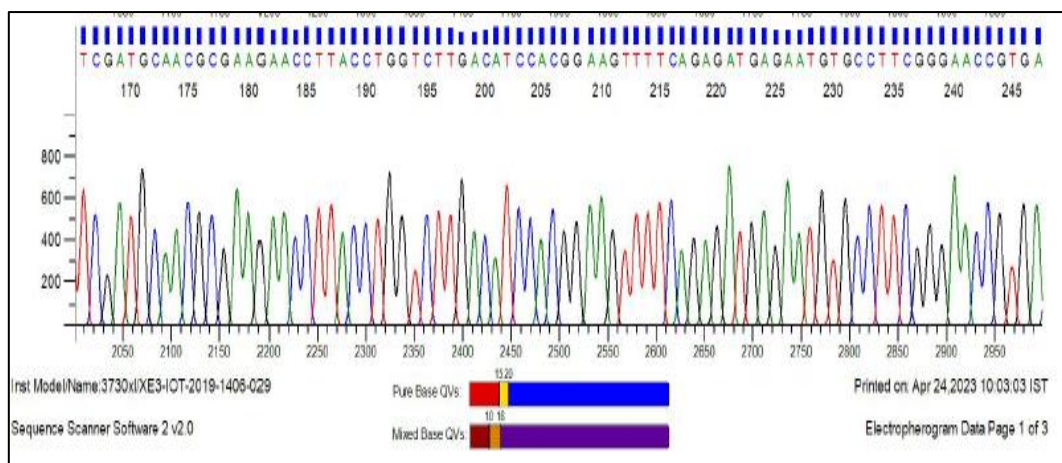
Graph 2: Depicting the ATGC peak for the sample OA12



Graph 3: Depicting the ATGC peak for the sample OA72



Graph 4: Depicting the ATGC peak for the sample OA54



Graph 5: Depicting the ATGC peak for the sample OA54

PHYLOGENIC TREE OF SELECTED SPECIES

A graphical depiction of the evolutionary history between a group of species or taxa over a given period of time is called an evolutionary tree, phylogenetic tree, or phylogeny tree.[1–2] Put differently, it represents an evolutionary tree or branching diagram that illustrates the links between different biological species or other entities based on the similarities and variances in their physical or genetic traits⁹².

In our study phylogenic tree analysis we found

- ***Enterococcus faecalis***, (Enterococcus faecalis strain DSM 100906, Enterococcus faecalis strain PPMi37) and so on.
- ***Enterococcus faecium***, (Enterococcus durans strain JCM 8725, Enterococcus hirae strain NBRC 3181) etc.
- ***Pseudomonas cedrina*** (Pseudomonas sp. EP25, Pseudomonas azotoformans strain EN12) and others.
- ***Pseudomonas fluorescens*** (Pseudomonas sp. strain Zea02, Pseudomonas cedrina strain Cl-10) and so on.
- ***Escherichia coli*** (Escherichia coli strain PYK20, Escherichia coli strain PYK9 and others

Pseudomonas cedrina

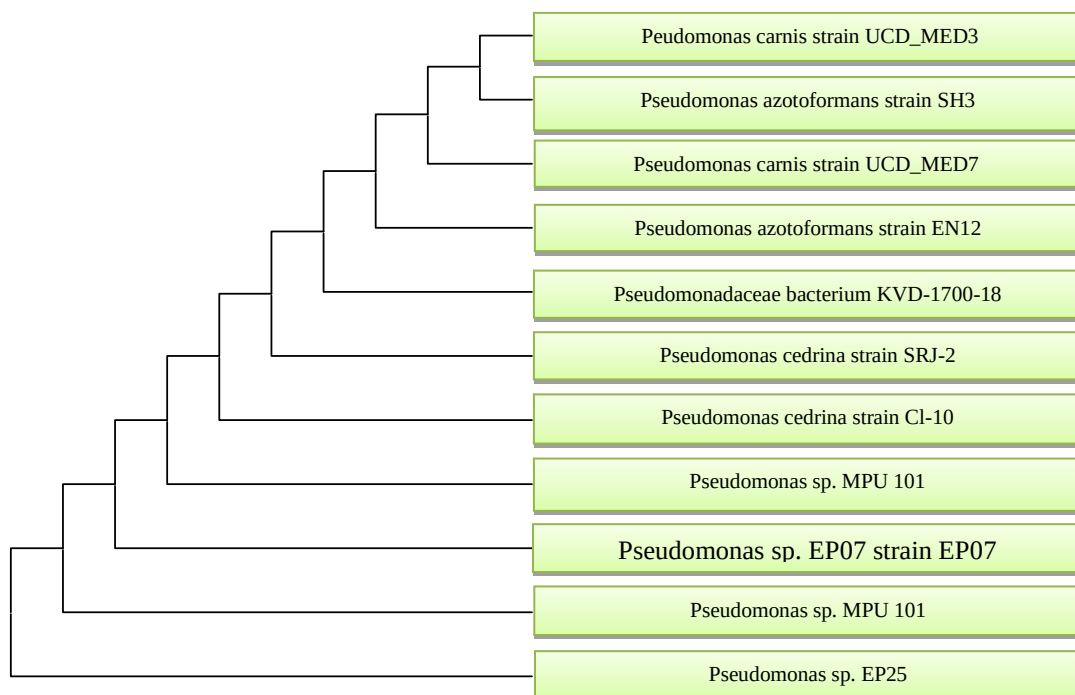


Fig 18 Phylogenic tree depicting the species of **Pseudomonas cedrina**

Pseudomonas Fluorescens

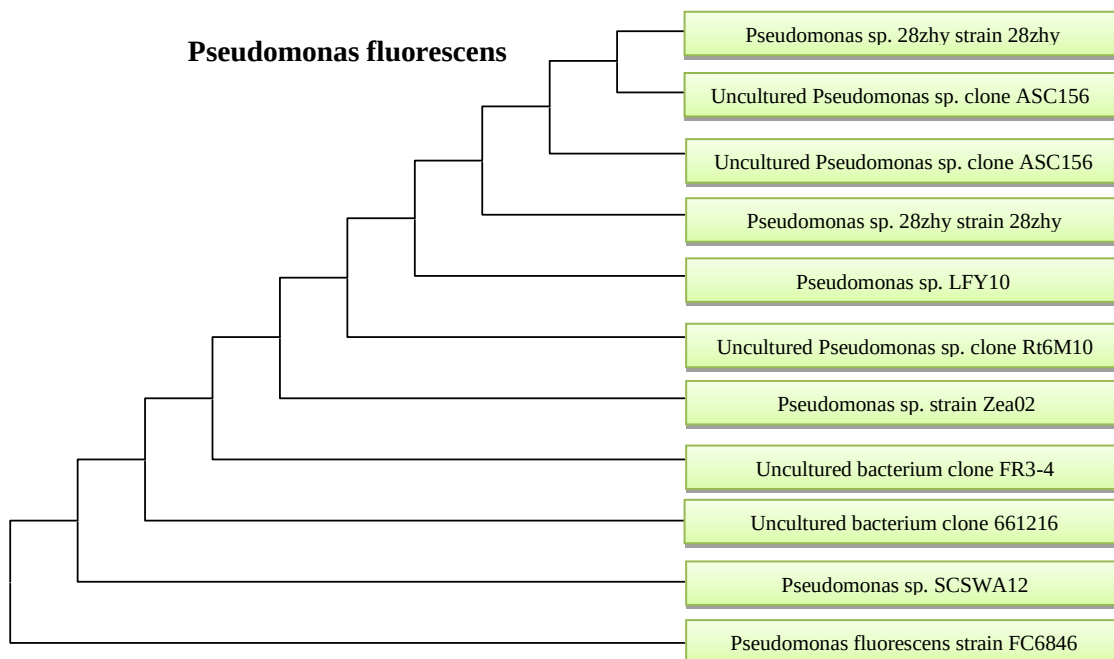


Fig 19 Phylogenic tree depicting the species of **Pseudomonas fluorescens**

Enterococcus faecium

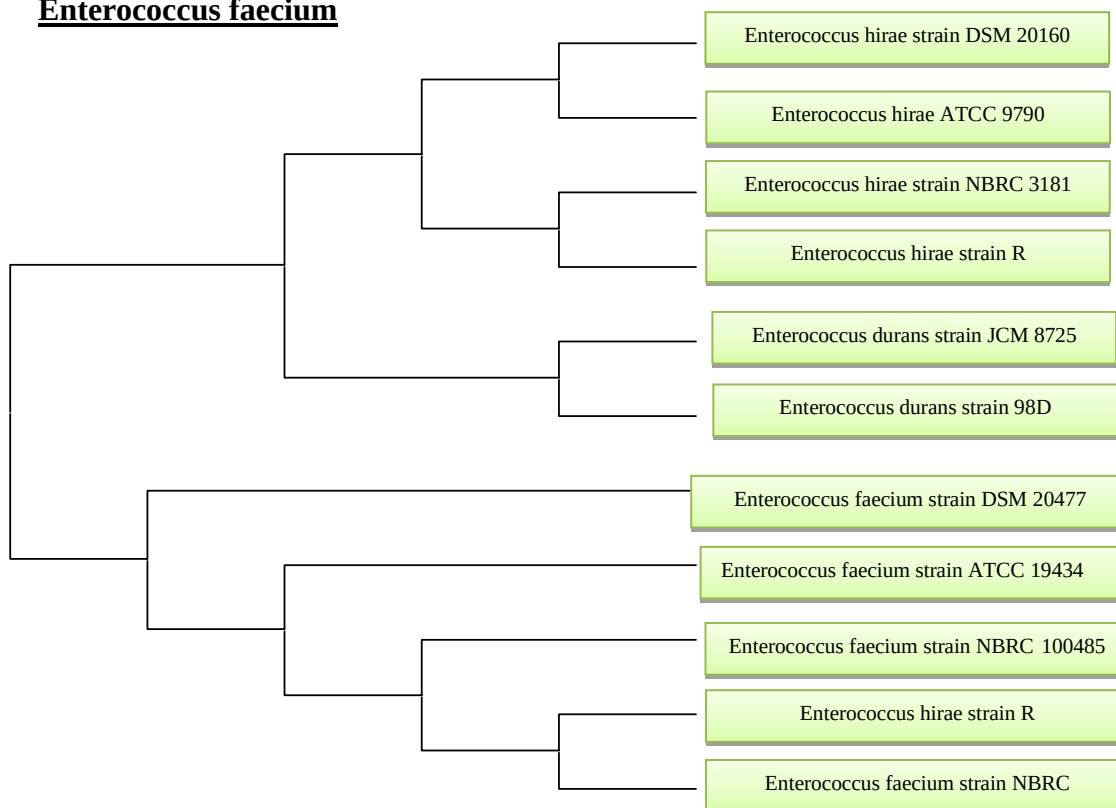


Fig 20 Phylogenetic tree depicting the species of *Enterococcus faecium*

Enterococcus faecalis

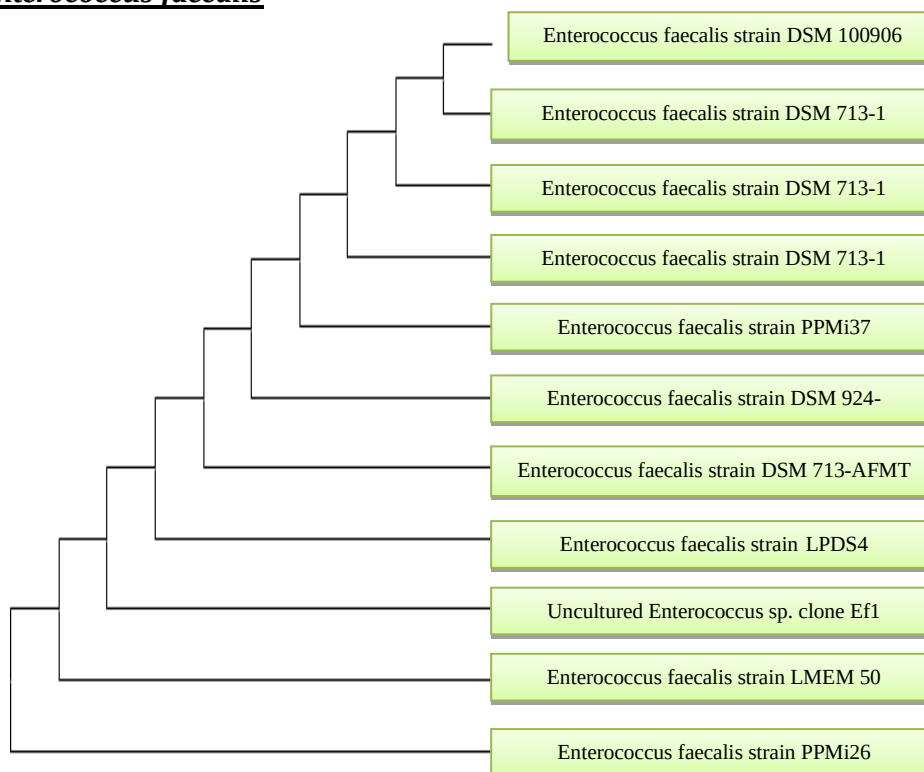


Fig 21 Phylogenetic tree depicting the species of *Enterococcus faecalis*

Escherichia coli

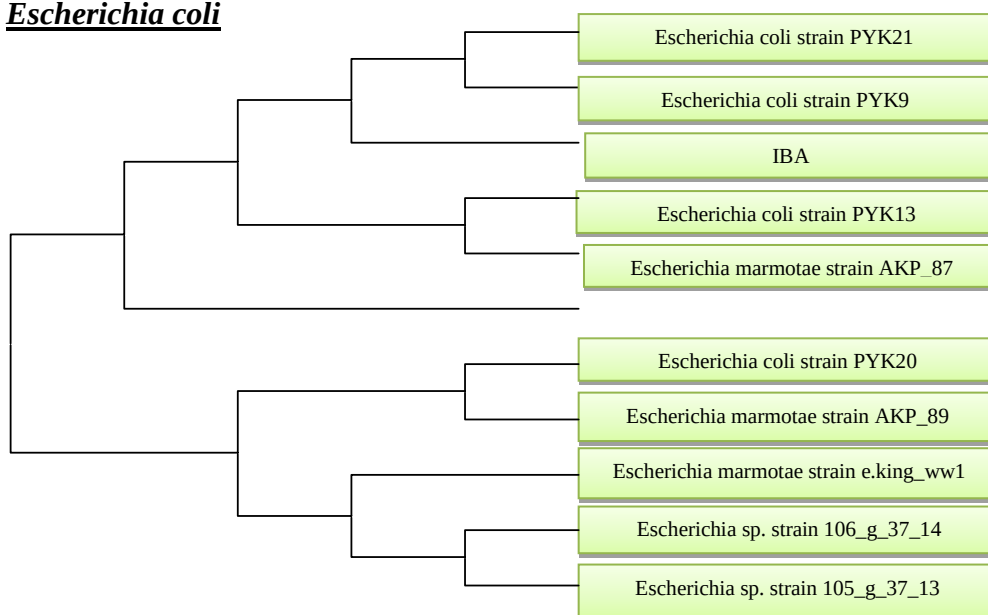


Fig 21 Phylogenic tree depicting the species of *Escherichia coli*

Table 5: Distribution of isolated pathogens bacteria among study group (n=200)

Flora	Sustained pregnancy (%) (n=100)	Flora	Spontaneous abortion (%) (n=100)
Lactobacillus inners	58	Escherichia Coli	48
Lactobacillus crispatus	32	Enterococcus faecalis	28
Escherichia coli	10	Enterococcus faecium	12
		Pseudomonas fluorescens	7
		Pseudomonas cedrina	5
Total	100		100

OUTCOME

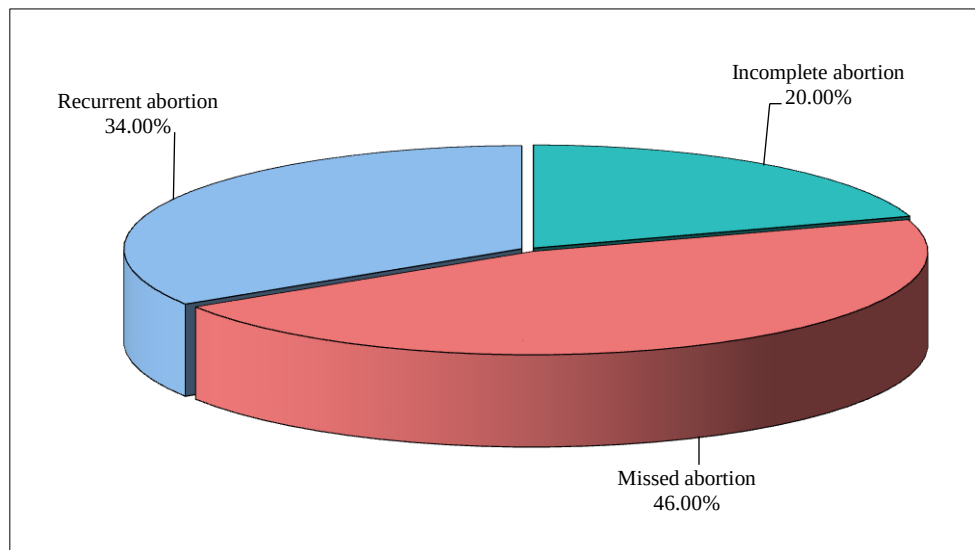
The pathogen identification rate was 13.15% (n=200) when 200 subjects' vaginal secretion cultures were examined. The bacterial detection rates were 19.96% in women who had an SA (N=100) and 7.93% in the women who maintained a pregnancy of the same gestation (N=100), which were significantly different ($p < 0.05$). In comparison to the women who continued their pregnancy, those with SA had a greater pathogen detection rate, while the women who continued pregnancy had less aberrant vaginal flora. Additionally, the pathogenic flora types in the women who experienced SA were more diverse. Within the SA group (N=100), the five most prevalent species were: 1) *Escherichia coli* (48%), 2) *Enterococcus faecalis* (28 %), 3) *Enterococcus faecium* (12%), 4) *Pseudomonas fluorescens* (7%), and 5) *Pseudomonas cedrina* (5%).

The most common species in the group who continued pregnancy (N=100) were 1) *Lactobacillus inners* (58%), 2) *Lactobacillus crispatus* (32%), and 3) *Escherichia coli* 10% ($p < 0.05$). Pregnant women had comparatively decreased detection rates of *Lactobacillus crispatus* and *Escherichia coli*. In the women who experienced SA, *Escherichia coli* accounted for the largest percentage.

SECTION B: SOCIODEMOGRAPHIC DETERMINANTS OF SPONTANEOUS ABORTION

The study also examine the association between sociodemographic factors and reproductive history with spontaneous abortion and its comparison with the mother those who have continued pregnancy with same gestational age attending the OPD (Obstetrics & Gynecology Department) of tertiary care hospital Belagavi

Each group had a total of 100 responses (Group 1: 100 and Group 2: 100). Women who experienced a spontaneous abortion before 20 weeks of pregnancy and who had been admitted to the hospital for a medical procedure were classified as Group 1. Group 2 consisted of women who visited the antenatal outpatient department (OPD) and continued their pregnancy with the same gestational age.



Graph 6: Pie Graph showing the Types of abortion in abortion group (n=100)

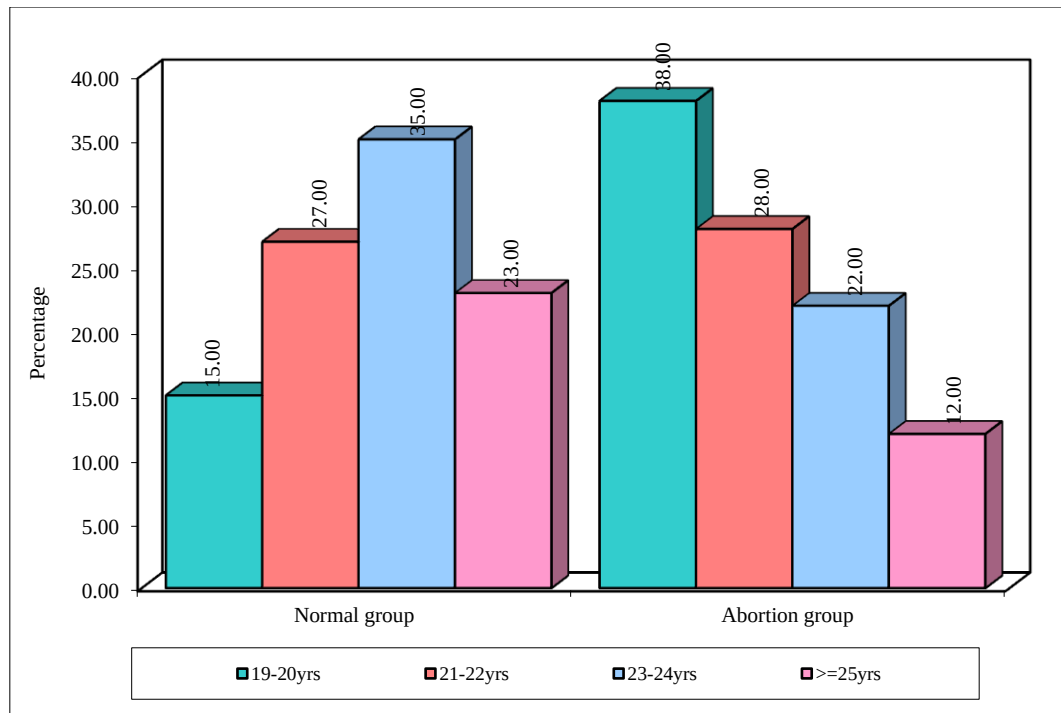
As mentioned in the figure above considering the type of abortion among mother who underwent abortion majority (46%) mothers had missed abortion, 34% mothers had recurrent abortion and 20% mothers had incomplete abortion.

Table 6: Association between abortions with Maternal Age**(n=200)**

Marital age	Normal group	%	Abortion group	%	Total	%	Chi-square	p-value
19-20yrs	15	15.00	38	38.00	53	26.50	8.0210	0.0460*
21-22yrs	27	27.00	28	28.00	55	27.50		
23-24yrs	35	35.00	22	22.00	57	28.50		
>=25yrs	23	23.00	12	12.00	35	17.50		
Total	100	100.00	100	100.00	200	100.00		

***p<0.05**

The chi square value 8.0210, with p value 0.0460* where p value is less than 0.05 shows that there is significant association between the marital age with the mothers those who underwent abortion and those continued pregnancy.



Graph 7: Bar Graph showing Comparison of normal group and abortion group with Maternal Age (n=200)

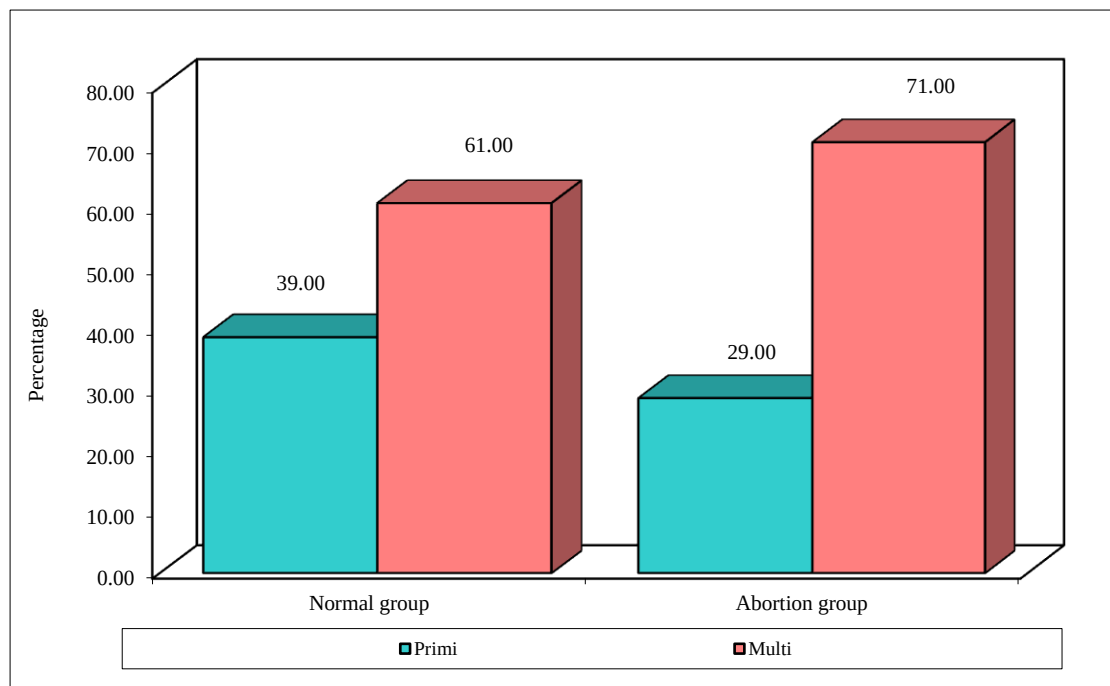
As shown in the above graph the mothers who underwent spontaneous abortion majority (38%) fall into the age group of 19 -20years when compared with mother who continued pregnancy majority (35%) of mother belong to the age group of 23-24years.

Table 7: Association between abortions with Parity

(n=200)

Parity	Normal group	%	Abortion group	%	Total	%	Chi-square	p-value
Primi	39	39.00	29	29.00	68	34.00	2.2280	0.1360
Multi	61	61.00	71	71.00	132	66.00		
Total	100	100.00	100	100.00	200	100.00		

The chi square value 2.2280, with p value 0.1360 where p value is more than 0.05 shows that there is no significant association between the parity with the mothers those who underwent abortion and those continued pregnancy



Graph 8: Bar Graph Showing the: Comparison of normal group and Abortion group with Parity

(n=200)

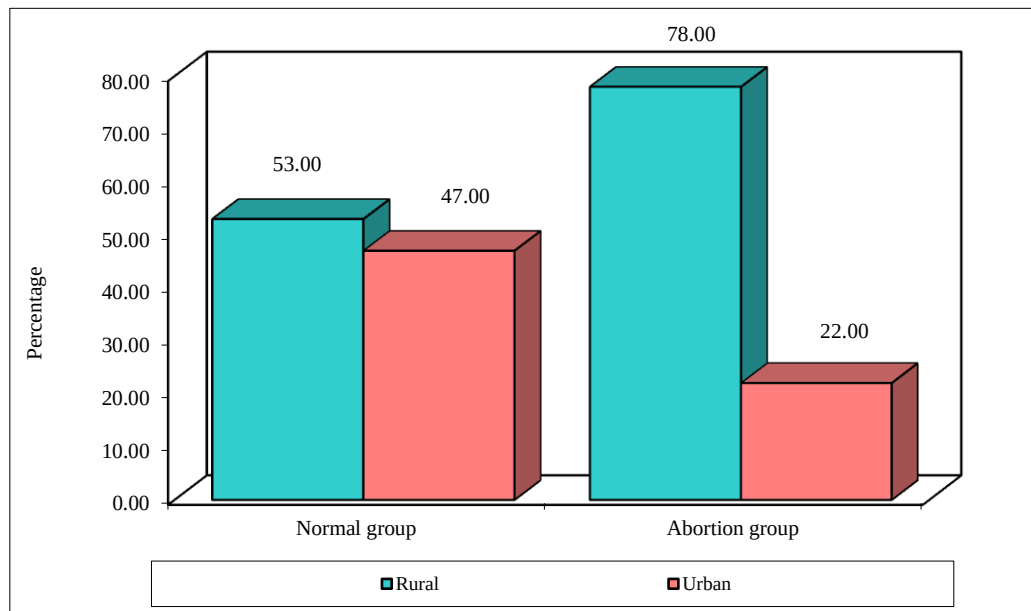
As revealed in the above graph the mother who underwent abortion majority (71%) were multi

Table 8: Association between abortions with Area of Residence (n=200)

Area of residence	Normal group	%	Abortion group	%	Total	%	Chi-square	p-value
Rural	53	53.00	78	78.00	131	65.50	15.7120	0.0001*
Urban	47	47.00	22	22.00	69	34.50		
Total	100	100.00	100	100.00	200	100.00		

***p<0.05**

The chi square value 15.7120, with p value 0.0001* where p value is less than 0.05 shows that there is significant association between the area of residence with the mothers those who underwent abortion and those continued pregnancy



Graph 9: Bar Graph showing the Comparison of normal group and abortion group with Area of Residence (n=200)

As revealed in the above graph the mother who underwent abortion majority (78%) belong to rural area when compared with mother who continued pregnancy majority (53%) belong to urban area

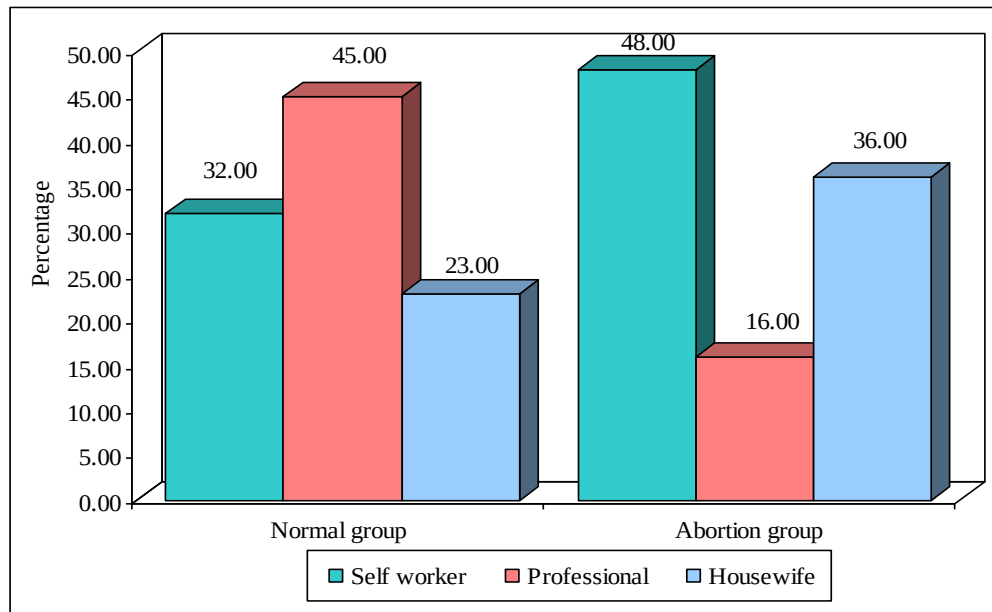
Table 9: Association between abortions with Occupations

(N=200)

Occupations	Normal group	%	Abortion group	%	Total	%	Chi-square	p-value
Self-worker	32	32.00	48	48.00	80	40.00	19.8510	0.0001*
Professional	45	45.00	16	16.00	61	30.50		
Housewife	23	23.00	36	36.00	59	29.50		
Total	100	100.00	100	100.00	200	100.00		

*p<0.05

The chi square value 19.8510, with p value 0.0001* where p value is less than 0.05 shows that there is significant association between the occupation of mother with the mothers those who underwent abortion and those continued pregnancy



Graph 10: Bar Graph showing Comparison of normal group and abortion group with Occupation **(n=200)**

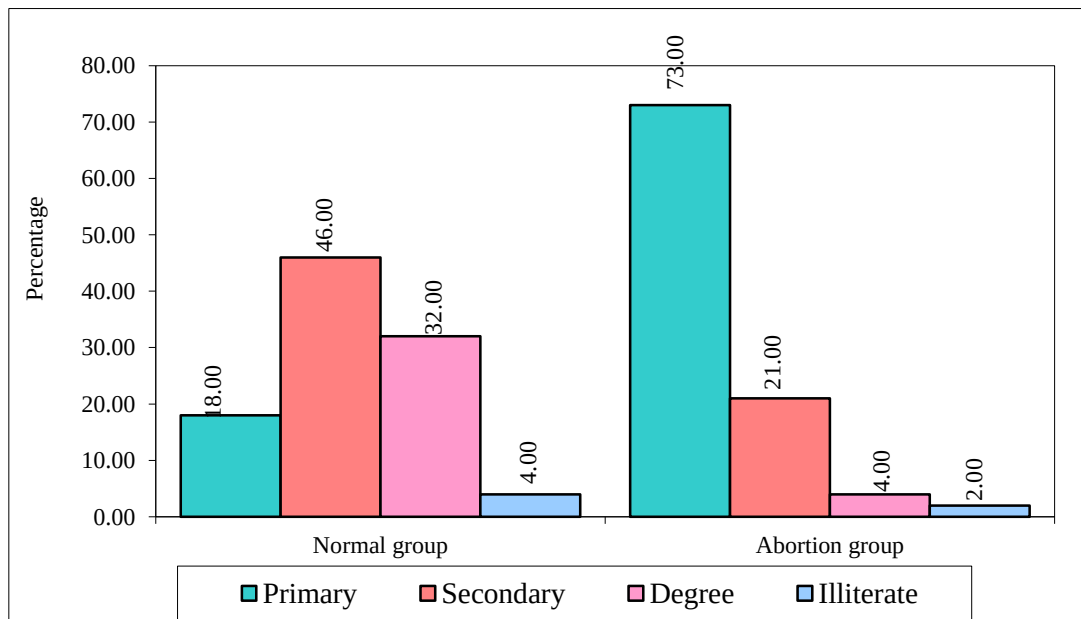
As mentioned in the above graph the mother who underwent abortion majority (48%) were self-occupied r when compared with mother who continued pregnancy majority (45%) were professionals

Table 10: Association between abortions with Maternal Education (n=200)

Maternal education	Normal group	%	Abortion group	%	Total	%	Chi-square	p-value
Primary	18	18.00	73	73.00	91	45.50	65.0150	0.0001*
Secondary	46	46.00	21	21.00	67	33.50		
Degree	32	32.00	4	4.00	36	18.00		
Illiterate	4	4.00	2	2.00	6	3.00		
Total	100	100.00	100	100.00	200	100.00		

*p<0.05

The chi square value 65.0150, with p value 0.0001* where p value is less than 0.05 shows that there is significant association between the maternal education with the mothers those who underwent abortion and those continued pregnancy



Graph 11: Bar Graph showing Comparison of normal group and abortion group with Maternal Education (n=200)

As mentioned in the above graph the mother who underwent abortion majority (73%) were illiterate when compared with mother who continued pregnancy majority (46%) had secondary education

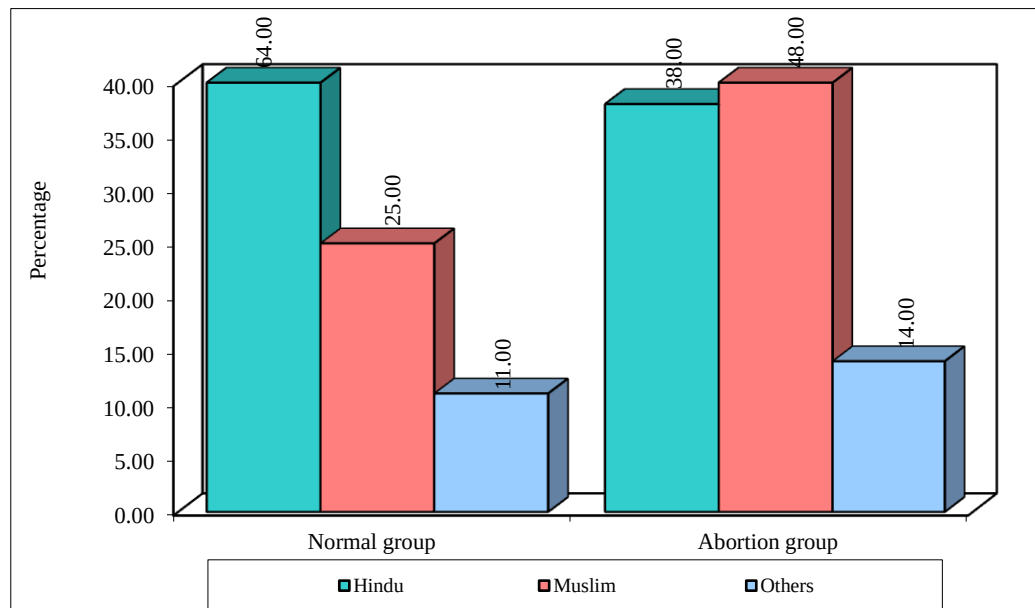
Table 11: Association between abortions with Religion

(n=200)

Religions	Normal group	%	Abortion group	%	Total	%	Chi-square	p-value
Hindu	64	44.00	38	38.00	102	51.00	27.6680	0.0001*
Muslim	25	45.00	48	48.00	73	36.05		
Others	11	11.00	14	14.00	25	12.05		
Total	100	100.00	100	100.00	200	100.00		

*p<0.05

The chi square value 27.6680 with p value 0.0001* where p value is less than 0.05 shows that there is significant association between the religion with the mothers those who underwent abortion and those continued pregnancy



Graph 12: Bar Graph showing the Comparison of normal group and abortion group with Religion

(n=200)

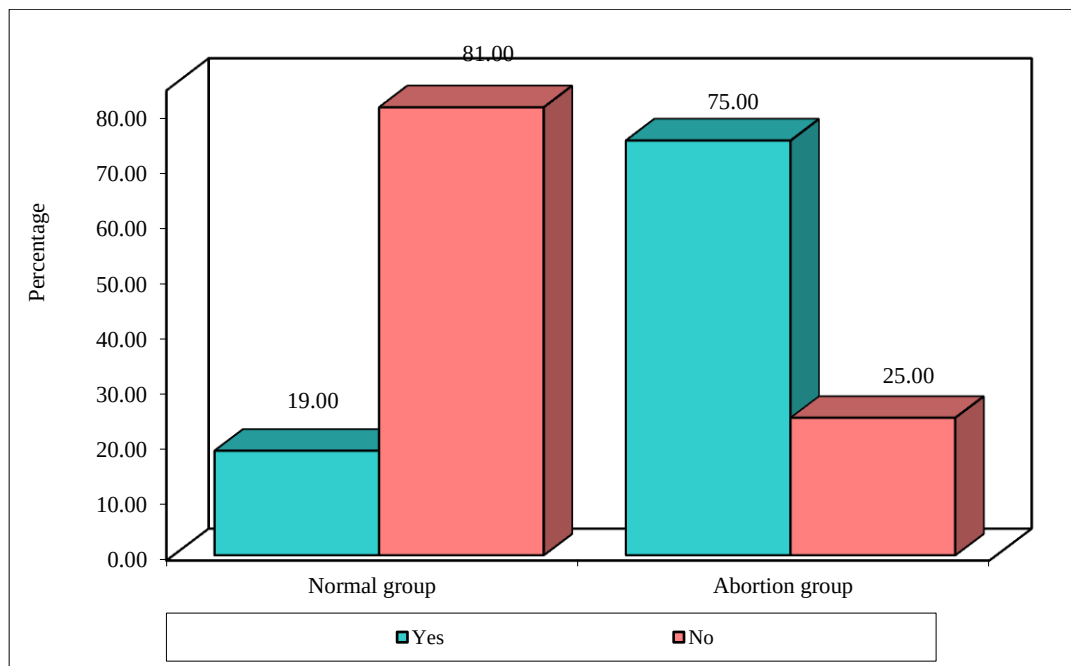
As mentioned in the above graph the mother who underwent abortion majority (48%) were from Muslim religion when compared with mother who continued pregnancy majority (64%) were from Hindu religion.

Table 12: Association between abortions with consanguineous marriage (n=200)

Consanguineous marriage	Normal group	%	Abort ion group	%	Total	%	Chi-square	p-value
Yes	19	19.00	75	75.00	94	47.00	63.1120	0.0001*
No	81	81.00	25	25.00	106	53.00		
Total	100	100.00	100	100.00	200	100.00		

*p<0.05

The chi square value 63.1120, with p value 0.0001* where p value is less than 0.05 shows that there is significant association between the consanguineous marriage with the mothers those who underwent abortion and those continued pregnancy



Graph 13: Comparison of normal group and abortion group with consanguineous marriage (n=200)

As mentioned in the above graph the mother who underwent abortion majority (75%) had nuclear consanguineous marriage when compared with mother who continued pregnancy majority (81%) didn't had consanguineous marriage

Table 13: Association between abortions with socio demographic characteristics
by simple logistics regression analysis (n=200)

Factors	Normal group	%	Abortion group	%	Unadjusted OR	95% CI for OR		p-value
						Lower	Upper	
Marital age								
19-20yrs	15	15.00	28	28.00	1.87	1.00	3.50	0.0500*
21-22yrs	35	35.00	38	38.00	1.09	0.69	1.72	0.7260
23-24yrs	27	27.00	22	22.00	0.82	0.46	1.43	0.4760
>=25yrs	23	23.00	12	12.00	Ref.			
Parity								
Primi	39	39.00	29	29.00	1.16	0.83	1.64	0.3850
Multi	61	61.00	71	71.00	Ref.			
Area of residence								
Rural	53	53.00	78	78.00	1.47	1.04	2.09	0.0300*
Urban	47	47.00	22	22.00	Ref.			
Occupations								
Self-worker	32	32.00	48	48.00	1.50	0.96	2.35	0.0760
Professional	45	45.00	16	16.00	0.36	0.20	0.63	0.0001*
Housewife	23	23.00	36	36.00	Ref.			
Maternal education								
Primary	18	18.00	73	73.00	Ref.			
Secondary	46	46.00	21	21.00	0.46	0.27	0.77	0.0030*
Degree	32	32.00	4	4.00	0.13	0.04	0.35	0.0001*
Illiterate	4	4.00	2	2.00	0.50	0.09	2.73	0.4230
Religions								
Hindu	44	44.00	80	80.00	Ref.			
Muslim	45	45.00	17	17.00	0.38	0.22	0.66	0.0010*
Others	11	11.00	3	3.00	0.27	0.08	0.98	0.0460*
Consignees marriage								
Yes	19	19.00	75	75.00	3.95	2.39	6.53	0.0001*
No	81	81.00	25	25.00	Ref.			

Risk of abortion with unadjusted odds ratio 1.87 times more in the age group of 19-20years as compare to the age of mothers ≥ 25 yrs , parity primi mother with unadjusted odds ratio 1.16 times had more risk of abortion when compared with multi mother, Area of residence the mother who stayed in rural area with unadjusted ratio 1.47 times more when compared with mother who stayed in urban, occupation the mothers who were self-workers with unadjusted odds ratio 1.50 times more when compared with housewife when looking into the education of mother illiterate mother with unadjusted odds ratio 0.50 times had risk of abortion when compared it with primary education,, in view with Religion muslims with unadjusted odds ratio 0.38 times more when compared with Hindu religion, consanguineous marriage with unadjusted odds ratio 3.95 had times more risk of abortion

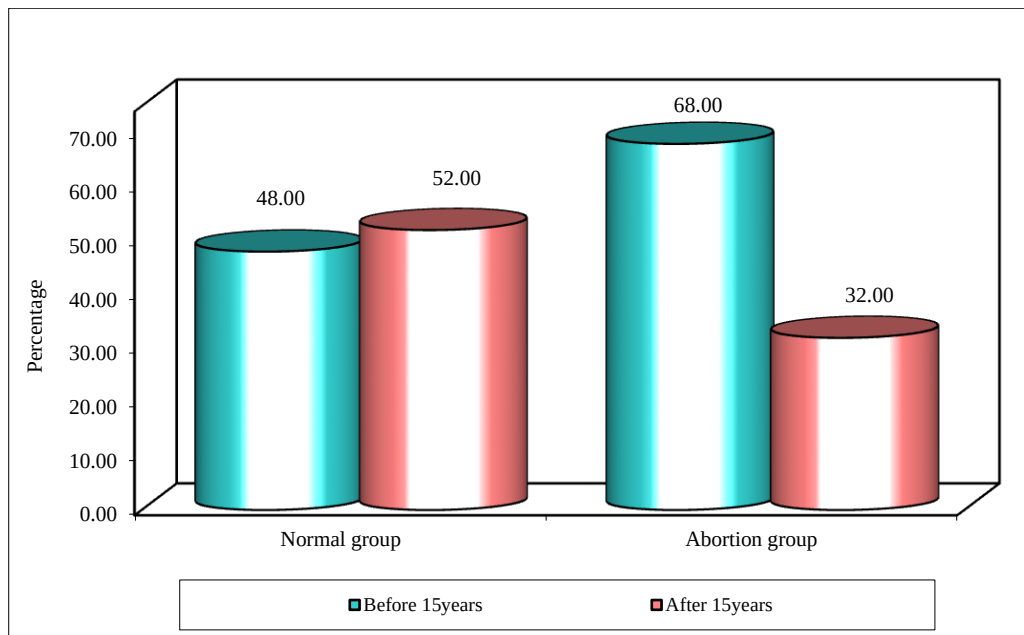
REPRODUCTIVE FACTORS

Table 14: Association between abortions with age at menarche (n=200)

Age at menarche	Normal group	%	Abort ion group	%	Total	%	Chi-square	p-value
Before 15years	48	48.00	68	68.00	116	58.00	8.2100	0.0040*
After 15years	52	52.00	32	32.00	84	42.00		
Total	100	100.00	100	100.00	200	100.00		

*p<0.05

The chi square value 8.2100, with p value 0.0040* where p value is less than 0.05 shows that there is significant association between age at menarche with the mothers those who underwent abortion and those continued pregnancy



Graph 14: Bar Graph showing the Comparison of normal group and abortion group with age at menarche (n=200)

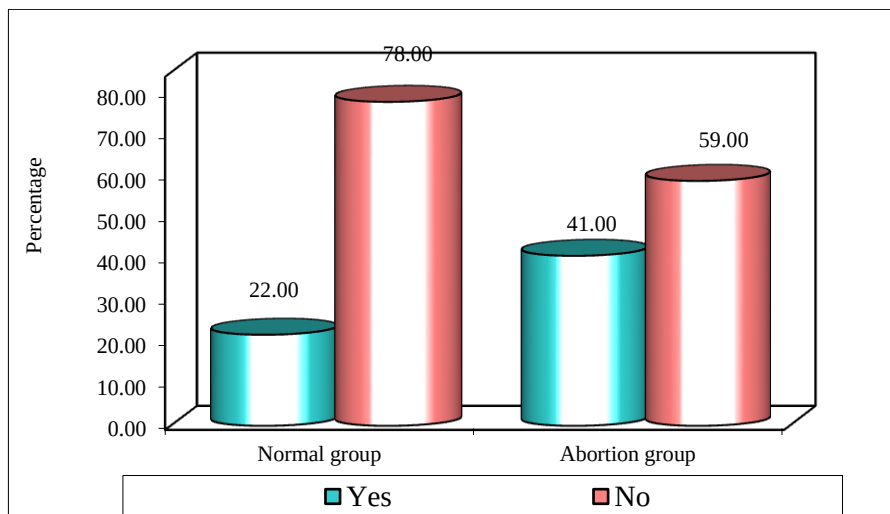
As mentioned in the above graph the mother who underwent abortion majority (68%) of mother attained menarche before 15 years when compared with mother who continued pregnancy majority (52%) attained menarche after 15 years.

Table 15: Association between abortions with any complication during last pregnancy (n+200)

Any complication during last pregnancy	Normal group	%	Abortion group	%	Total	%	Chi-square	p-value
Yes	22	22.00	41	41.00	63	31.50	8.3650	0.0040*
No	78	78.00	59	59.00	137	68.50		
Total	100	100.00	100	100.00	200	100.00		

*p<0.05

The chi square value 8.3650 with p value 0.0040* where p value is less than 0.05 shows that there is significant association between complication during last pregnancy with the mothers those who underwent abortion and those continued pregnancy



Graph 15: Bar Graph showing the Comparison of normal group and abortion group with any complication during last pregnancy (n=200)

As mentioned in the above graph the mother who underwent abortion majority (59%) of mother's had complication in previous pregnancy when compared with mother who continued pregnancy majority (78%) of mother's didn't had complication in previous pregnancy

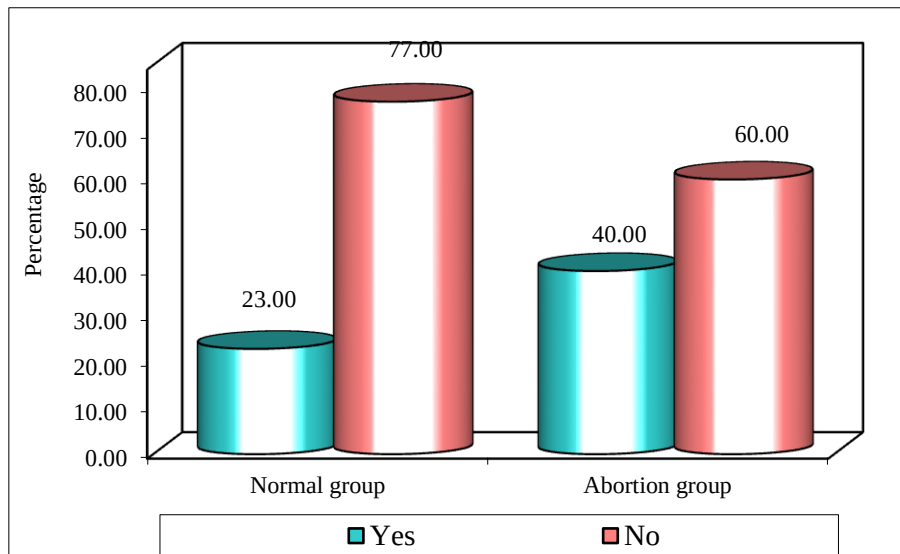
Table 16: Association between abortions with history of previous Abortion

(n=200)

History of previous abortion	Normal group	%	Abort ion group	%	Total	%	Chi-square	p-value
Yes	23	23.00	40	40.00	63	31.50	6.6970	0.0100*
No	77	77.00	60	60.00	137	68.50		
Total	100	100.00	100	100.00	200	100.00		

*p<0.05

The chi square value 6.6970 with p value 0.0100* where p value is less than 0.05 shows that there is significant association between history of previous abortion with the mothers those who underwent abortion and those continued pregnancy



Graph 16: Bar Graph showing the Comparison of normal group and abortion group with history of previous abortion (n=200)

As stated in the above graph the mother who underwent abortion majority (60%) of mother's were with previous abortion when compared with mother who continued pregnancy majority (77%) of mother's didn't had history of previous abortion.

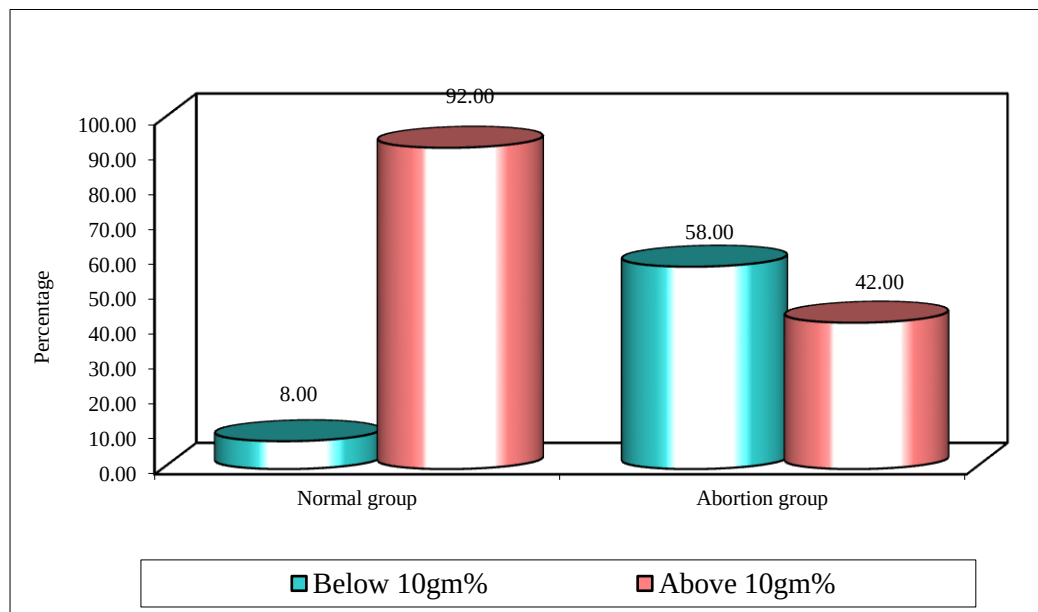
Table 17: Association between abortions with levels of Hb%

(n=200)

Hb%	Normal group	%	Abortion group	%	Total	%	Chi-square	p-value
Below 10 gm%	8	8.00	58	58.00	66	33	5.7010	0.0170*
Above 10gm%	92	92.00	42	42.00	134	67		
Total	100	100.00	100	100.00	200	100.00		

*p<0.05

The chi square value 5.7010 with p value 0.0170* where p value is less than 0.05 shows that there is significant association between Hb% of mother with those who underwent abortion and those continued pregnancy



Graph 17: Bar Graph showing the Comparison of normal group and abortion group with levels of hb%

(n=200)

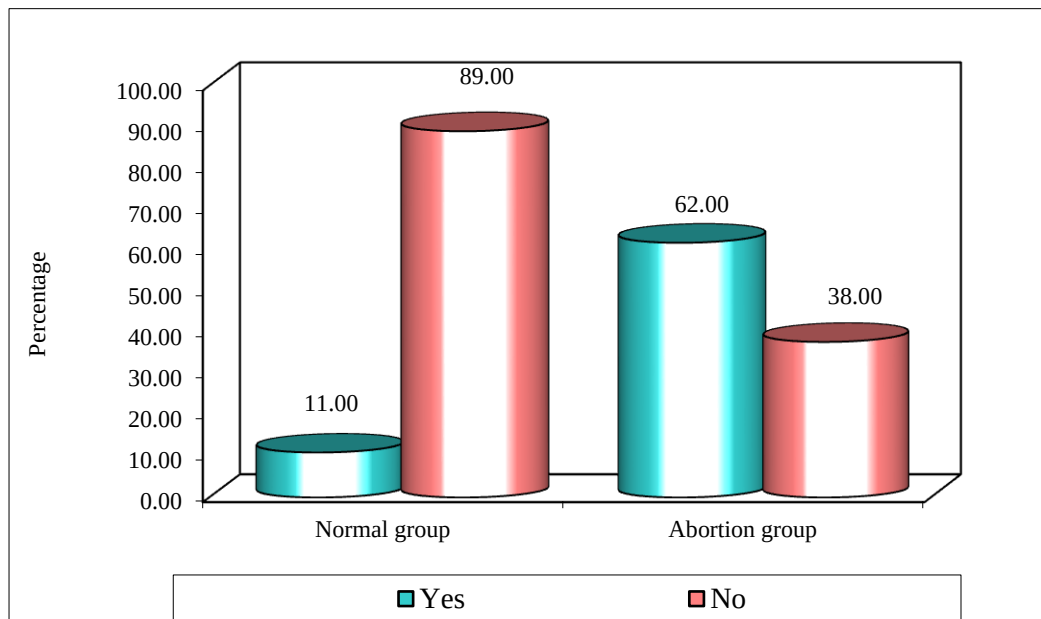
As stated in the above graph, the mother who underwent abortion majority (58%) of mother's Hb was below 10gm% when compared with mother who continued pregnancy majority (92%) of mother's Hb was above 10gm%.

Table 18: Association between abortions with Maternal infection (n=200)

Maternal infection	Normal group	%	Abortion group	%	Total	%	Chi-square	p-value
Yes	11	11.00	62	62.00	73	46.50	101.316	0.0001*
No	89	89.00	38	38.00	127	53.50		
Total	100	100.00	100	100.00	200	100.00		

*p<0.05

The chi square value 101.316 with p value 0.0001* where p value is less than 0.05 shows that there is significant association between maternal infection of mother with those who underwent abortion and those continued pregnancy.



Graph 18: Bar Graph showing the Comparisons of normal group and abortion group with maternal infection (n=200)

As detailed in the above graph, the mother who underwent abortion majority (62%) of mother's had maternal infection when compared with mother who continued pregnancy majority (89%) of mother's didn't have maternal infection.

Table 19: Association between abortions with pregnancy/reproductive related variables by simple logistic regression analysis (n=200)

Pregnancy variables	Normal group	%	Abort ion group	%	Unadj usted OR	95% CI for OR		p-value
						Lower	Upper	
Age at menarche								
Before 15years	48	48.00	68	68.00	1.42	0.98	2.05	0.0650
After 15years	52	52.00	32	32.00	Ref.			
Any complication during last pregnancy								
Yes	22	22.00	41	41.00	1.86	1.11	3.13	0.0180*
No	78	78.00	59	59.00	Ref.			
History of previous abortion								
Yes	23	23.00	40	40.00	1.74	1.04	2.91	0.0340*
No	77	77.00	60	60.00	Ref.			
Hb%								
Above 10gm%	8	8.00	1	1.00	Ref.			
Below 10gm%	92	92.00	99	99.00	1.08	0.81	1.43	0.6130
Maternal infection								
Yes	11	11.00	82	82.00	7.46	3.97	13.99	0.0001*
No	89	89.00	18	18.00	Ref.			

*p<0.05

Risk of abortion with unadjusted odds ratio 1.42 times more in the mothers those who attained menarche before 15 years compare to the mother who attained menarche after 15 years , the mother who had complication during last pregnancy with unadjusted odds ratio 1.86 times more risk of abortion when compared with those who didn't had complication , the mother those who had history of previous abortion with unadjusted ratio 1.74 times more risk when compared with mother who didn't had abortion , in view with Hb% the mothers those who had Hb below 10gm% with unadjusted odds ratio 1.08 times more risk of abortion when compared with the mothers those who had Hb above 10gm% ,the mothers those who had infection during pregnancy with unadjusted odds ratio 7.46 times had more risk of abortion when compared with the mother those who didn't had.

CHAPTER V

DISCUSSION

This chapter deals with the discussions and a brief framework of the study findings, conclusions, implications, and recommendations. Discussions are either supported or contested by the literature or critical analysis.

In our study we sought to determine whether a particular organism may be associated with SA, by comparing the vaginal microbiomes of women who experienced an abortion spontaneously within 20 weeks of gestation and those who maintained their pregnancy with the same gestational age.

- The vaginal flora of the women that had an SA was more varied and demonstrated specific species (i.e., *Enterococcus faecalis*, *Pseudomonas fluorescens*, *Enterococcus faecium*, *Escherichia coli*, *Pseudomonas cedrina*). Our results are consistent with those of previous investigators. Al-dorri (2016) found that 35 (39.32%) of those who experienced SA had *Listeria* infection in the second trimester, and 54 (60.68%) in the first trimester.
- *Enterococcus faecalis* is one of the main constituents of the flora in human intestinal tracts, and in our study the *Enterococcus faecalis* detection rate was 28% (n=100). Schlüter and colleagues (2009) speculated that one of the primary components of the intestinal flora in both human and animal digestive systems is *E. faecalis*. *E. faecalis* has the capacity to build biofilm in the vagina, as found.⁹³

Given that the vaginal and urinary tracts are adjacent, Vaginal discharge culture results in the SA group showed that *E. faecalis* was the most prevalent result at 3.17% (48/1,513), significantly greater than that of the NP and control groups ($P < 0.05$).

- Barcaite et al. (2012) collected less vaginal swabs from 827 neonates and 998 pregnant women. The colonization rates of *Escherichia coli* in the neonates and women were 14.4% and 19.9%, respectively. According to the results of the current study, 48% of women who experienced SA had an *Escherichia coli* detection rate, which is consistent with the findings of Barcaite and colleagues' (2012) inquiry in which the SA group's detection rate was 2.51%. which relates to the findings of the present study in which the *Escherichia coli* detection rate was 48% in women who experienced SA.⁹⁴
- In present study *Lactobacillus inners* 58% (n=100), was highest prevalent bacteria among women who continued pregnancy. The results coincides with research done by Dimitrios Nasioudis¹, Larry J. Forney et al which found that 3.8% of first-time mothers tested positive for *Lactobacillus iners*,⁹⁵
- According to the current study the proportion of *Lactobacillus crispatus* was 32% (n=100). among women who carried on with their pregnancies, the findings align with the study carried out by (Barcaite et al., 2012) whereas the percentage of women who experienced their first conception contained *Lactobacillus crispatus* (76.4%).
- An overgrowth of aerobic bacterial flora leads to a shift in the vaginal microbiota, which is characteristic of aerobic vaginitis (AV). Normally, lactobacilli perform this function. An association between AV and Group B *Streptococcus* (GBS), specifically *S. aureus*, *E. coli*, and *E. faecalis* was demonstrated by the evidence.⁹⁶

- Up to 20% of expectant mothers experience BV, which has been linked to SA.[16] Although a watery discharge with a fishy odor is frequently the defining feature of this illness, 50% of the women who have this infection may not have any symptoms at all or just have minor ones. A growing body of research indicated that BV in pregnancy was linked to poor perinatal outcomes, even though the majority of women with BV in early pregnancy would probably not experience a chronic infection later in pregnancy
- Study conducted by Xiau-Li LU et al findings of the study inferred that 425 samples (13.15%) out of 3,233 vaginal discharge samples were positive for a pathogen. In the SA, NP, and control groups, the pathogen detection rates were 19.96% (302/1,513), 7.93% (72/908), and 6.28% (51/812), respectively. The five most prevalent bacteria were: Uu, C. albicans, Candida tropicalis, GBS, and E. faecalis in the NP group; Candida glabrata, Uu, E. coli, Gardnerella vaginalis, and C. albicans in the control group; and Enterococcus faecalis, Candida albicans, Escherichia coli, group B Streptococcus (GBS), and Ureaplasma urealyticum (Uu) in the SA group; and Uu, C. albicans, GBS, in the NP group .the finding coincides with the findings of preseny study⁹⁷
- Consequently, researcher postulated that infection could cause Spontaneous abortion because the highest number of infectious pathogens was found in those women's significant samples of vaginal discharge from the Spontaneous abortion and non-pregnant women, and Bacterial vaginosis is considered a risk factor for Spontaneous abortion (Nasioudis & Forney, 2017).

SECTION B: SOCIO DEMOGRAPHIC AND REPRODUCTIVE DETERMINANTS OF SPONTANEOUS ABORTION

- In this study, we explored the relationship between sociodemographic characteristics and variables associated to reproduction and spontaneous abortion. The results of the study showed that there was a statistically significant correlation between the mother's occupation, age at marriage, place of residence, mother's education, religion and consanguineous marriage. It also showed a statistically significant correlation with other reproductive factors, including age at menarche, complications during the previous pregnancy, history of abortion, her level of hemoglobin percentage, and maternal infection.
- In contrast to Zheng, D., Li, C., Wu, T., & Tang, K.'s (2017) study. SA risk was 1.68 times higher in rural areas than in urban areas (AOR = 1.68, 95%CI: 1.54–1.84). Compared to women with low incomes, women with high incomes had a lower risk of SA (AOR = 0.90, 95%CI: 0.84–0.97). Women with higher educational attainment showed a reduced prevalence of SA (AOR = 0.90, 95%CI: 0.82–0.98) compared to those with lower educational attainment.⁹⁸
- When compared to workers in agricultural and related industries, the risk of SA was shown to be lower only in industrial workers (AOR = 0.59, 95%CI: 0.53–0.66) and professional workers (AOR = 0.75, 95%CI: 0.66–0.84). Following stratification based on rural/urban characteristics, there was a greater correlation between income and SA in urban areas (AOR = 0.88, 95%CI: 0.78–0.99) compared to rural areas (AOR = 0.92, 95%CI: 0.84–1.00). Urban areas had a higher association between education and SA (AOR = 0.66,

95%CI: 0.55–0.78) than rural areas (AOR = 1.05, 95%CI: 0.34–1.17), and there was no difference in the impact of occupation on SA among women in either grouping.

- In comparison to the current study, the risk of abortion was 1.87 times higher in the 19–20 year age group, the risk of abortion was 1.16 times higher for parity primi mothers, and the risk of abortion was 1.47 times higher for mothers who lived in rural areas compared to mothers who lived in urban areas.
- With considering occupation, mothers who worked independently had an unadjusted odds ratio 1.50 times higher than those who were housewives. When it concerned education, illiterate mothers had an unadjusted odds ratio 0.50 times higher risk of abortion. When it came to religion, Muslims had an unadjusted odds ratio 0.38 times higher than Hindus. Taking into account the type of family, mothers who were part of nuclear families had an unadjusted odds ratio 1.04 times higher than those who were housewives, and mothers in consanguineous marriages had an unadjusted odds ratio 3.95 times higher risk of abortion.

CHAPTER VI

CONCLUSION

In conclusion, we speculate that vaginal infection may play a role in SA as evidenced by the organisms *Enterococcus faecalis*, *Pseudomonas fluorescens*, *Enterococcus faecium*, *Escherichia coli*, and *Pseudomonas cedrina* that were abundant in the vaginal flora of the women who experienced SA when compared with women who continued their pregnancy.

Future research to better understand the relationship between pregnancy and vaginal flora will enable the creation of new diagnostic tools, treatments, and preventive measures as well as prompt identification and treatment to improve one's odds of maintaining their pregnancy.

Women who had spontaneous abortions had a vaginal microbiome that was more varied and irregularly distributed with bacteria. The bacteria identified could affect the women's pregnancy outcome and perhaps result in spontaneous abortion.

It is vital for nurses to provide education to women who are in the fertile period regarding prenatal screening, sexually transmitted infections, and perineal care.

The need for additional care, support, and oversight of health promotion activities for women with such socioeconomic and reproductive variables will have a substantial impact on policy and program development. Assessing pregnant mothers for modifiable risk factors and increasing awareness among them can help lower the rate of pregnancy loss.

Implication for Nursing**a. Nursing Practice**

Previous research as well as findings from this study provides evidence that bacterial vaginosis may be a risk factor for SA. As a result, routine screening for these microorganisms for women who are of reproductive age should be included as a routine practice to an antenatal visit. Attention should be given to women who are in reproductive age to provide education about potential adverse pregnancy outcomes caused by invasion of these microorganisms into vaginal flora during pregnancy. A nurse may make a significant impact in several areas related to obstetric care, including educational counselling, implementing therapeutic and preventive measures, and communicating with expectant mothers and their families. As the vaginal microbiome is directly impacted by sex, nutrition/diet, and behavioural health nurses can educate pregnant individuals regarding perineal care, dietary regimens, as well as the causes, early signs, and prevention of vaginal infections during pregnancy.

Clinical Implications

- Nurses can provide education to pregnant women about prevention of vaginal infections through perineal care to increase their awareness of the adverse effects of infection during pregnancy.
- By taking a detailed health history, nurses can assess pregnant women for signs and symptoms of vaginal infections as part of routine prenatal care.
- In rural areas and/or regions with decreased access to obstetric providers, nursing education should be strengthened to help nurses and advance practice nurses know how to detect and treat such cases early in a woman's pregnancy.

b. Nursing Education

Nurses play an integral role in providing and advocating for best practices for safe and effective care that meet the health, emotional, and social needs of the mother, and family. The nursing curriculum should enable nursing students to develop advanced knowledge and acquire skills in the practice of maternal healthcare. The study findings suggest that the course content in the curriculum should cover a range of vaginal microbiome and its association with adverse pregnancy outcome and management of morbidity measurement techniques in maternal health

In-service education programs, workshops and seminars on methods of imparting effective antenatal care, measures to prevent vaginal infection and associated perinatal morbidity and measures to prevent adverse pregnancy outcome should be conducted for nursing personnel so that they can upgrade their knowledge and skills and apply it in their practice. Nursing students should also be encouraged for the effective utilization of research-based practice in the clinical areas.

c. Nursing Administration

Nurse administrators currently have challenging responsibilities that require them to remain up to date on new developments, technology, and practices. In order to prevent difficulties and enhance patient well-being, the nurse administrator should offer midwife in-service training programs that emphasize the value of educating moms before pregnancy and delivery for optimal birth outcomes. The causes of poor pregnancy outcome like miscarriage and the prompt therapy of both avoidable and non-preventable variables are strikingly little understood.

d. Nursing Research

Nursing research is needed in the areas of when and how to educate couples about disease-risk factors that are both avoidable and unpreventable those are linked to vaginal infections and unfavourable pregnancy outcome. When it comes to teaching new mothers and their partners about symptoms to look out for and when to seek help, there is inconsistent information.

Research clarifies how new tools and evaluations can be used to start various initiatives that assist moms and their families in preventing spontaneous abortions as a result severe vaginal infections. Health workers must feel comfortable and knowledgeable in order to handle the unfavourable pregnancy outcome in an appropriate manner.

It is imperative that nurses receive the confidence, compassion, and confidence to handle these situations.

Strengths

While the study sample was within the acceptable range, the results were more clearly expressed because a comparison of the vaginal flora of women who experienced SA and those whose pregnancies continued was made.

The findings indicate that distinct organisms were found in both groups: vaginal swabs from women with term pregnancies displayed normal flora, whereas those from women who experienced SA displayed abnormal flora.

This is significant because understanding aberrant vaginal flora may help with early infection identification and treatment, which could prevent SA and contribute to positive pregnancy outcomes.

Limitations

The results cannot be extrapolated to a broader community due to the study's confinement to a specific area. Although the study sample size was sufficient to detect differences between groups, increasing the study's overall sample size may have provided a more thorough analysis. To ascertain the causal links and contributory elements, further study with a longitudinal case-control study is recommended.

More research is required to better understand the relationship between vaginal flora, the course of pregnancy, and therapeutic approaches that may help prevent miscarriages. Thus, further research should focus on determining the timing and mechanism of the establishment of high-risk vaginal microbial communities. Additionally, research on other variables that may contribute to SA should be conducted.

CHAPTER VII

SUMMARY

In order to evaluate the vaginal microbiome and socio demographic determinants of spontaneous abortion, the investigator started the current investigation.

Objectives

1. To compare the vaginal microbiome in mothers who have undergone spontaneous abortion and those who have continued pregnancy with same gestational age
2. To determine the socio demographic determinant of spontaneous abortion and compare it with the socio demographic determinants of mothers those who have continued pregnancy with same gestational age.

Cross-sectional comparative research approach. The study population was women who experienced an SA at a gestational age of less than 20 weeks and women who maintained their pregnancies at the same gestational age. The study was conducted at tertiary care hospital in North Karnataka, India.in accordance with the ethical standard The study complied with the General Data Protection Regulation and the principles and rules of protection of personal data.

A convenience sampling technique was used to recruit women who experienced SA and was admitted into the hospital for further care. We also recruited women of the same gestational weeks who maintained pregnancy and attended their antenatal outpatient department for routine visits. The study excluded women who received vaginal antimicrobial treatment or antibiotics that might disrupt the vaginal

flora, as well as those who experienced SA due to coexisting medical conditions, such heart disease or pregnancy-induced hypertension. A total of 200 participants were included in the study; 100 being women who experienced SAs at 20 weeks' gestation and 100 who continued pregnancy with same gestational age. Approval from the Institutional Ethics Committee was obtained. Participants were recruited from May 2021 to April 2023.

Potential participants were given a detailed explanation of the procedure, and after consent was obtained, vaginal swabs were obtained. The vaginal swab was collected from the posterior vaginal fornix. The swabs were taken to the lab for processing within an hour of being collected. Biochemical analysis, which includes an IMViC test (i.e., indole, methyl red, Voges-Proskauer, and citrate), was performed to identify an organism in the coliform group. Then, Gram staining was performed to distinguish between different types of bacteria, based on the various characteristics of the bacterial cell wall. The sample was then processed for DNA extraction according to the QIAamp DNA Mini Kit methodology (QIAamp® DSP DNA Mini Kit Instructions for Use Handbook., January 2021). After extraction, the sample was kept at 4°C for further examination. The samples were prepared for PCR analysis.

The sample size was computed using 95% confidence interval 5% error in presence of microbiome load and 10% as attrition. Two hundred women provided enough data to allow for a comparison of the microbiome characteristics between groups of women who sustained their pregnancies and those who had SAs. Subject with respect to microbiome community found in the vaginal niche was employed in metagenomics research to characterize species diversity by using the Shannon diversity index. The significant difference was calculated by the Shannon diversity index at $p < 0.05$.

SECTION A VAGINAL MICROBIOME AND ITS RELATION WITH MOTHER THOSE WHO HAVE UNDERWENT SPONTANEOUS ABORTION AND THOSE WHO CONTINUE PREGNANCY WITH SAME GESTATIONAL AGE

Biochemical Testing

The MViC test is a set of four distinct biochemical assays used to distinguish and identify bacteria, particularly those belonging to the Enterobacteriaceae family. It is mostly used to identify Gram-negative bacteria, however it can (and is) used to identify any sort of bacteria. It is essential for recognizing and separating Enterobacteriaceae family members

Of the vaginal swabs from women who experienced SA (N=100), 52% were indole negative, 52% were methyl positive, 65% were Voges proskauer positive, and 54% were citrate positive. Similarly, of swabs from women who had continued pregnancy (N=100), 60% were indole negative, 55% were methyl red negative, 58% were Voges proskauer positive and 68% were citrate positive

Gram Staining

Smears were prepared on glass microscope slide then a series of stains were applied to perform a gram stain

Gram staining analysis inferred that in the women who had SA (N=100) 56% were gram positive. 44% were gram negative, 45% appeared to be cocci, and 28% appeared to be Bacilli. Among the samples from women who maintained their pregnancies (N=100), 62% of the samples were gram positive, 38% were Gram Negative, 72% appeared to have cocci, and 28% seemed bacilli. Further the sample was processed for DNA Isolation

DNA Isolation

The sample was treated further to extract DNA.

The sample was then processed according to the QIAamp DNA Mini Kit methodology, and DNA extraction was carried out.

PCR Analysis

The chosen samples were prepared for PCR analysis; the specifics are described below.

Outcome

The pathogen identification rate was 13.15% (n=200) when 200 subjects' vaginal secretion cultures were examined. The bacterial detection rates were 19.96% in women who had an SA (N=100) and 7.93% in the women who maintained a pregnancy of the same gestation (N=100), which were significantly different ($p < 0.05$). In comparison to the women who continued their pregnancy, those with SA had a greater pathogen detection rate, while the women who continued pregnancy had less aberrant vaginal flora. Additionally, the pathogenic flora types in the women who experienced SA were more diverse. Within the SA group (N=100), the five most prevalent species were: 1) ***Escherichia coli* (48%), 2) *Enterococcus faecalis* (28 %), 3) *Enterococcus faecium* (12%), 4) *Pseudomonas fluorescens* (7%), and 5) *Pseudomonas cedrina* (5%).**

The most common species in the group who continued pregnancy (N=100) were 1) ***Lactobacillus inners* (58%), 2) *Lactobacillus crispatus* (32%), and 3) *Escherichia coli* 10% ($p < 0.05$).** Pregnant women had comparatively decreased detection rates of *Lactobacillus crispatus* and *Escherichia coli*. In the women

SECTION B: SOCIODEMOGRAPHIC DETERMINANTS OF SPONTANEOUS ABORTION

The study also examine the association between sociodemographic factors and reproductive history with spontaneous abortion and its comparison with the mother those who have continued pregnancy with same gestational age attending the OPD (Obstetrics & Gynecology Department) of tertiary care hospital Belagavi

- Considering the type of abortion majority (46%) mothers had missed abortion, 34% mothers had recurrent abortion and 20% mothers had incomplete abortion.
- The mothers who underwent abortion majority (38%) fall into the age group of 19 -20years when compared with mother who continued pregnancy majority (35%) of mother belong to the age group of 23-24years.
- The mother who underwent abortion majority (71%) were multi
- The mother who underwent abortion majority (78%) belong to rural area when compared with mother who continued pregnancy majority (53%) belong to urban area
- The mother who underwent abortion majority (48%) were self-occupied r when compared with mother who continued pregnancy majority (45%) were professionals
- The mother who underwent abortion majority (73%) were illiterate when compared with mother who continued pregnancy majority (46%) had secondary education
- The mother who underwent abortion majority (48%) were from Muslim religion when compared with mother who continued pregnancy majority (64%) were from Hindu religion.

- The mother who underwent abortion majority (75%) had nuclear consanguineous marriage when compared with mother who continued pregnancy majority (81%) didn't had consanguineous marriage

Reproductive factors

- Mother who underwent abortion majority (68%) of mother attained menarche before 15 years when compared with mother who continued pregnancy majority (52%) attained menarche after 15 years
- Graph the mother who underwent abortion majority (59%) of mother's had complication in previous pregnancy when compared with mother who continued pregnancy majority (78%) of mother's didn't had complication in previous pregnancy
- The mother who underwent abortion majority (60%) of mother's were with previous abortion when compared with mother who continued pregnancy majority (77%) of mother's didn't had history of previous abortion.
- The mother who underwent abortion majority (58%) of mother's Hb was below 10gm% when compared with mother who continued pregnancy majority (921%) of mother's Hb was above 10gm%.
- The mother who underwent abortion majority (62%) of mother's had maternal infection when compared with mother who continued pregnancy majority (89%) of mother's didn't has maternal infection.

CHAPTER VIII

RECOMMENDATIONS AND STRATEGIES TO PREVENT SPONTANEOUS ABORTION ASSOCIATED WITH VAGINAL DYSBIOSIS

Access to care	Strategies to address modifiable risk factors include
Identifying women with modifiable risk factors to prevent spontaneous abortion, each maternity care professional works both individually and together to address these.	• Identification of genital infections both before and during pregnancy. • Antibiotic use (systemic or oral).⁹⁹ • Using topical drugs on the vagina • The kind and frequency of sexual interactions. • Spacing method used. • Total number of partners in sex a lifetime). • Practices related to personal hygiene (application of antiseptics and disinfectants to the vaginal area, as well as douching and feminine sprays and wipes). • Status of parity. • Result of a prior pregnancy. • Kind of vaginal discharge that is produced. • Seek Early Prenatal Care: As soon as a mother finds out she is pregnant, she should make an appointment with a healthcare professional to receive early prenatal care. This will assist in determining any health problems or hazards that could result in an spontaneous abortion

Strategies to prevent spontaneous abortion	• Mother should take proactive measures to cure any vaginal infection that may alter the typical ecosystem and habitat of the vagina. • As maintaining good vaginal hygiene is crucial, concentrate on using water and light, unscented soap for a gentle cleanse. Douching the vagina might upset the natural equilibrium, so avoid doing so will reduce the infection rates. Advice mother to choose breathable, loose-fitting clothing and put on cotton underwear. • Refrain from using vaginal washes that could alter the natural ecosystem and environment of the vagina. • Prevent alcohol consumption, drug use, and smoking: These factors increase the risk of miscarriage. • Douches and other "feminine hygiene products" could disrupt the delicate balance of bacteria in vagina by eliminating some of the beneficial bacteria that are meant to prevent infections. And avoid feminine products that include fragrance, like Tampons, pads, sprays, bubble baths, and soaps • Keep up a healthy diet: Eating a well-balanced diet rich in lean proteins, healthy grains, and a range of fruits and vegetables may help lower the chance of miscarriage.
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	• In order to reduce the risk of STDs, make sure both partners get tested and engage in safe sexual practices. use barrier techniques to lower the risk of STIs. • Maintaining overall well-being can help the body fight infections, and this can be achieved through eating a balanced diet. According to the expert, cutting back on excessive sugar consumption is especially crucial because high blood sugar levels might encourage yeast overgrowth.
Ensure that all midwives and clinicians receive training on the following topics so they can effectively screen women and are informed about available community resources and care pathways:	• Perineal health • Sexual health • Nutritional health

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- .

ANNEXURES

ANNEXURE I ETHICAL CLEARANCE LETTER



KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Formerly known as KLE University)
(Deemed-to-be-University established u/s 3 of the UGC Act, 1956)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JNMC Campus, Nehru Nagar, Belagavi-590 010, Karnataka State, India
☎: 0831-2444444 FAX: 0831-2493777 Web: <http://www.kledeemeduniversity.edu.in> E-mail: info@kledeemeduniversity.edu.in

Ref.No.KAHER/EC/20-21/ 001/12

4th September 2020

To,
Ms. Babita Belgundkar
Part-Time Ph.D. Research Scholar,
2019-20 Batch, Faculty of Nursing,
KAHER, Belagavi.

Dear Research Scholar,

The KAHER Ethics Committee on Human Subjects for Ph.D. Research Project met on **Thursday, the 20th February, 2020** to consider your application for approval of the research project **"Vaginal Microbiome And Socio-Demographic Determinants Of Spontaneous Abortion In Teaching Hospitals Of North Karnataka."**

As there are no ethical issues involved in your proposed research project, the committee has provided approval for this research project.

You are requested to report to Ethical Committee of the following:

1. Any deviation from or change of the protocol.
2. Any changes in study documents.


(Dr. Anita Dalal)

Member-Secretary
Ethical Committee (Human) for Ph. D. Research
KAHER, Belagavi.


(Dr. B.C. Kotintot)

Chairman
Ethical Committee (Human) for Ph. D. Research
KAHER, Belagavi.

CC to: - The Director Research Foundation, KAHER, Belagavi.
- The Director Academic Affairs, KAHER, Belagavi.
- The Registrar, KAHER, Belagavi.
- Special Officer to Hon. Vice Chancellor, KAHER, Belagavi.

ANNEXURE II PERMISSION LETTER FROM HOSPITAL AUTHORITY

LETTER SEEKING PERMISSION TO CONDUCT RESEARCH STUDY

From,

Mrs. Babita Belgundkar
Ph.D Scholar, Faculty of Nursing
KAHER, Belagavi

To,

Medical Director
KLE's Dr. Prabhakar Kore Hospital and MRC
Belagavi, Karnataka

Forwarded Through: Dr. Sangeeta N Kharde, Vice-Principal, KAHER Institute of Nursing Sciences,
Belagavi

Subject: Permission to conduct research study

Respected Sir/Madam,

With reference to the subject cited above, I would like to request you to kindly grant me permission to conduct a research study in your esteemed hospital for my Ph.D. work. I am pursuing my Ph.D at KLE Academy of Higher Education and Research, Belagavi under the guidance of Dr. Sangeeta N Kharde, Vice-Principal and HOD, Department of OBG Nursing, KAHER Institute of Nursing Sciences, Belagavi. The topic of my study is "Vaginal Microbiome and socio demographic determinants of spontaneous abortion in tertiary care hospitals of North Karnataka". Samples for my study are mothers admitted for spontaneous abortion. The data collected will be kept confidential and used only for my Ph.D study work. Hence I request you to kindly permit me to conduct study at your esteemed hospital.

Kindly do the needful.

Thanking You,

Date: 26-05-22

Place: Belagavi

Guide

S. Kharde

Prof. Dr. Sangeeta Kharde
Vice Principal and HOD Department of OBG Nursing
KAHER, INS

Medical Director & Chief Executive
KLES Dr. Prabhakar Kore Hospital &
Medical Research Centre, BELAGAVI

Yours faithfully,

Mrs. Babita Belgundkar

Principal
KAHER Institute of Nursing Sciences
BELAGAVI

ANNEXURE III KANNADA CONSENT

ಬೆಳಗಾವಿ ಕರ್ನಾಟಕ ತೃತೀಯ ಆರೈಕೆ ಆಸ್ಪತ್ರೆಗಳಲ್ಲಿ ಸ್ವಾಭಾವಿಕ ಗರ್ಭಪಾತದ ಯೋನಿ

ಮೈಕ್ರೋಬಯೋಮ್ ಮತ್ತು ಸೋಶಿಯೋ ಡೆಮೋಗ್ರಾಫಿಕ್ ಡಿಟರ್ಮಿನಂಟ್ಸ್

ಸಂಶೋಧನಾ ವಿದ್ವಾಂಸರು: ಶ್ರೀಮತಿ ಬಬಿತಾ ಬೆಳಗುಂದಕರ

ಮೇಲ್ವಿಚಾರಕರು: ಪ್ರೊ (ಡಾ.) ಸಂಗೀತಾ ಖರ್ಡೆ

ಮಾಹಿತಿ ಹಾಳೆ:

ಪರಿಚಯ:

ಬೆಳಗಾವಿಯ ಕೆಎಲ್‌ಇ ವಿಶ್ವವಿದ್ಯಾನಿಲಯದ ಸಂಶೋಧನಾ ವಿದ್ವಾಂಸರಾದ ಶ್ರೀಮತಿ ಬಬಿತಾ ಬೆಳಗುಂದಕರ್ ಅವರು ಕೆಎಲ್‌ಇ ಆಸ್ಪತ್ರೆಗಳಲ್ಲಿ ನಡೆಸುತ್ತಿರುವ “ಯೋನಿ ಸೂಕ್ಷ್ಮಜೀವಿ ಮತ್ತು ಸಮಾಜಶಾಸ್ತ್ರೀಯ ನಿರ್ಣಯಕಾರರು ಉತ್ತರ ಕರ್ನಾಟಕದ ಬೋಧನಾ ಆಸ್ಪತ್ರೆಗಳಲ್ಲಿ ಸ್ವಾಭಾವಿಕ ಗರ್ಭಪಾತ” ಎಂಬ ಶೀರ್ಷಿಕೆಯ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಮ್ಮತಿಸುವಂತೆ ನಾವು ನಿಮ್ಮನ್ನು ವಿನಂತಿಸುತ್ತಿದ್ದೇವೆ. : ಪ್ರೊ (ಡಾ.) ಸಂಗೀತಾ ಖರ್ಡೆ, ಪ್ರಾಧ್ಯಾಪಕರು

ಕಾರ್ಯವಿಧಾನದ ವಿವರಣೆ:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನೀವು ಸಾಮಾನ್ಯ ಆರೋಗ್ಯ ಮಾಹಿತಿ, ಸಾಮಾಜಿಕ-ಜನಸಂಖ್ಯಾ ವಿವರಗಳ ಕುರಿತು ಕೆಲವು ಸಿದ್ಧಪಡಿಸಿದ ಪ್ರಶ್ನೆಗಳಿಗೆ ಉತ್ತರಿಸಬೇಕು ಮತ್ತು ನಂತರ ನಿಮಗೆ ಆರಾಮದಾಯಕವಾಗಿಸುವ ಮೂಲಕ ಯೋನಿ ಸ್ಕ್ರೀಯರ್ ಅನ್ನು ತೆಗೆದುಕೊಳ್ಳಲಾಗುತ್ತದೆ. ಈ ಸಂಪೂರ್ಣ ವಿಧಾನವು 20-30 ನಿಮಿಷಗಳನ್ನು ತೆಗೆದುಕೊಳ್ಳುತ್ತದೆ.

ಸಂಭಾವ್ಯ ಪ್ರಯೋಜನಗಳು:

ಸ್ವಾಭಾವಿಕ ಗರ್ಭಪಾತವು ಅನೇಕ ಗಂಭೀರ ಶಾರೀರಿಕ ಮತ್ತು ಮಾನಸಿಕ ಪರಿಣಾಮಗಳಿಗೆ ಕಾರಣವಾಗಬಹುದು ಮತ್ತು ಅದರ ಕಾರಣವು ಸೋಂಕಾಗಿರಬಹುದು, ಇದು ಯೋನಿ ಮೈಕ್ರೋಬಯೋಮ್ ಮತ್ತು ಸೋಶಿಯೋಡೆಮೋಗ್ರಾಫಿಕ್ ಡಿಟರ್ಮಿನಂಟ್‌ಗಳ ಸ್ವಾಭಾವಿಕ

ಗರ್ಭಪಾತವನ್ನು ಅರ್ಥಮಾಡಿಕೊಳ್ಳುವ ಮೂಲಕ ತಡೆಯಬಹುದು ತಾಯಿಯ ಕಾಯಿಲೆ ಮತ್ತು ಮರಣವನ್ನು ತಡೆಯಲು ಸಹಾಯ ಮಾಡುತ್ತದೆ.

ಸಂಭವನೀಯ ಅಪಾಯಗಳು:

ಅಧ್ಯಯನವನ್ನು ನಡೆಸಲು ಬಳಸಲಾದ ಉಪಕರಣಗಳು ಸುರಕ್ಷಿತವಾಗಿರುತ್ತವೆ ಮತ್ತು ನಿಮಗೆ ಯಾವುದೇ ಹಾನಿಯನ್ನುಂಟುಮಾಡುವ ಸಾಧ್ಯತೆಯಿಲ್ಲ.

ಗೌಪ್ಯತೆ:

ನಿಮ್ಮ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ ಮತ್ತು ನಿಮ್ಮ ಗುರುತು ಯಾರಿಗೂ ತಿಳಿಯದಂತೆ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಕೋಡ್ ಮಾಡಲಾಗುವುದು.

ಹಿಂತೆಗೆದುಕೊಳ್ಳುವಿಕೆ:

ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವಿಕೆಯು ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ. ನೀವು ಯಾವುದೇ ಹಂತದಲ್ಲಿ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸದಿದ್ದರೆ ನೀವು ನಿರಾಕರಿಸಬಹುದು ಇದು ಆರೋಗ್ಯ ವಿಷಯಗಳಿಗೆ ಸಂಬಂಧಿಸಿದಂತೆ ತಾಯಿಯ ಮೇಲೆ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ.

ಭಾಗವಹಿಸುವಿಕೆಯ ವೆಚ್ಚ:

ಅಧ್ಯಯನದ ವೆಚ್ಚವನ್ನು ಸಂಶೋಧಕರು ಭರಿಸುತ್ತಾರೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮಗೆ ಯಾವುದೇ ಹೆಚ್ಚುವರಿ ವೆಚ್ಚ ಇರುವುದಿಲ್ಲ.

ಭಾಗವಹಿಸುವಿಕೆಯ ಪಾವತಿ:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮಗೆ (ನಿಮ್ಮ ಮಗುವಿಗೆ) ಯಾವುದೇ ಪ್ರೋತ್ಸಾಹ ಇರುವುದಿಲ್ಲ.

ಸಂಪರ್ಕ ಮಾಹಿತಿ

ಈ ಸಾಹಸೋದ್ಯಮದಲ್ಲಿ ನಾನು ನಿಮಗೆ ಮಾಡಲು ಆಸಕ್ತಿ ಹೊಂದಿರುವ ಸಂಶೋಧನೆಯ ಬಗ್ಗೆ ಮಾಹಿತಿಯನ್ನು ಒದಗಿಸಿದ್ದೇನೆ. ನಾನು ಭಾಗವಹಿಸುವಿಕೆ, ಅಪಾಯಗಳು ಮತ್ತು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವ ಪ್ರಯೋಜನಗಳ ಬಗ್ಗೆ ಮಾಹಿತಿಯನ್ನು ನೀಡಿದ್ದೇನೆ. ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆ

ಕಡ್ಡಾಯವಲ್ಲ. ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ತ್ಯಜಿಸಬಹುದು. ನಿಮಗೆ ಯಾವುದೇ ಸಂದೇಹಗಳು ಅಥವಾ ಪ್ರಶ್ನೆಗಳಿದ್ದರೆ ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಶ್ರೀಮತಿ ಬಬಿತಾ ಬೆಳಗುಂದಕರ್, ಸಂಶೋಧನಾ ವಿದ್ವಾಂಸ, KAHER, ಮೊಬೈಲ್ ಸಂಖ್ಯೆ: 7892017669 ಅನ್ನು ಸಂಪರ್ಕಿಸಬಹುದು.

ನಾನು ಕೆಳಗೆ ಸಹಿ ಮಾಡುವ ಮೂಲಕ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನನ್ನ ಒಪ್ಪಿಗೆಯನ್ನು ನೀಡುತ್ತಿದ್ದೇನೆ.

ಭಾಗವಹಿಸುವವರ ಚಿಹ್ನೆ

ಹೆಸರು

ದಿನಾಂಕ

ತನಿಖಾಧಿಕಾರಿಯ ಚಿಹ್ನೆ

ಹೆಸರು

ದಿನಾಂಕ

ಸಮ್ಮತಿ ನಮೂನೆ

ನಾನು ಈ ಒಪ್ಪಿಗೆಯನ್ನು ಓದಿದ್ದೇನೆ ಮತ್ತು ನಾನು ಅದನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ. ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಿಸಿದಂತೆ ನನ್ನ ಪ್ರಶ್ನೆಯನ್ನು ಕೇಳಲು ನನಗೆ ಅವಕಾಶವನ್ನು ನೀಡಲಾಗಿದೆ

ಭಾಗವಹಿಸುವವರ ಸಹಿ: _____

ಸಂಶೋಧಕರ ಸಹಿ: _____

ದಿನಾಂಕ:

ಸ್ಥಳ:

ಅನಕ್ಷರಸ್ವರಾಗಿದ್ದರೆ

ಸಂಭಾವ್ಯ ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಒಪ್ಪಿಗೆಯ ನಮೂನೆಯ ನಿಖರವಾದ ಓದುವಿಕೆಯನ್ನು ನಾನು ನೋಡಿದ್ದೇನೆ ಮತ್ತು ವ್ಯಕ್ತಿಯು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಅವಕಾಶವನ್ನು ಹೊಂದಿದ್ದೇನೆ. ವ್ಯಕ್ತಿಯು ಮುಕ್ತವಾಗಿ ಒಪ್ಪಿಗೆ ನೀಡಿದ್ದಾರೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ.

ಸಾಕ್ಷಿಯ ಹೆಸರು _____

ಭಾಗವಹಿಸುವವರ ಹೆಬ್ಬರಳು ಮುದ್ರೆ

ಸಾಕ್ಷಿಯ ಸಹಿ _____

ದಿನಾಂಕ:

ಸ್ಥಳ

ಸಂಶೋಧಕರ ಹೇಳಿಕೆ

ಸಂಶೋಧಕರ ಹೇಳಿಕೆ

ಸಂಭಾವ್ಯ ಭಾಗವಹಿಸುವವರಿಗೆ ನಾನು ಮಾಹಿತಿ ಹಾಳೆಯನ್ನು ನಿಖರವಾಗಿ ಓದಿದ್ದೇನೆ ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯದ ಅತ್ಯುತ್ತಮವಾಗಿ ಭಾಗವಹಿಸುವವರು ಮೇಲೆ ತಿಳಿಸಿದ ಕಾರ್ಯವಿಧಾನವನ್ನು ಅರ್ಥಮಾಡಿಕೊಳ್ಳುತ್ತಾರೆ ಎಂದು ಖಚಿತಪಡಿಸಿಕೊಳ್ಳಿ.

ಅಧ್ಯಯನದ ಕುರಿತು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಭಾಗವಹಿಸುವವರಿಗೆ ಅವಕಾಶ ನೀಡಲಾಗಿದೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ ಮತ್ತು ಭಾಗವಹಿಸುವವರು ಕೇಳಿದ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ಸರಿಯಾಗಿ ಮತ್ತು ನನ್ನ ಸಾಮರ್ಥ್ಯಕ್ಕೆ ತಕ್ಕಂತೆ ಉತ್ತರಿಸಲಾಗಿದೆ. ಸಮ್ಮತಿಯನ್ನು ನೀಡುವಂತೆ ವ್ಯಕ್ತಿಯನ್ನು ಒತ್ತಾಯಿಸಲಾಗಿಲ್ಲ ಮತ್ತು ಒಪ್ಪಿಗೆಯನ್ನು ಮುಕ್ತವಾಗಿ ಮತ್ತು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ನೀಡಲಾಗಿದೆ ಎಂದು ನಾನು ದೃಢೀಕರಿಸುತ್ತೇನೆ.

ಭಾಗವಹಿಸುವವರಿಗೆ ಈ ತಿಳುವಳಿಕೆಯುಳ್ಳ ಒಪ್ಪಿಗೆ ನಮೂನೆಯ ಪ್ರತಿಯನ್ನು ಒದಗಿಸಲಾಗಿದೆ.

ಸಂಶೋಧಕರ ಹೆಸರು / ಸಮ್ಮತಿಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿ_____

ಸಂಶೋಧಕರ ಸಹಿ/ಸಮ್ಮತಿಯನ್ನು ತೆಗೆದುಕೊಳ್ಳುವ ವ್ಯಕ್ತಿ_____

ದಿನಾಂಕ _____

ANNEXURE IV

वेजाइनल मायक्रोबायोम आणि सोशियो डेमोग्राफिक निर्धारक तृतीयक केअर हॉस्पिटल्समध्ये उत्स्फूर्त

गर्भपाताचे निर्धारक बेळगावी कर्नाटक

रिसर्च स्कॉलर : सौ बबिता बेळगुंदकर

पर्यवेक्षक: प्रा.(डॉ.) संगीता खर्डे

माहिती पत्रक:

परिचय:

केएलई विद्यापीठ, बेळगावी येथील रिसर्च स्कॉलर श्रीमती बबिता बेळगुंदकर यांच्या मार्गदर्शनाखाली आयोजित केएलई हॉस्पिटलमधील “योनील मायक्रोबायोम आणि सोशियोडेमोग्राफिक डिटरमिनंट्स ऑफ स्पॉटेनियस एबोर्शन ऑफ टीचिंग हॉस्पिटल्स ऑफ नॉर्थ कर्नाटक” या अभ्यासामध्ये सहभागी होण्यासाठी आम्ही तुम्हाला विनंती करत आहोत. : प्रा.(डॉ.) संगीता खर्डे, प्रा

प्रक्रियेचे स्पष्टीकरण:

या अभ्यासात तुम्हाला सामान्य आरोग्य माहिती, सामाजिक-जनसांख्यिकीय तपशीलांबद्दल काही तयार केलेल्या प्रश्नांची उत्तरे द्यावी लागतील आणि नंतर तुम्हाला सोयीस्कर बनवून योनि स्मीअर घेण्यात येईल. या संपूर्ण प्रक्रियेस 20-30 मिनिटे लागतील.

संभाव्य फायदे:

उत्स्फूर्त गर्भपात अनेक गंभीर शारीरिक आणि मानसिक परिणामांना कारणीभूत ठरू शकतो आणि त्याचे कारण संक्रमण असू शकते जे योनि मायक्रोबायोम आणि उत्स्फूर्त गर्भपाताचे सोशियोडेमोग्राफिक निर्धारक

समजून घेतल्यास प्रतिबंधित केले जाऊ शकते, ज्यामुळे माता विकृती आणि मृत्यू टाळण्यास मदत होऊ शकते.

संभाव्य धोके:

अभ्यास आयोजित करण्यासाठी वापरण्यात आलेली साधने सुरक्षित आहेत आणि त्यामुळे तुमचे कोणतेही नुकसान होण्याची शक्यता नाही.

गोपनीयता:

तुमची ओळख उघड होणार नाही आणि सर्व माहिती संकलित केली जाईल जेणेकरून तुमची ओळख कोणाला कळणार नाही

नकार

अभ्यासात सहभाग ऐच्छिक आहे. तुम्हाला कोणत्याही वेळी अभ्यासात भाग घ्यायचा नसेल तर तुम्ही नाकारू शकता ज्यामुळे आरोग्याच्या बाबतीत आईवर परिणाम होणार नाही.

सहभागाची किंमत:

अभ्यासाचा खर्च संशोधक उचलेल. या अभ्यासात सहभागी होण्यासाठी तुम्हाला कोणताही अतिरिक्त खर्च लागणार नाही.

सहभागाचे पैसे

या अभ्यासात सहभागी होण्यासाठी तुम्हाला (तुमच्या बाळाला) कोणतेही प्रोत्साहन दिले जाणार नाही

संपर्क माहिती

या उपक्रमात मी तुम्हाला संशोधनाची माहिती पुरवली आहे ज्यामध्ये मला स्वारस्य आहे. मी अभ्यासात सहभाग, धोके आणि फायद्यांची माहिती देखील दिली आहे. तुमचा सहभाग अनिवार्य नाही. तुम्ही कोणत्याही वेळी सोडू शकता. जर तुम्हाला काही शंका किंवा प्रश्न असतील तर तुम्ही श्रीमती बबिता बेळगुंदकर, रिसर्च स्कॉलर, काहेर, मोबाईल नंबर: 7892017669 यांच्याशी कधीही संपर्क साधू शकता.

मी खाली स्वाक्षरी करून अभ्यासात सहभागी होण्यासाठी माझी संमती देत आहे.

_____	_____	_____
सहभागीचे स्वाक्षरी	नाव	तारीख
_____	_____	_____
अन्वेषकाचे स्वाक्षरी	नाव	तारीख

संमती फॉर्म

मी हा संमती फॉर्म वाचला आहे आणि मला समजला आहे. मला अभ्यासाबाबत माझे प्रश्न विचारण्याची संधी देण्यात आली आहे

सहभागीचे स्वाक्षरी _____

अन्वेषकाचे स्वाक्षरी _____

तारीख

जागा

निरक्षर असल्यास

मी संभाव्य सहभागीला संमती फॉर्मचे अचूक वाचन करताना पाहिले आहे आणि त्या व्यक्तीला प्रश्न विचारण्याची संधी मिळाली आहे. मी पुष्टी करतो की व्यक्तीने मुक्तपणे संमती दिली आहे.

साक्षीचे नाव _____

सहभागी व्यक्तीचा अंगठा

साक्षीदाराची स्वाक्षरी _____

तारीख

जागा

संशोधकाचे विधान

मी पुष्टी करतो की सहभागीला अभ्यासाविषयी प्रश्न विचारण्याची संधी देण्यात आली होती आणि सहभागीने विचारलेल्या सर्व प्रश्नांची उत्तरे माझ्या क्षमतेनुसार अचूक आणि सर्वोत्तम दिली गेली आहेत.

मी पुष्टी करतो की संमती देण्यासाठी व्यक्तीवर जबरदस्ती केली गेली नाही आणि संमती मुक्तपणे आणि स्वेच्छेने दिली गेली आहे.

या माहितीपूर्ण संमती फॉर्मची एक प्रत सहभागींना प्रदान करण्यात आली आहे

संशोधक/संमती घेणाऱ्या व्यक्तीचे _____

संशोधक/संमती घेणाऱ्या व्यक्तीची स्वाक्षरी _____

तारीख _____

ANNEXURE IV

DATA COLLECTION PROFORMA

BASELINE INFORMATION	Code No
1. Maternal age at enrollment:	8. Marital Status: a. ☐ Married b. ☐ Unmarried
2. Gestational Age:	9. Type of family : a. ☐ Rural b. ☐ Urban
3. Parity: a. ☐ Primi b. ☐ Multi	10. Diet: a. ☐ Vegetarian b. ☐ Non Vegetarian
4. Area of Residence: a. ☐ Rural/ b. ☐ Urban Home Address.	11. Monthly Income: a. ☐ More than 10,000/month b. ☐ less than 10,000/month
5. Occupation : a. ☐ Self worker b. ☐ Government servant c. ☐ House wife If ye specify :	12. Past obstetrical History : - Period of gestation during last pregnancy a. ☐ less than 20weeks b. ☐ More than 20weeks - Any Complication during last pregnancy a. ☐ Yes b. ☐ No If yes please specify - History of previous abortion if yes No of Abortion
6. Maternal education : a. ☐ Primary b. ☐ Secondary c. ☐ Degree d. ☐ Illiterate	13. Did mother have any antenatal care? a. ☐ Yes b. ☐ No c. ☐ Don't Know If yes, how many antenatal visits did she have?
7. Religion : a. ☐ Hindu b. ☐ Muslim c. ☐ Christian d. ☐ Sikh	

14. Any medication taken during pregnancy:

a. ☐ Yesb. ☐ No

If yes please specify

15. Haemoglobin % :

.....

16. History of Contraceptive used:

a. ☐ Yesb. ☐ No

If yes please specify

17. Did mother receive any of the following during this pregnancy?

YES NO DK

a. Tetanus toxoid vaccine..... 1 ☐ 2 ☐ 3 ☐b. Antenatal Screening 1 ☐ 2 ☐ 3 ☐c. Iron..... 1 ☐ 2 ☐ 3 ☐d. Vitamins, calcium 1 ☐ 2 ☐ 3 ☐e. HIV test..... 1 ☐ 2 ☐ 3 ☐f. Blood pressure measurement 1 ☐ 2 ☐ 3 ☐g. Urine test for protein 1 ☐ 2 ☐ 3 ☐h. Ultrasound for gestational age/other 1 ☐ 2 ☐ 3 ☐

If yes for ultrasound

18. Maternal infection..... a. Yes b. No c. Don't know

If Yes →Specify

19. If other information might be helpful in determining cause of spontaneous abortion please describe...

20. Mode of Transportation to hospital.....

ANNEXURE V PUBLICATION



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Vaginal Microbiota and Spontaneous Abortion

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 Acceptance Rate

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- CINAHL
- PubMed/Medline
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CLINICAL EVALUATION & IMPROVEMENT

childbearing



A Cross-Sectional Comparative Study of Vaginal Microbiota and Spontaneous Abortion at a Tertiary Care Hospital in North Karnataka, India

Babita Belgundkar, Sangeeta Kharde, Suneel Dodamani & Shivani Tendulkar

ABSTRACT

Objective: To compare the vaginal microbiome in women who have undergone spontaneous abortion with a gestational age of less than 20 weeks to that of women who have a continued pregnancy of the same gestational age.

Design: Cross-sectional comparative research design.

Setting: The study was conducted in a tertiary care hospital of North Karnataka, India. In this rural area, many women lack awareness of prenatal care and women's health information. Given rising rates of spontaneous abortion, studying the impact of the vaginal microbiome on pregnancy outcomes is crucial for raising local awareness.

Participants: Two hundred women; 100 experienced spontaneous abortion within 20 weeks' gestation, and 100 maintained pregnancy with the same gestational age.

Measures: Vaginal swab was used to detect participants' vaginal microbiota. DNA was extracted from the vaginal samples;

the 16S ribosomal RNA sequencing method was used to identify the organisms present.

Results: A pathogen detection rate of 13.15% ($n = 200$ women) was found in vaginal cultures. The percentages of bacterial detection in women who had spontaneous abortion and those whose pregnancies continued were 19.9% and 7.9%, respectively ($p < .05$). Compared to women who sustained their pregnancy, women who experienced spontaneous abortion had five common species: *Escherichia coli* (48%), *Enterococcus faecalis* (28%), *Enterococcus faecium* (12%), *Pseudomonas fluorescens* (7%), and *Pseudomonas cedrina* (5%); the most prevalent species among those with sustained pregnancy were *Lactobacillus iners* (58%), *Lactobacillus crispatus* (32%), and *E. coli* (10%; $p < .05$).

Conclusion: The vaginal microbiomes of women with spontaneous abortion were more varied and irregularly

Vaginal Microbiota and Spontaneous Abortion

distributed with bacteria. The bacteria identified could affect a woman's pregnancy outcome and perhaps be associated with spontaneous abortion. It is vital for nurses to provide education to women who are in the fertile period regarding prenatal screening, sexually transmitted infections, and perineal care.

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KEYWORDS: bacterial vaginosis, DNA sequencing, microbiological analysis, pregnancy, spontaneous abortion, vaginal infection, vaginal microbiome, vaginal microbiota

Spontaneous abortion (SA) is the termination of a pregnancy without medical or mechanical intervention before the fetus is sufficiently developed to survive (Cunningham et al., 2005). Also called *miscarriage* in lay terms, SA is intrauterine pregnancy loss before 20 weeks of gestation or 139 days, counting from the first day of the last normal menses (Patki & Chauhan, 2016). The American College of Obstetricians and Gynecologists (2018) estimates that SA is the most common form of pregnancy loss. Globally, it is estimated that as many as 26% of all pregnancies end in SA and that 80% of early pregnancy loss occurs in the first trimester (Dugas & Slane, 2022).

India has documented significant rates of SA. An Indian household survey conducted in Bihar, India, revealed that the rate of miscarriage was 46 per 1,000 pregnancies (4.6%) and typically occurred later in pregnancy (Dhaded, et al., 2018). Conversely, an epidemiologic investigation carried out in the major Indian cities revealed that as many as 32% of Indian women experienced recurrent SAs, with the majority (80%) of SAs occurring during the first 3 months of pregnancy (Kuppusamy et al., 2023). The study revealed a greater prevalence of recurrent SA among Indian women compared to Western data (Patki & Chauhan, 2016).

Numerous factors are linked to SA. Age, clotting disorders, immunologic factors, infections, and genetic disorders are the significant risk factors associated with recurrent SA in Indian women (Patki & Chauhan, 2016). Additionally, women whose first pregnancy resulted in miscarriage are more likely to experience another miscarriage during their second pregnancy compared to those with term pregnancies. More specifically, infections have been linked to 66% of late losses and 15% of early miscarriages (Giakoumelou et al., 2016).

The health of a woman and fetus throughout pregnancy can be influenced by the diversity of vaginal microbiota. Pregnant women's microbiomes are more stable and less diverse than

those of nonpregnant women (Freitas, 2018). Vaginal microbiota can be affected by urogenital infections, diet, sexual behavior, medicinal treatments, and hormonal fluctuations. Possible causes of SA include toxoplasmosis, bacterial vaginosis, rubella, cytomegalovirus, herpes simplex, *Lactobacillus*, and chlamydia (Giakoumelou et al., 2016). Consequently, it is imperative that the specific organism be identified. In a prospective study (Nasioudis et al., 2017) of 155 women who were seen at an outpatient obstetric service, the vaginal microbiome differed between women with a first or subsequent pregnancy. *Lactobacillus crispatus* was prevalent in 76.4% of primigravid women, 50% of those with a history of prior abortion, and 22.2% of individuals with a prior birth ($p \leq .01$). Conversely, *Lactobacillus iners* prevalence was observed in 3.8% of primigravid women, compared to 19.2% ($p = .03$) and 20.8% ($p = .03$) in those with a history of prior SA or birth, respectively. The prevalence of *Gardnerella* as the predominant bacterial genus increased from 3.8% in primigravid women to 15.4% and 14.3% in individuals with a history of prior SA or birth, respectively ($p > .05$). Moreover, prevalence of *L. iners* was significantly associated with a history of SA ($p \leq .02$). Researchers in Iraq (Hussien et al., 2020) analyzed 80 deep vaginal swabs from 40 women with recurrent SA and 40 women who maintained their pregnancies to evaluate the prevalence of vaginitis due to bacterial causes and its relationship with SA. Findings revealed that the greatest rates of isolated bacteria from the high vaginal swab culture of women who experienced SA were for *Gardnerella vaginalis* (36.36%), *Escherichia coli* (27.27%), and *Staphylococcus aureus* (22.73%); the isolated bacteria species with the greatest rate from women who maintained their pregnancies was *E. coli* (50%).

The composition of the vaginal microbiome and its influence on pregnancy outcomes, specifically SA, varies with pregnancy history. Worldwide, there is evidence that preventable infections may account for up to 15% of early (within 13 weeks of gestation) SAs and up to 66% of late SAs (13-22 weeks of gestation) (Giakoumelou et al., 2016). If researchers can identify the microbes that are correlated with SA, routine screening and treatment for these organisms could decrease the incidence of SA and can be made mandatory for pregnant women. This will allow for early detection and management of vaginitis and, possibly, a successful pregnancy outcome. Therefore, the aim of this study was to identify and compare the vaginal microbiomes of women experiencing SA to the microbiomes of those who have a continued pregnancy of the same gestational age.

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CLINICAL IMPLICATIONS

- Nurses can provide education to pregnant women about prevention of vaginal infections to increase their awareness of the adverse effects of infection during pregnancy.
- By taking a detailed health history, nurses can assess pregnant women for signs and symptoms of vaginal infections as part of routine prenatal care.
- In rural areas and/or regions with decreased access to obstetric providers, nursing education should be strengthened to help nurses and advance practice nurses know how to detect and treat such cases early in a woman's pregnancy.

Methods

We used a cross-sectional comparative research approach. The study population was women who experienced an SA at a gestational age of less than 20 weeks and women who maintained their pregnancies at the same gestational age. The study was conducted at a tertiary care hospital in North Karnataka, India; approval from the Institutional Ethics Committee was obtained, and the research was conducted in accordance with the General Data Protection Regulation and the principles and rules of protection of personal data.

Participant Recruitment

A convenience sampling technique was used to recruit women who experienced SA and were admitted to the hospital for further care. We also recruited women with pregnancies of the same number of gestational weeks who maintained pregnancy and attended their antenatal outpatient department for routine visits. The study excluded women who received vaginal antimicrobial treatment or antibiotics that might disrupt the vaginal flora, as well as those who experienced SA due to coexisting medical conditions, such as heart disease or pregnancy-induced hypertension. A total of 200 women expressed interest in the study, and all were included in the study; 100 women experienced SAs at 20 weeks of gestation, and 100 had continued pregnancies with the same gestational age. Participants were recruited from May 2021 to April 2023.

Data Collection

Potential participants were given a detailed explanation of the procedure, and after consent was provided, vaginal swabs were obtained. The vaginal swab was collected from the posterior vaginal fornix. The swabs were taken to the laboratory for processing within an hour of being collected. The samples were cultured for 24 hours on blood nutrition and MacConkey agar. In accordance with the manufacturer's instructions, the media were prepared and sterilized. After being solidified, the produced medium was used for susceptibility testing, identification, determining the viable count, and isolation. After that, the inoculated plates were kept in an incubator at 37 °C for a whole day. Each inoculated plate had approximately 10 to 15 colonies. From , a single colony was picked and processed for

further biochemical analysis, which includes an IMViC test (i.e., indole, methyl red, Voges–Proskauer, and citrate) was performed to identify an organism in the *coliform* group. Then, Gram staining was performed to distinguish between different types of bacteria based on the various characteristics of the bacterial cell wall. When bacteria exhibit a thick layer of peptidoglycan, they are classified as Gram positive, whereas those with a thin layer are classified as Gram negative and stain red to pink. The sample was then processed for DNA extraction according to the QIAamp DNA Mini Kit methodology (Qiagen, 2021). After extraction, the sample was kept at 4 °C for further examination. The samples were prepared for polymerase chain reaction analysis (PCR).

Statistical Analysis

The sample size was computed using the 95% confidence interval, 5% error in the presence of the microbiome load, and 10% as attrition. Two hundred women provided enough data to allow for a comparison of the microbiome characteristics between groups of women who sustained their pregnancies and those who had SAs. Metagenomics, specifically the Shannon diversity index, was used to measure the diversity of the bacteria identified in the vagina. The significant difference was calculated by the Shannon diversity index at $p < .05$.

Pregnant women's microbiomes are more stable and less diverse than those of nonpregnant women

Results

Of the vaginal swabs from women who experienced SA ($n = 100$), 60% were indole negative, 55% were methyl red negative, 58% were Voges–Proskauer positive, and 68% were citrate positive. Similarly, of swabs from women who had a continued pregnancy ($n = 100$), 52% were indole negative, 52% were methyl red positive, 65% were Voges–Proskauer positive, and 54% were citrate positive. Gram staining analysis inferred that in the women who had SA ($n = 100$), 62% of the samples were Gram positive, 38% were Gram negative, 72% appeared to be cocci, and 28% appeared to be bacilli. Among the samples from women who maintained their pregnancies ($n = 100$), 56% were Gram positive, 44% were Gram negative, 45% appeared to be cocci, and 55% appeared to be bacilli (see Table 1).

DNA Isolation

DNA extraction was conducted using the QIAamp DNA Mini Kit methodology. After extraction, the sample was stored at 4 °C for further examination. The samples were prepared for polymerase chain reaction analysis (see Table 2).

The pathogen identification rate was 13.15% when 200 participants' vaginal secretion cultures were examined. The

TABLE 1 RESULTS OF BIOCHEMICAL TESTING AND GRAM STAINING (N = 200)

Test	Sustained Pregnancy, % (n = 100)		Spontaneous Abortion, % (n = 100)	
Biochemical test	+	–	+	–
Indole	40	60	48	52
Methyl red	45	55	52	48
Voges-Proskauer	58	42	35	65
Citrate	68	32	54	46
Gram staining				
Gram positive	62		56	
Gram negative	38		44	
Cocci	72		45	
Bacilli	28		55	

bacterial detection rates were 19.96% in women who had an SA (n = 100) and 7.93% in the women who maintained a pregnancy of the same gestation (n = 100), which were significantly different ($p < .05$). In comparison to the women whose pregnancies continued, those with SA had a greater pathogen detection rate, whereas the women with continued pregnancies had less aberrant vaginal flora. Additionally, the pathogenic flora types in the women who experienced SA were more diverse.

Within the SA group (n = 100), the five most prevalent species were *E. coli* (48%), *Enterococcus faecalis* (28%), *Enterococcus faecium* (12%), *Pseudomonas fluorescens* (7%), and *Pseudomonas cedrina* (5%). The most common species in the group with continued pregnancy (n = 100) were *L. iners* (58%), *L. crispatus* (32%), and *E. coli* 10% ($p < .05$). Pregnant women had comparatively decreased detection rates of *L. crispatus* and *E. coli*. In the women who experienced SA, *E. coli* accounted for the largest percentage.

Discussion

With this study, we sought to determine whether a particular organism may be associated with SA by comparing the vaginal microbiomes of women who experienced an abortion spontaneously within 20 weeks of gestation and those who maintained their pregnancies with the same gestational age. The vaginal flora of women who experienced SA were more varied and demonstrated specific species (i.e., *E. faecalis*, *P. fluorescens*, *E. faecium*, *E. coli*, and *P. cedrina*). Our results are consistent with those of previous investigators. Al-dorri (2016) found that 35 (39.32%) of those who experienced SA had *Listeria* infection in the second trimester and 54 (60.68%) in the first trimester. *E. faecalis* is one of the main constituents of the flora in human intestinal tracts, and in our study, the *E. faecalis* detection rate was 28% (n = 100 women with spontaneous abortion). Schlüter and colleagues (2009) speculated that *E. faecalis* found in the vagina likely originates from the intestinal tract due to the proximity of the urinary and

TABLE 2 DISTRIBUTION OF ISOLATED PATHOGEN BACTERIA AMONG THE STUDY GROUP (N = 200)

Flora	Sustained Pregnancy, % (n = 100)	Flora	Spontaneous Abortion, % (n = 100)
<i>Lactobacillus inners</i>	58	<i>Escherichia coli</i>	48
<i>Lactobacillus crispatus</i>	32	<i>Enterococcus faecalis</i>	28
<i>Escherichia coli</i>	10	<i>Enterococcus faecium</i>	12
		<i>Pseudomonas fluorescens</i>	7
		<i>Pseudomonas cedrina</i>	5
Total	100		100

genital tracts. Among the results of vaginal secretion culture in the SA group, *E. faecalis* was the most common and had a significantly greater occurrence compared to the nonpregnant control groups ($p < .05$; Schlüter et al., 2009).

Lower vaginal swabs were obtained by Barcaite et al. (2012) from 827 newborns and 998 pregnant women. The women's and neonates' *E. coli* colonization rates were 19.9% and 14.4%, respectively. Most neonates with *E. coli* infections were infected by the feces of their mothers, not by vaginal fluid. In Barcaite and colleagues' investigation (2012), the SA group's *E. coli* detection rate was 2.51%, which relates to the findings of the present study, in which the *E. coli* detection rate was 48% in women who experienced SA. In the present study, *L. iners* (58%) was the bacteria with the greatest prevalence among women whose pregnancies continued ($n = 100$). The results add to those of Nasioudis et al. (2017), who found that 3.8% of women who were pregnant for the first time tested positive for *L. iners*. According to the current study, the proportion of *Lactobacillus crispatus* was 32% among 100 women who carried on with their pregnancies. These findings align with the study carried out by Barcaite et al. (2012) wherein the proportion of *Lactobacillus crispatus* was 76.4% among women who had their first conception.

Strengths

Although the study sample was within the acceptable range, the results were more clearly expressed because a comparison of the vaginal flora of women who experienced SA and those whose pregnancies continued was made. The findings indicate that distinct organisms were found in both groups: vaginal swabs from women with term pregnancies displayed normal flora, whereas those from women who experienced SA displayed abnormal flora. This is significant because understanding aberrant vaginal flora may help with early infection identification and treatment, which could prevent SA and contribute to positive pregnancy outcomes.

Limitations

This study has some limitations. The results cannot be extrapolated to a broader community due to the study's confinement to a specific area. Although the study sample size was sufficient to detect differences between groups, increasing the study's overall sample size may have provided a more thorough analysis. To ascertain the causal links and contributory elements, further study with a longitudinal case-control study is recommended. More research is required to better understand the relationship between vaginal flora, the course of pregnancy, and therapeutic approaches that may help prevent miscarriages. Thus, further research should focus on determining the timing and mechanism of the establishment of high-risk vaginal microbial communities.

Additionally, research on other variables that may contribute to SA should be conducted.

Implications for Practice

Previous research as well as findings from this study provide evidence that bacterial vaginosis may be a risk factor for SA. As a result, we recommend that routine screening and treatment for these microorganisms for women in India who are of reproductive age be included as a routine practice to an antenatal visit. Particular attention should be given to women living in rural areas to provide education about potential adverse pregnancy outcomes caused by invasion of these microorganisms into vaginal flora during pregnancy. Nurses can make a significant impact in several areas related to obstetric care, including educational counseling, implementing therapeutic and preventive measures, and communicating with pregnant women and their families. Because the vaginal microbiome is directly affected by sexual activity, nutrition/diet, and behavioral health (Lewis et al., 2017), nurses can provide health education on perineal care and dietary modifications as well as the causes, early signs, and prevention of vaginal infections during pregnancy.

Nurses can provide health education on perineal care and dietary modifications as well as the causes, early signs, and prevention of vaginal infections during pregnancy

Conclusion

In conclusion, we speculate that vaginal infection may play a role in SA because the organisms *E. faecalis*, *P. fluorescens*, *E. faecium*, *E. coli*, and *P. cedrina* were abundant in the vaginal flora of women who experienced SA when compared with women who did not experience SA. Future research to better understand the relationship between pregnancy and vaginal flora will enable the creation of new diagnostic tools, treatments, and preventive measures as well as prompt identification and treatment to improve one's odds of maintaining a pregnancy.

Acknowledgment

We are grateful to Dr. Tyagi, Dr. Jawali, and the entire staff at the Basic Science and Research Centre for their invaluable assistance with microbiological analysis and for their substantial scientific contributions to the work.

Author Disclosures

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Vaginal Microbiota and Spontaneous Abortion

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None. **NWH**



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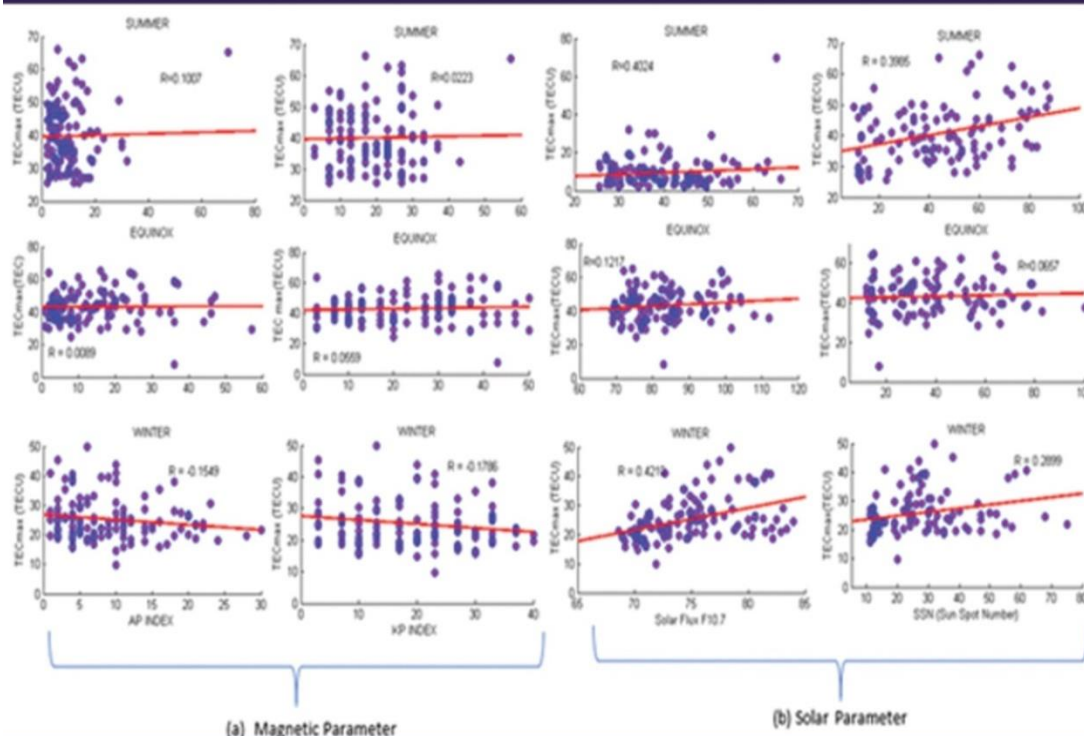


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The figures (a) and (b) illustrate the seasonal variation in TECmax and its corresponding solar and magnetic activities. These figures also display the correlation coefficient (R) for reference (after Amita Pal, Richa Trivedi, Amit Jain, Sudhir Jain; page x-y)

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RESEARCH ARTICLE

Socio-demographic and reproductive determinants of spontaneous abortion- A cross-sectional comparative research at a tertiary care hospital in North Karnataka, India

Belgundkar Babita^{1*}, Kharde Sangeeta¹, Dodamani Suneel²

Abstract

The current study aims to explore the relationship between Spontaneous Abortion and socioeconomic and reproductive factors. Cross-sectional comparative research was conducted. The study collected data from 200 women aged 19 to 35, consisting of 2 groups. Group 1 mothers who had undergone spontaneous and group 2 mothers who continued pregnancy with the same gestational week and the socio-demographic and reproductive factors were compared. Participants were interviewed using a structured questionnaire on socio-demographics, and reproductive factors were collected. Risk of abortion with an unadjusted odds ratio 1.87 times more in the age group of 19 to 20 years, parity primi mother with an unadjusted odds ratio 1.16 times had more risk of abortion, Area of residence the mother who stayed in a rural area with unadjusted ratio 1.47 times more risk of abortion, occupation the mothers who were self-workers with unadjusted odds ratio 1.50 times more risk of abortion, when looking into the education of mother illiterate mother with unadjusted odds ratio 0.50 times had a risk of abortion, in view with Religion muslims with unadjusted odds ratio 0.38 times more risk of abortion considering the type of family the mother who belong to a nuclear family with unadjusted odds ratio 1.04 times had more risk of abortion, consanguineous marriage with unadjusted odds ratio 3.95 had times more risk of abortion

Conclusion: Our study shed light on clarifying whether certain socio-demographic and reproductive determinants do increase miscarriage risk or whether screening of pregnant women for treatable determinants would improve the rate of pregnancy loss.

Keywords: Cross-sectional, comparative analysis, Socio-demographic and Reproductive, Determinants, Spontaneous abortion.

Introduction

Miscarriage is defined as spontaneous abortion (SA) without medical or mechanical means to terminate a pregnancy before the fetus is sufficiently developed to survive. In other

words, miscarriage is early pregnancy loss before the 20th week of gestation, or 139 days, counting from the first day of the last normal menses (Patki, A., & Chauhan, N., 2016).

Abortion is defined by the World Health Organization (WHO) as "the expulsion or extraction from its mother of a fetus or embryo weighing less than 500 grams. (Shakhatreh, 2022)

The American College of Obstetricians and Gynecologists (ACOG) estimates it is the most common form of pregnancy loss. It is estimated that globally as many as 26% of all pregnancies end in miscarriage and up to 10% of clinically recognized pregnancies. Moreover, 80% of early pregnancy loss occurs in the first trimester. (Dugas C. & Slane V.H., 2022). India has documented significant rates of SA. An Indian household survey conducted in Bihar, India, revealed that the rate of miscarriage was 46 per 1000 pregnancies (4.6%) and typically occurred later in pregnancy (Dhaded, S. M. Somannavar M. S, Jacob., 2017)

Conversely, an epidemiological investigation carried out in the major Indian cities revealed that as many as 32% of Indian women experienced recurrent spontaneous miscarriages. The majority (80%) of SAs occur throughout the first three months of pregnancy. The study revealed

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a higher prevalence of recurrent spontaneous abortion among Indian women as compared to Western data. (Patki A. & Chauhan N., 2016).

Overall, 10 to 20% of clinically recognized pregnancies will end in early pregnancy loss (American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Gynecology, 2018).

Numerous risk factors, including chromosomal abnormalities and fetal deformities, chronic illnesses in mothers, uterine disorders, immunological factors, and infections, are associated with spontaneous abortion in many cases (Alves C, Jenkins SM, Rapp A. Early Pregnancy Loss Spontaneous Abortion Updated, 2023). Yet, a number of modifiable risk factors for spontaneous abortion were also found to exist: increased smoking, alcohol and caffeine consumption, obesity, and older parenteral age (Rasch V, 2003). Nevertheless, the therapeutic care of these individuals faces significant challenges because the etiology of around 50% of recurrent pregnancy loss cases is still unclear. Because of its identified etiology, recurrent pregnancy loss is generally considered to be a complicated illness (Cao, C., et al., 2022).

Lack of a thorough grasp of risk factors, differential diagnosis, and inadequate management planning can all make accurate diagnosis difficult, especially in the early stages of pregnancy.

There hasn't been any agreement reached yet, despite the fact that few studies have particularly looked into the connection between socioeconomic and reproductive factors and the occurrence of spontaneous abortion.

Hence, our research aims to elucidate if certain socio-demographic and reproductive factors are associated with a higher risk of miscarriage or if screening pregnant women for modifiable factors might lead to a lower pregnancy loss rate. The objectives of the Present study are

- To compare the socio-demographic and reproductive determinants of mothers who undergone spontaneous abortion and those who continued pregnancy with the same gestational age

Methodology

A hospital-based, cross-sectional comparative study was conducted to examine the association between socio-demographic factors and reproductive history with mothers who underwent spontaneous abortion on one arm and another who continued pregnancy with the same gestational age enrolled in the study. A total of 200 respondents were included. Mothers who recently experienced a spontaneous abortion within 20 weeks of pregnancy and had been admitted to the hospital for a procedure or therapy were considered and mothers who prolonged their pregnancy with the same gestational age and went to the prenatal outpatient department (OPD) were recruited. The samples were chosen using a convenient sampling technique.

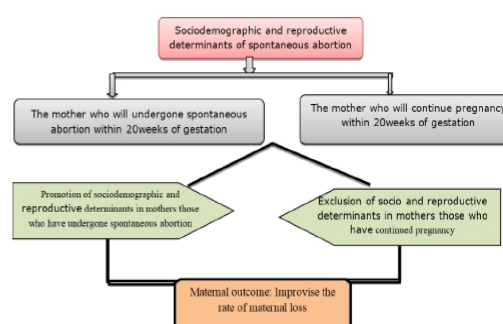


Figure 1: Framework

After receiving institutional ethics committee approval, data collection was carried out from both groups. Prior to data collection, each respondent provided their informed consent. The study's participation by respondents was completely optional, and they were free to stop at any time without providing a reason.

Data were gathered for 12 months in 2022 utilizing a face-to-face interviewing technique and the structured interview schedule's Kannada and Hindi versions. The questionnaires on socio-demographic factors, such as education, occupation, family, monthly family income, and reproductive history (such as prior history of abortion, prior history of stillbirth, prior history of premature delivery, and prior ANC checkup), were developed based on the review of the literature. Before gathering the data, the questionnaire's face and content validity were confirmed.

Statistical Analysis

The data were gathered and entered into SPSS version 16. Additionally, an odds ratio with 95% confidence intervals was computed to quantify the risk between two groups after the data were analyzed using descriptive statistics, chi-square, and logistic regression and the Fisher exact test.

Conceptual Frame Work

A conceptual framework directs this investigation (Figure 1). According to this framework, spontaneous abortion is directly impacted by certain socio-demographic determinants. According to this conceptual framework, we can anticipate that the socio-demographic determinants may, in turn, either encourage or exclude some determinants affecting the consequences for maternal outcome.

Results

A total of 200 respondents in each group were included (Group 1:100 and Group 2: 100). Group 1 was defined as women who had a recent spontaneous abortion before 22 weeks of pregnancy and had been hospitalized for a medical procedure or treatment. Group 2 were women who continued pregnancy with the same gestational age and who had visited the antenatal outpatient department (OPD) (Figure 2).

Table 1 indicates the chi-square value of respected determinants with *p-value* where *p-value* is less than 0.05 hence it showed that there is a significant association between the marital age, area of residence, occupation of mother, maternal education, religion, consanguineous marriage, monthly income with the mother those who underwent abortion and those continued pregnancy (Figure 3).

Table 2 put an insight on the risk of abortion with unadjusted odds ratio 1.87 times more and with adjusted odds ratio 13.18 times more in the age group of 19 to 20 years, parity primi mother (Figure 4) with unadjusted odds ratio 1.16 times and with adjusted odds ratio 0.17 times had more risk of abortion, area of residence the mother who stayed in rural area with unadjusted ratio 1.47 times more and with unadjusted ratio 2.42 times (Figure 5), occupation the mothers who were self-workers with unadjusted odds ratio 1.50 times and with adjusted odds ratio 0.90 times more (Figure 6), when looking into the education of mother illiterate mother with unadjusted odds ratio 0.50 times and with adjusted odds ratio 0.18 times had risk of abortion (Figure 7), in view with religion muslims with unadjusted odds ratio 0.38 and with adjusted odds ratio 0.16 times more (Figure 8), considering the type of family the mother who belong to nuclear family with unadjusted odds ratio 1.04 times and with adjusted odds ratio 1.84 had more risk of abortion (Figure 9), consanguineous marriage with unadjusted odds

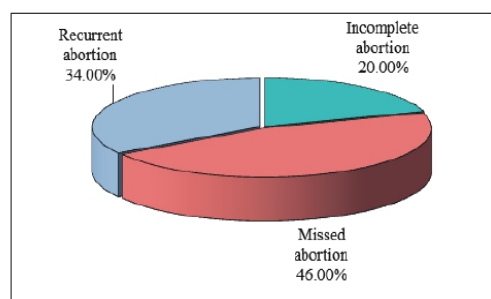


Figure 2: Types of abortion in abortion group

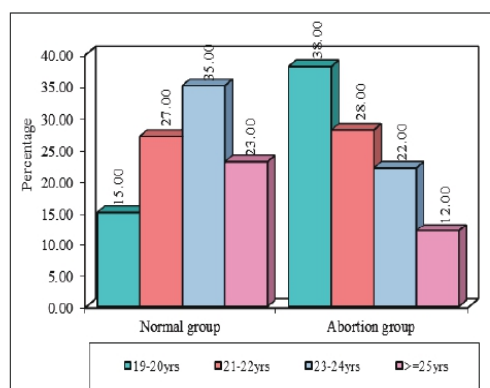


Figure 3: Comparison of normal group and abortion group with maternal age

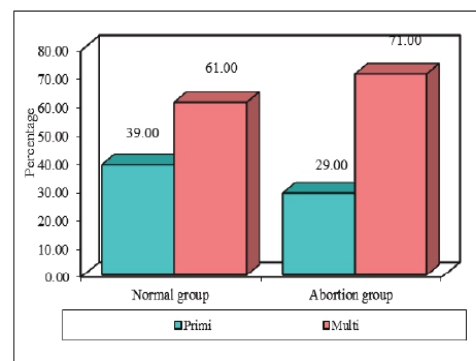


Figure 4: Comparison of normal group and abortion group with parity

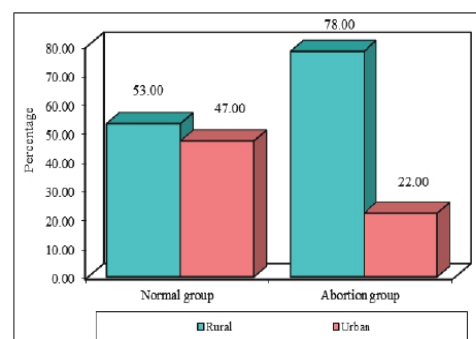


Figure 5: Comparison of normal group and abortion group with area of residence

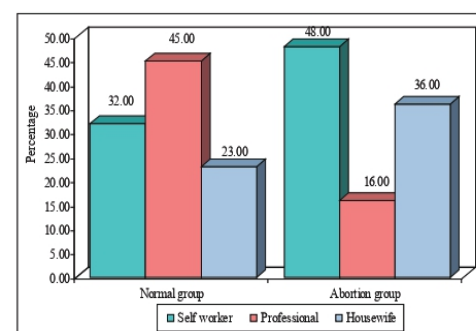


Figure 6: Comparison of normal group and abortion group with occupations

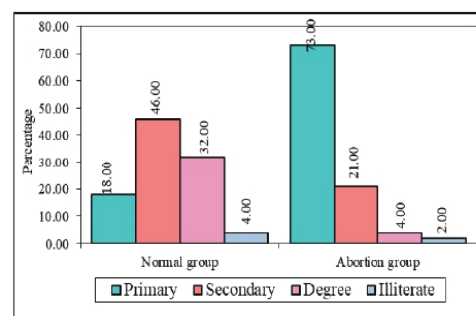


Figure 7: Comparison of normal group and abortion group with maternal education

Table 1: Association between abortions with socio-demographic factors.

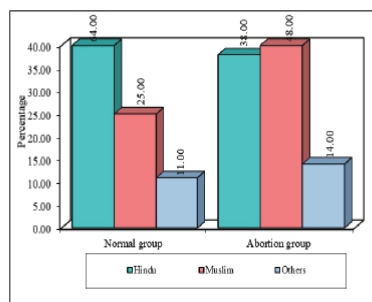
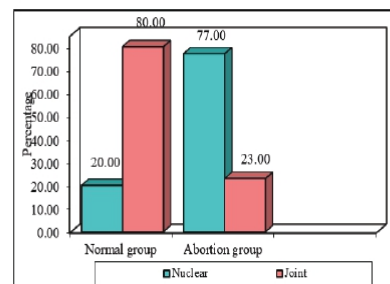
*p<0.05

<i>Marital age</i>	<i>Normal group</i>	<i>%</i>	<i>Abortion group</i>	<i>%</i>	<i>Total</i>	<i>%</i>	<i>Chi-square</i>	<i>p-value</i>
19-20yrs	15	15.00	38	38	53	26.50	8.0210	0.0460*
21-22yrs	27	27.00	28	28	55	27.50		
23-24yrs	35	35.00	22	22	57	28.50		
>=25yrs	23	23.00	12	12	35	17.50		
Total	100	100	100	10	20	100		
<i>Parity</i>								
Primi	39	39.00	29	29	68	34.00	2.2280	0.1360
Multi	61	61.00	71	71	132	66		
Total	100	100	100	10	20	100		
<i>Area of residence</i>								
Rural	53	53.00	78	78	131	65.50	15.7120	0.0001*
Urban	47	47.00	22	22	69	34.50		
Total	100	100	100	10	200	100		
<i>Occupations</i>								
Self-worker	32	32.00	48	48	80	40.00	19.8510	0.0001*
Professional	45	45.00	16	16	61	30.50		
Housewife	23	23.00	36	36	59	29.50		
Total	100	100	100	10	20	100		
<i>Maternal Education</i>								
Primary	18	18.00	73	73	91	45.50	65.0150	0.0001*
Secondary	46	46.00	21	21	67	33.50		
Degree	32	32.00	4	4	36	18.00		
Illiterate	4	4.00	2	2	6	3.00		
Total	100	100	100	10	20	100		
<i>Religions</i>								
Hindu	64	44.00	38	38	102	51.00	27.6680	0.0001*
Muslim	25	45.00	48	48	73	36.05		
Others	11	11.00	14	14	25	12.05		
Total	100	100	100	10	20	100		
<i>Type of fa</i>								
Nuclear	20	20.00	77	77	43	21.50	0.2670	0.6060
Joint	80	80.00	23	23	157	78.50		
Total	100	100	100	10	20	100		
<i>Consanguineous marriage</i>								
Yes	19	19.00	75	75	94	47	63.1120	0.0001*
No	81	81.00	25	25	106	53.00		
Total	100	100	100	10	200	100		
<i>Monthly income</i>								
>10000	79	79.00	62	62	141	70.50	6.9480	0.0080*
<10000	21	21.00	38	38	59	29.50		
Total	100	100	100	10	200	100		

Table 2: Association between abortions with socio-demographic characteristics by logistic regression analysis

Factors	Unadjusted OR	95% CI for OR		p-value	Adjusted OR	95% CI for OR		p-value
		Lower	Upper			Lower	Upper	
Marital age (years)								
19–20	1.87	1.00	3.50	0.0500*	13.18	2.02	86.00	0.0070*
21–22	1.09	0.69	1.72	0.7260	1.53	0.40	5.96	0.5370
23–24	0.82	0.46	1.43	0.4760	1.05	0.29	3.86	0.9420
>=25	Ref.				Ref.			
Parity								
Primi	1.16	0.83	1.64	0.3850	0.17	0.04	0.76	0.0210*
Multi	Ref.				Ref.			
Area of residence								
Rural	1.47	1.04	2.09	0.0300*	2.42	0.95	6.12	0.0630
Urban	Ref.				Ref.			
Occupations								
Self-worker	1.50	0.96	2.35	0.0760	0.90	0.31	2.64	0.8490
Professional	0.36	0.20	0.63	0.0001*	0.21	0.06	0.71	0.0120*
Housewife	Ref.				Ref.			
Maternal education								
Primary	Ref.				Ref.			
Secondary	0.46	0.27	0.77	0.0030*	0.18	0.07	0.48	0.0010
Degree	0.13	0.04	0.35	0.0001*	0.02	0.00	0.11	0.0001
Illiterate	0.50	0.09	2.73	0.4230	0.08	0.01	1.00	0.0500*
Religions								
Hindu	Ref.				Ref.			
Muslim	0.38	0.22	0.66	0.0010*	0.16	0.05	0.50	0.0020*
Others	0.27	0.08	0.98	0.0460*	0.04	0.00	0.42	0.0070*
Type of family								
Nuclear	1.04	0.76	1.42	0.8110	1.84	0.65	5.19	0.2490
Joint	Ref.				Ref.			
Consanguineous marriage								
Yes	3.95	2.39	6.53	0.0001*	27.07	8.21	89.28	0.0001*
No	Ref.				Ref.			
Monthly income								
>10000	Ref.				Ref.			
<10000	1.81	1.06	3.08	0.0290*	0.56	0.18	1.74	0.3140

*p<0.05

**Figure 8:** Comparison of normal group and abortion group with religions**Figure 9:** Comparison of the normal group and abortion group with type of family

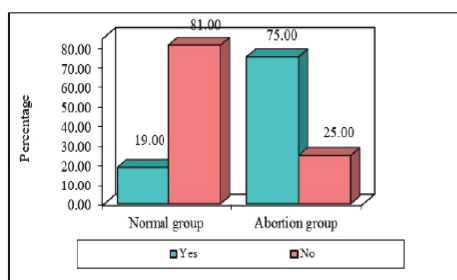


Figure 10: Comparison of normal group and abortion group with consanguineous marriage

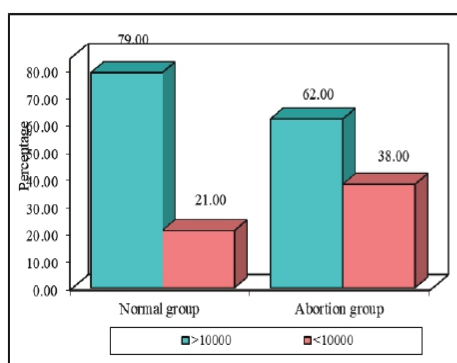


Figure 11: Comparison of normal group and abortion group with monthly income

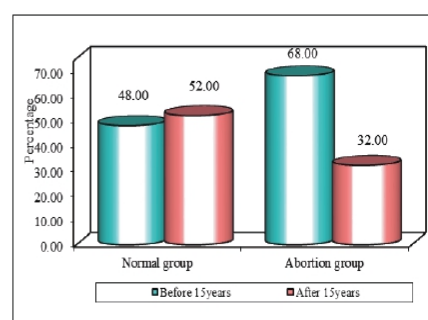


Figure 12: Comparison of normal group and abortion group with age at menarche

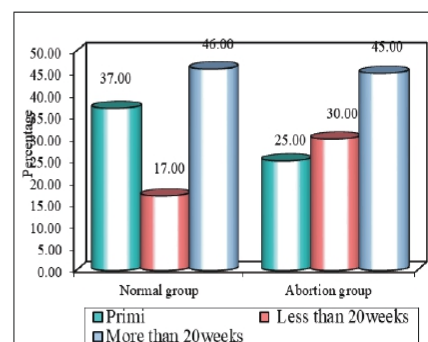


Figure 13: Comparison of normal group and abortion group with Period of gestation during last pregnancy

ratio 3.95 and with adjusted odds ratio 2.77 had times more risk of abortion (Figure 10). Comparison of normal group and abortion group with monthly income (Figure 11).

Reproductive Factors

Table 3 demonstrates the chi-square value of reproductive factors with their respective *p-value* where *p-value* was less than 0.05 and shows that there is a significant association between age at menarche (Figure 12), complication during last pregnancy (Figures 13 and 14), history of previous abortion (Figure 15), any medication taken during pregnancy, Hb% of mother, lifestyle factors of mother, receiving of tetanus toxoid, antenatal screening of mother, maternal infection of mother with mother those who underwent abortion and those continued pregnancy (Figures 12-23).

Table 4 illustrates the risk of abortion with unadjusted odds ratio 1.42 and with adjusted odds ratio 0.53 times more in the mothers those who attained menarche before 15 years, the mother who had complication during last pregnancy with unadjusted odds ratio 1.86 and with adjusted odds ratio 0.36 times more risk of abortion, the mother those who had history of previous abortion with unadjusted ratio 1.74 and with adjusted ratio 1.70 times more risk, risk of abortion with unadjusted odds ratio 1.04 and with adjusted odds ratio 0.33 times more in the mothers those who didn't had antenatal care, the mothers those who took medication during pregnancy with unadjusted

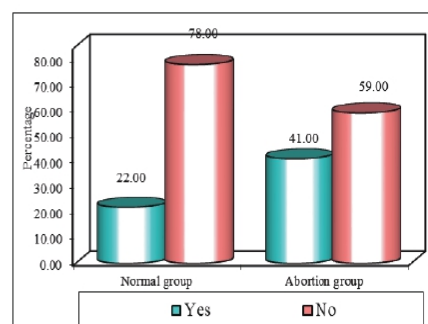


Figure 14: Comparison of the normal group and abortion group with any complication during the last pregnancy

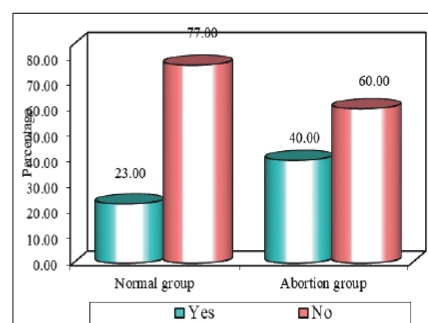


Figure 15: Comparison of the normal group and abortion group with a history of previous abortion

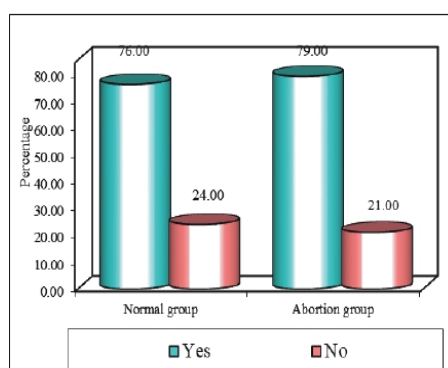


Figure 16: Comparison of the normal group and abortion group with the mother had any antenatal care

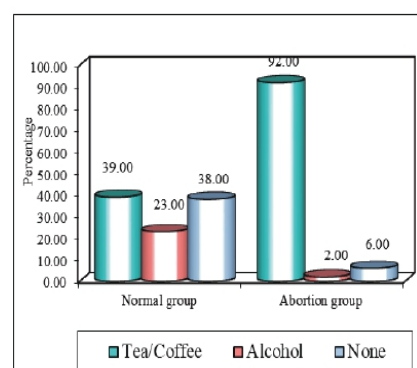


Figure 20: Comparison of normal group and abortion group with Any lifestyle factors

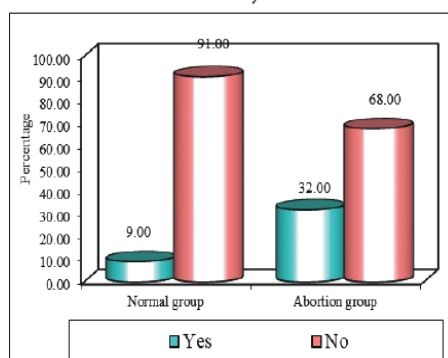


Figure 17: Comparison of normal group and abortion group with any medication taken during pregnancy

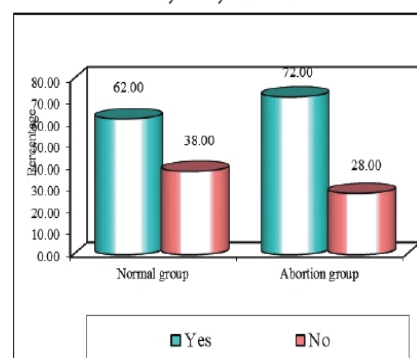


Figure 21: Comparison of normal group and abortion group with received tetanus toxoid

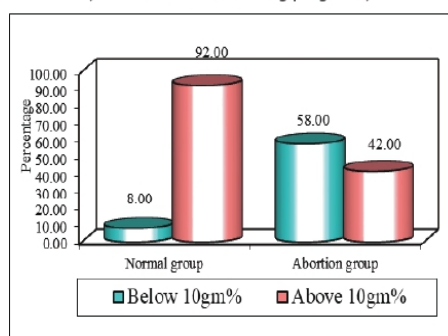


Figure 18: Comparison of normal group and abortion group with levels of Hb%

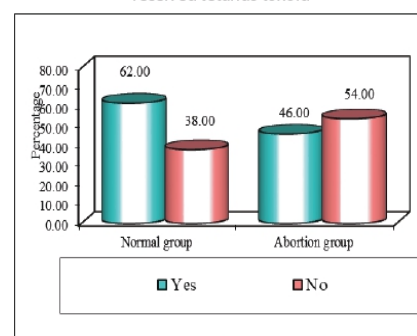


Figure 22: Comparison of normal group and abortion group with antenatal screening

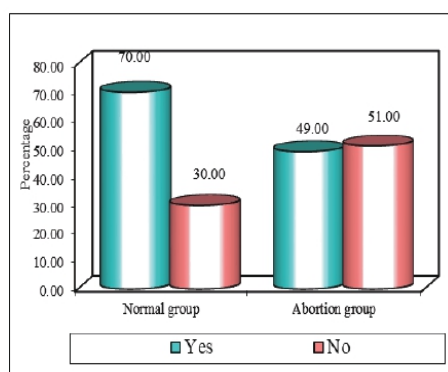


Figure 19: Comparison of normal group and abortion group with history of contraceptive used

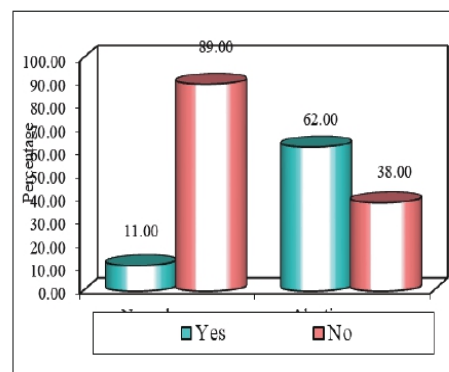


Figure 23: Comparison of normal group and abortion group with maternal infection

Table 3: Association between abortions with Reproductive factors

<i>Age at menarche</i>	<i>Normal group</i>	<i>%</i>	<i>Abortion group</i>	<i>%</i>	<i>Total</i>	<i>%</i>	<i>Chi-square</i>	<i>p-value</i>
Before 15 years	48	48	68	68	116	58	8.2100	0.0040*
After 15 years	52	52	32	32	84	42		
Total	100	100	100	100	200	100		
<i>Period of gestation during last pregnancy</i>								
Primi	37	37	25	25	62	31	5.9290	0.0520
Less than 20 weeks	17	17	30	30	47	23		
More than 20 weeks	46	46	45	45	91	45		
Total	100	100	100	100	200	100		
<i>Any complication during last pregnancy</i>								
Yes	22	22	41	41	63	31.50	8.3650	0.0040*
No	78	78	59	59	137	68.50		
Total	100	100	100	100	200	100		
<i>History of previous abortion</i>								
Yes	23	23	40	40	63	31.50	6.6970	0.0100*
No	77	77	60	60	137	68.50		
Total	100	100	100	100	200	100		
<i>Any Medication taken during pregnancy</i>								
Yes	9	9	32	32	41	20.50	16.2290	0.0001*
No	91	91	68	68	159	79.50		
Total	100	100	100	100	200	100		
<i>Hb%</i>								
Below 10 gm%	8	8	58	58	66	33	5.7010	0.0170*
Above 10 gm%	92	92	42	42	134	67		
Total	100	100	100	100	200	100.00		
<i>History of contraceptive used</i>								
Yes	70	70	49	49	119	59.50	9.1500	0.0020*
No	30	30	51	51	81	40.50		
Total	100	100	100	100	200	100.00		
<i>Any lifestyle factors</i>								
Tea/Coffee	39	39	92	92	131	65.50	62.3550	0.0001*
Alcohol	23	23	2	2	25	12.50		
None	38	38	6	6	44	22.00		
Total	100	100	100	100	200	100		
<i>Received tetanus toxoid</i>								
Yes	62	62	72	72	134	67	12.0000	0.0010*
No	38	38	28	28	66	33		
Total	100	100	100	100	200	100		
<i>Antenatal Screening</i>								
Yes	62	62	46	46	108	51	42.3370	0.0001*
No	38	38	54	54	92	49		
Total	100	100	100	100	200	100		
<i>Maternal infection</i>								
Yes	11	11	62	62	73	46.50	101.316	0.0001*
No	89	89	38	38	127	53.50		
Total	100	100	100	100	200	100.00		

Table 4: Association between abortions with pregnancy/reproductive-related variables by logistic regression analysis

Pregnancy variables	Unadjusted OR	95% CI for OR		p-value	Adjusted OR	95% CI for OR		p-value
		Lower	Upper			Lower	Upper	
Age at menarche								
Before 15 years	1.42	0.98	2.05	0.0650	0.53	0.16	1.73	0.2950
After 15 years	Ref.				Ref.			
Period of gestation during last pregnancy								
Primi	0.68	0.41	1.12	0.1300	6.18	1.03	37.08	0.0460*
Less than 20 weeks	1.77	0.97	3.20	0.0610	5.02	0.52	48.55	0.1640
More than 20 weeks	Ref.				Ref.			
Any complication during last pregnancy								
Yes	1.86	1.11	3.13	0.0180*	0.36	0.07	1.83	0.2170
No	Ref.				Ref.			
History of previous abortion								
Yes	1.74	1.04	2.91	0.0340*	1.70	0.23	12.74	0.6070
No	Ref.				Ref.			
Did the mother had any antenatal care								
Yes	1.04	0.76	1.42	0.8100	0.33	0.07	1.47	0.1440
No	Ref.				Ref.			
Any Medication taken during pregnancy								
Yes	3.56	1.70	7.45	0.0010*	8.69	1.31	57.47	0.0250*
No	Ref.				Ref.			
Hb%								
Normal	Ref.				Ref.			
Abnormal	1.08	0.81	1.43	0.6130	0.02	0.00	0.17	0.0001*
History of contraceptive used								
Yes	1.43	0.99	2.06	0.0500*	0.45	0.14	1.46	0.1830
No	Ref.				Ref.			
Any lifestyle factors								
Tea/Coffee	2.36	1.62	3.43	0.0001*	7.15	1.67	30.63	0.0080*
Alcohol	0.09	0.02	0.37	0.0010*	-	-	-	0.9960
None	Ref.				Ref.			
Received tetanus toxoid								
Yes	1.50	1.04	2.16	0.0300*	1.75	0.50	6.14	0.3820
No	Ref.				Ref.			
Antenatal Screening								
Yes	2.64	1.71	4.08	0.0001*	6.75	1.87	24.31	0.0040*
No	Ref.				Ref.			
Maternal infection								
Yes	7.46	3.97	13.99	0.0001*	154.68	26.55	901.19	0.0001*
No	Ref.				Ref.			
Modes of transportation								
Ambulance	-	-	-	0.9980	-	-	-	0.9960
Bus	1.37	0.96	1.97	0.0850	0.76	0.13	4.44	0.7630
Car	0.25	0.12	0.54	0.0001*	0.13	0.01	1.45	0.0980
Bike	Ref.				Ref.			

*p<0.05

odds ratio 3.56 and with adjusted odds ratio 8.69 times more risk of abortion, in view with Hb% the mothers those who had Hb below 10 gm% with unadjusted odds ratio 1.08 times and with adjusted odds ratio 1.02 more risk of abortion when compared with the mothers those who had Hb above 10 gm% considering the history of contraceptive used the mother those who didn't use contraceptives with unadjusted odds ratio 1.43 and with times had more risk of abortion, considering the lifestyle factors the mother those who had habit of tea and coffee with unadjusted odds ratio 2.36 and with adjusted odds ratio 1.75 times had more risk of abortion, the mother those who had not taken tetanus toxoid with unadjusted odds ratio 1.50 and with adjusted odds ratio 1.75 times had more risk of abortion, the mothers those who didn't had antenatal screening with unadjusted odds ratio 2.64 and with adjusted odds ratio 6.75 times had more risk of abortion, the mothers those who had infection during pregnancy with unadjusted odds ratio 7.46 and with adjusted odds ratio 154.68 times had more risk of abortion.

Discussion

In this study, we examined the association between socio-demographic factors and reproductive-related variables with Spontaneous abortion. The study revealed that there was a statistically significant association between the marital age, area of residence, occupation of the mother, maternal education, religion, consanguineous marriage, monthly income and other reproductive factors such as age at menarche, any complication during last pregnancy, history of previous abortion, did mother had any antenatal care, any medication taken during last pregnancy, level of Hb%, history of contraceptive used, any lifestyle factor, received tetanus toxoid, antenatal screening, maternal infection.

When compared with the study done by Zheng, D., Li, C., Wu, T., & Tang, K. (2017). The risk of SA in rural areas was 1.68 times greater than in urban areas (AOR=1.68, 95%CI: 1.54–1.84). Women with high income had a decreased risk of SA when compared with that of women with low income (AOR=0.90, 95%CI: 0.84–0.97). Compared with women in low educational attainment, women in higher educational attainment had a lower prevalence of SA (AOR=0.90, 95%CI: 0.82–0.98). The risk of SA only reduced in factory worker (AOR=0.59, 95%CI: 0.53–0.66) and professional worker (AOR=0.75, 95%CI: 0.66–0.84) compared with agriculture and related workers. After stratifying by rural/urban, the association between income and SA in urban (AOR=0.88, 95%CI: 0.78–0.99) was stronger than that in rural (AOR=0.92, 95%CI: 0.84–1.00). Association between education and SA was found in urban (AOR=0.66, 95%CI: 0.55–0.78) but not in rural (AOR=1.05, 95%CI: 0.34–1.17), and there was no difference on how occupation impacted SA among women between the two subgroups. (Zheng, D., Li, C., Wu, T., & Tang, K. 2017).

When comparing with the current study risk of abortion with an unadjusted odds ratio 1.87 times more in the

age group of 19 to 20 years, parity primi mother with an unadjusted odds ratio 1.16 times had more risk of abortion, Area of residence the mother who stayed in a rural area with unadjusted ratio 1.47 times more when compared with mother who stayed in the urban, occupation the mothers who were self-workers with unadjusted odds ratio 1.50 times more when compared with housewife when looking into the education of mother illiterate mother with unadjusted odds ratio 0.50 times had risk of abortion, in view with religion Muslims with unadjusted odds ratio 0.38 times more when compared with Hindu religion considering the type of family the mother who belong to a nuclear family with unadjusted odds ratio 1.04 times had more risk of abortion, consanguineous marriage with unadjusted odds ratio 3.95 had times more risk of abortion

Conclusion

This study indicates that the most common unfavorable pregnancy outcome is still spontaneous abortions. The researchers found significant relationships between the socioeconomic status of women, reproductive factors, and the risks of spontaneous abortion. These factors included maternal age, area of residence, religion, occupation, type of family consanguineous marriage, and monthly income. Reproductive elements at the age of menarche, any difficulties with the previous pregnancy. Previous abortion history, medications given while pregnant, history of contraception usage, and any lifestyle modifications pregnancy screening. Moreover, maternal infection was connected to spontaneous.

Policy and program development will be significantly impacted by the fact that women with such socioeconomic and reproductive determinants need more care, support, and oversight of health promotion initiatives. Pregnancy loss rates can be reduced by evaluating expectant mothers for modifiable risk factors and raising awareness among them.

Acknowledgement

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