

Endometrial Microbiome & Development of Gut Microbiota in Foetus

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ABSTRACT

The human microbiota influences physiology, disease, and metabolic reproduction that maintains major function of human physiological properties. Similarly, there has been a debate regarding the endometrial or the uterine microbiota has been one of the major properties of microbiome that influences the physiological development of gut microbiota in foetus. However, origin of microbiome under uterine cavity and placental development has been a question of debate where various assistance suggests on the microbial diffusion and osmosis through the vaginal vaults to the labial commissure. On the contrary, with the detailed study of various clinical aspects and literature justified that the rapid development of microbiome especially with the *Lactobacillus*-Dominant groups covers around 95% of the gut development. Apart from this, previous study suggested reserved from the Human Microbial Project suggested that Normal vaginal delivery led to the growth of *Lactobacillus* and *Bifidobacterium* spp., *Enterobacteriaceae*, *Bacteriodes fragilis*, and *16srRNA based bacteria*. On the contrary, the C-section found growth of *Clostridia*, *Bacteriodes Spp.*, *Staphylococcus* and *Streptococci*. Similarly, the full-term pregnancy and pre-term pregnancy leads to development of different shades of bacteria in the foetal gut. Thus, these review structure as well as the clinical study verified the aspects of various debates regarding the existence of microbiome across the endometrium and surpassing of these throughout the foetal gut in natal development phase.

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KEYWORDS: *Pregnancy, Natal development, Human Microbiome, Lactobacillus, Gynaecology, Human uterus, Vaults of vagina*

INTRODUCTION

Foetus development within the uterine environment has been one of the crucial processes for the growth, maturation, and development of a human beings. In nexus with this, the gestation period from fertilisation to the birth goes through different stages of development in combination with certain factors that applies on life-building opportunities even in the post-natal period. Similarly, the development of gut microbiota has been even one of the essential perspectives that even occur during the embryological period through the osmoregulation of endometrial microbiome. This aspect has been still focused in a hypothetical approach where the need of study for the development of gut microbiota within the foetus has been still a major query. The 21st century has been the era of materialistic thoughts where elementary measurement of any living material could be easily

performed without hassle; however, non-materialistic analysis such as through the changes of sub-cognitive aptitude requires an altered understanding of functional properties. Similarly, the understanding of microbiological occurrence across the endometrium and further the development of gut microbiota of an individual has been one the perspective needs for the current research faculty.

According to Toson *et al.*, (2022) changes across the female genital tract microbiome have been consistently correlated to the gynaecological and obstetrical pathologies and tract dysbiosis that could impact the reproductive outcomes during the fertility treatment. In contrast with this, Reschini *et al.* (2022) identified that there has been growing evidence from the sequencing-based methods for bacterial detection

that currently are the cornerstone of the assessment in low-biomass anatomical sites. Furthermore, the clinical analysis of the foetus suffering from different ARDS in 38th week identifies an osmolar coefficient within the transfer of certain commensals to the uterine barriers of the amniotic sac. Furthermore, the study of Moreno *et al.* (2022), suggested that humans have co-evolved as holobionts with the microbial companions where low-abundance bacterial community or low-biomass microbiota with *Lactobacillus*-dominance have been easily identified across the uterine cavity. These literatures hence classify the occurrence of endometrial microbiota that has been leading to the development of gut microbiota among the foetus.

Aim of the research: This research aims to focus on the identification of endometrium microbiota as well as the development of gut microbiota in the foetus.

Objectives of the research:

- To identify the list of microbes and their dominance in endometrium
- To develop a literature correlative study on microbial occurrence across the pregnant women
- To identify the persistent development of microbes among the foetus
- To clarify the differential understanding and evaluate the identification of different ranges of microbiota

Hypothesis:

H₀= There has been no relation of endometrium microbiota with the gut microbiota of foetus

H₁= There has been a correlation of endometrium microbiota in pregnant women with the gut microbiota of foetus

Methods & Materials:

The research has been directly based on the interpretivism research philosophy based on which the qualitative review of different sets of literature along with the consideration of clinical correlation from pregnant women. In this study, multicentre prospective observational study has been applied within the maternal age ≤ 40 years of age as well as focuses on the understanding of literature accordingly. The main perspective of this study has been based on the identification of different sets of microbes that occurs across the developmental period.

The clinical qualitative study has been based on the application of PRISMA and Boolean Algebra based on which advanced Boolean search was applied to qualitatively advance the findings in research. In this scenario, various types of data have been evaluated as well as correlated with the research aims. Further, the application of Advanced search was focused to include full-text articles through the keywords such as “Endometrium Microbiota”, “Gut Microbiota”, “Pregnancy”, and many more. Majorly articles were collected from different identified journals appropriately based on which the application of study could be applied in future clinical application.

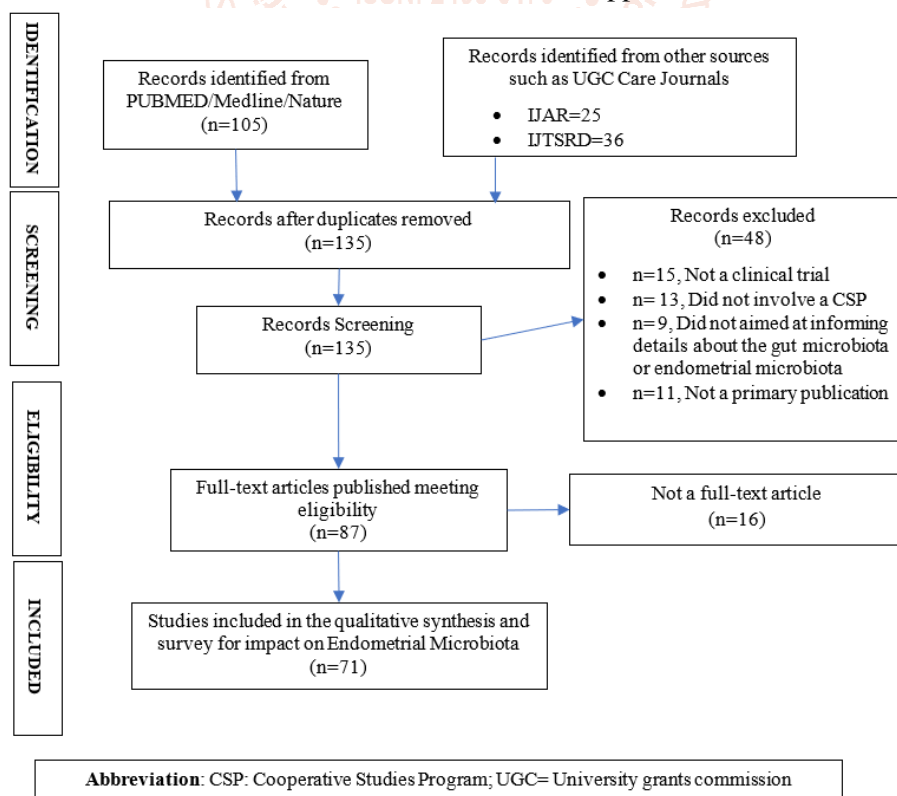


Figure 1: PRISMA

From the above figure, it could be evaluated that PRISMA or the Preferred Reporting Items for Systemic Reviews and Meta-Analyses based on which evidence-based minimum set of items for reporting in systemic review and meta-analyses has been applied in the research. Thus, going through the 27-item checklist and a 4-phase flow diagram, around 71 full-text articles was effectively calculated and applied in the research for better understanding and development of conclusion.

Literature Review

Anatomy of Female Reproductive System & microbiome

Female reproductive system is one of the major complicated organ systems in the body comprising of different sets of organs such as ovaries, fallopian tube, uterus, cervix, and vagina. Female genital organs are the uterus, vagina, and ovaries that are also referred to as the internal organs; while, the external organs are the external genitalia (mons pubis, labia majora and minora, clitoris), vestibule and vestibular organs.

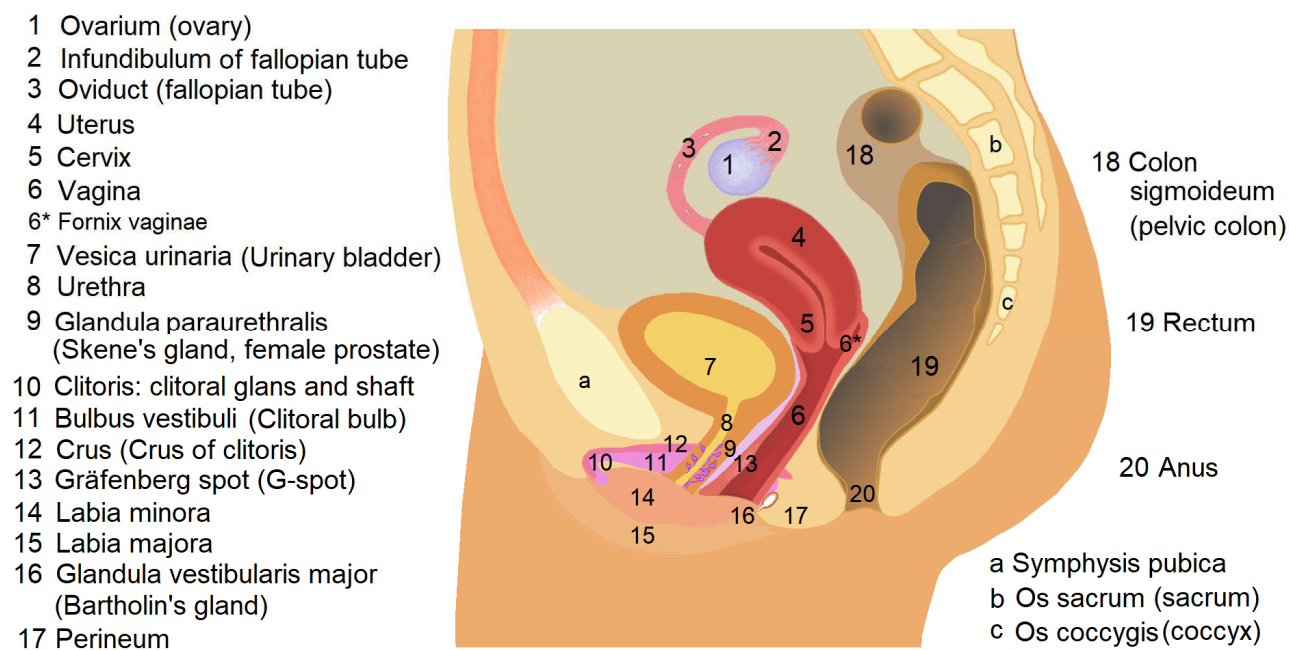


Figure 2: Female genital organs
(Source: Wikipedia)

In reference to the above image, it could be defined that the female reproductive organs are placed in the pelvic regions in a non-peritoneal structure. According to Mitra (2011), uterus, thick and muscular organ, is the pelvic organ lying between the rectum behind and the bladder in front. It projects into the vagina and opens in it by an orifice called the *external os* where the uterine tubes are connected at its supero-lateral angle with a length of 7.5 cm, breadth 5cm, thickness 2.5cm, and weighs around 30-40gms. In accordance with Ghosh (2010), the uterus is itself subdivided into three parts fundus, body and cervix where fundus resembles lying above the intramural part of the uterine tube, body resembles part between cervix and fundus where the junction between the body and cervix is called as the isthmus. On the other hand, from recent studies it has been evaluated that there have been different types of microbes in the uterus comprising mainly of bacteria (85%), fungi (10%), viruses (5%), and archaea (0.3%) (Toson *et al.*, 2022). Similarly, it has been even evident that the communities of *Clostridium botulinum*, *Hydrogenophaga sp.*, *Klebsiella pneumoniae*, and *Pasteurella multocida* are the most evident colonies in the uterine region.

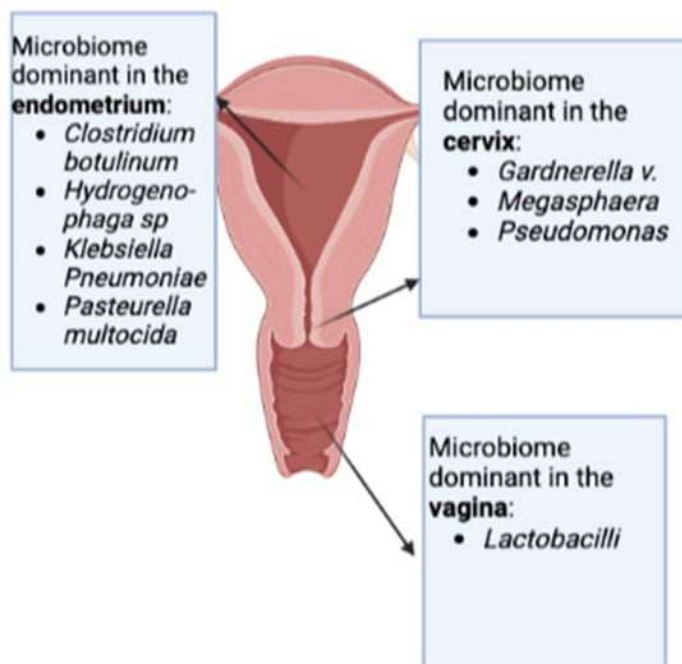


Figure 3: Microbes in different regions of the female reproductive organs

(Source: Inversetti *et al.*, 2023)

According to Inversetti *et al.* (2023), the application of DNA sequencing across the different regions elevated on varied genetic persists, where evident RNA evaluated on the presence of various dominant microbiome across the region. The other internal organs such as vagina has been the portion exposed to the outer environment where fornix or the vaults of vagina have been the major input source for the commensals in the internal organs. However, healthy living and existence of microbes especially the *Lactobacilli spp.* across the region has been essential in order to maintain effective pH ratio of the internal organs effectively. Similarly, Kunz *et al.* (1996), evaluated that the occurrence of microbes across the upper genital tract has been guided by the spontaneous uterine contractions and the osmolar shift, with the highest frequency around the time of ovulation and during orgasms that could participate even in the transportation of spermatozoa to the fallopian tubes. Thus, from these extensive studies of literature, it has been evaluated that the presence of varied bacteria across the upper genital tract and the lower genital tract has been manifesting on the maintenance of preferred homeostasis. In addition to this, it is of uttermost importance to better categories and characterise the role of these microbiota in close contact with the embryo during its development. In nexus with this, endometrium milieu may even play a significant role in regards to the significant different in anatomical, physiological, and immune response as compared to the vagina.

Vaginal vaults or fornix

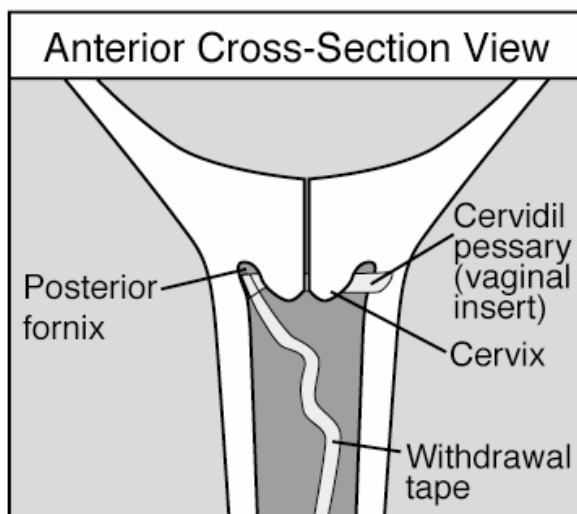


Figure 4: Fornix in Vagina

(Source: Aquino, 2020)

According to Aquino (2020), the vaginal fornix or fornices has been the widest part at the end of the vaginal canal that forms a dome shaped around the cervix that can expand to accommodate penetration during the sexual intercourse as well as it even serves as a storage area for sperm after intercourse. However, the vaginal fornix consists of a series of recesses or pockets that surround the cervix where it has a slightly different shape and depth that allows for flexibility and movement during sexual activity.

Fornix	Description
Anterior fornix	It is located towards the front of the vagina and closest to the pubic bone. It has been often referred to as the deep spot or the A spot in consideration with its sensitivity and potential for pleasurable sensations.
Posterior fornix	It is the back side of vaginal fornix that is situated towards the rear of the body close by the G spot. It is closer to the cul-de-sac or rectum as well as can be pleasurable to stimulate during sexual activities.
Lateral fornix	It is located on each side of the cervix forming a circular shape around it. These fornices could be even stimulated during sexual activity and play a role in providing pleasure and support.

Table 1: Vaginal fornices and its anatomical positioning

The vaginal fornix as well as the other vault related regions of the vagina develops a specific floral pattern of microbial niche. The discovery of Albert Doderlein in 1892 focused on the specific presence of different sets of human flora that implies of the individual's overall health. The primary colonizing bacteria of a health individual are of the genus of *Lactobacillus spp* to form around 90-95% of the overall niche. This class of the bacteria corresponds to the long-term colonization of the vagina adhering to the vaginal epithelial cells.

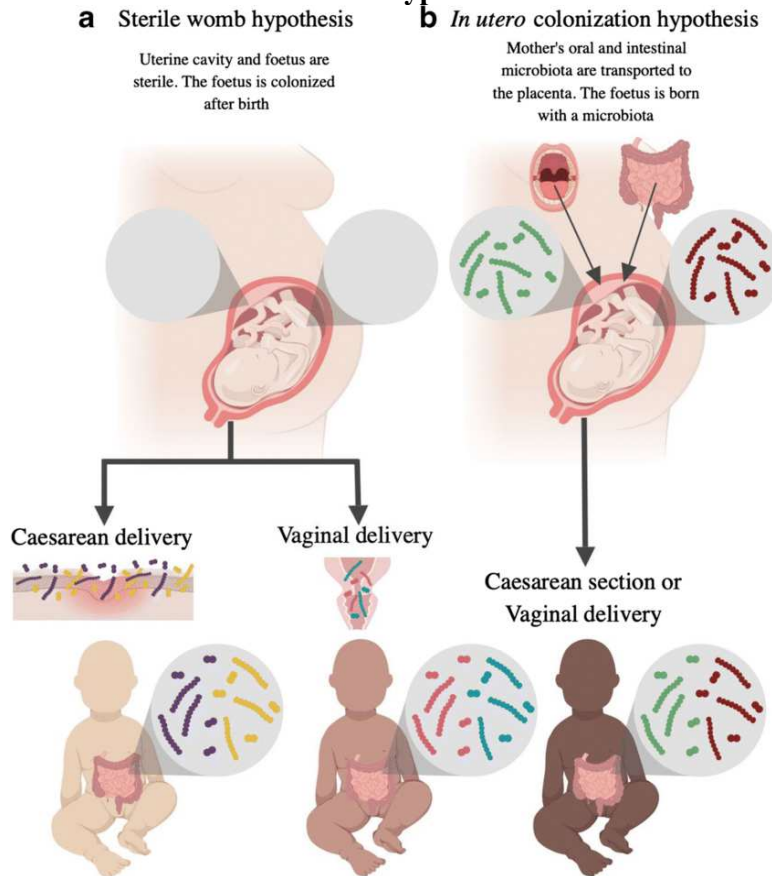
Until the discovery in late 20th century, uterus was thought to be a sterile organ protected by the cervical mucus from ascending bacteria present in the vaginal milieu, especially during pregnancy. According to Peric *et al.* (2019), the mucosal epithelial cells cervix forms a barrier by producing a highly hydrated gel covering the cervical surface and protects them against chemical and mechanical injuries as well as microbial attacks. These cells even secrete many defensive substances like mucins, defensins, protegrins, lysozyme, histatins, and nitric oxide. However, Hansen *et al.* (2014) illustrated that 'sterile womb' hypothesis has been challenged by varied studies and it has been found that microbes were nevertheless able to ascend through the cervical mucus plug along with some small labelled particles to actively transport from posterior vaginal fornix, through cervix and uterus and then back to the fallopian tubes and dominant follicle during follicular and luteal phase of menstruation.

Metagenomic studies performed on endometrial samples

Study	Target	Sample type	Major microbial genera
Franasiak <i>et al.</i> , (2016)	33 patients going through IVF	Catheter tips used for embryo transfer	Species of <i>Flavobacterium</i> , <i>Lactobacilli</i> , <i>Limnohabitans</i> , <i>Polynucleobacter</i> , <i>Bdellovibrio</i> , <i>Chryseobacterium</i> , <i>Spirochaeta</i> , and <i>Clostridium</i> .
Verstraelen <i>et al.</i> (2016)	19 Hysteroscopy patients	Endometrial sample	Dominant <i>Bacteroides spp.</i> along with <i>Lactobacillus crispatus</i> , <i>Pelomonas spp.</i> , <i>Prevotella spp.</i> , <i>Atopobium Vaginae</i> , and <i>Mobiluncus curtisii</i>
Chen <i>et al.</i> (2017)	110 pregnant non-infected patients	Endometrial samples	30.6% <i>Lactobacillus spp.</i> , 9.09% <i>Pseudomonas spp.</i> , 9.07% <i>Acinetobacter spp.</i> , 7.29% <i>Vagococcus spp.</i> , 5% <i>Sphingobium spp.</i>

Table 2: Metagenomic study of Endometrial microbiota

In analysis of the above tabular sheets, it could be analysed that pregnancy and foetal development are not only composed of a certain sterile environment rather it has been guided by the vaginal to Upper Genital tract niche that enriches the bacterial growth in the healthy foetus for further accumulation in post-natal development. Similarly, the illustration of sterile womb hypothesis could be elicited where the bacterial growth could be evident at various stages from infected to infertile as well as in healthy pregnant women.

Sterile womb hypothesis V/s In-utero colonisation hypothesis**Figure 5: Illustration of gut microbial development vs sterile womb hypothesis**(Source: Rodriguez *et al.*, 2019)

The sterile womb hypothesis has been accepted in the early 18th century that argues the environment of uterus and the foetus is completely devoid of any microbial infestation. On the contrary, it even guides that the gut microbiota only origins from the presence of microbes in the Caesarean delivery or the vaginal delivery subsiding the character of differentiated gut health in natal stages. On the contrary, the studies by Avelar Rodriguez *et al.* (2019), evaluated that in preterm neonates decreased number of *Bifidobacterium* spp. has been effectively evident and their microbiota exhibits less diversity as compared with term neonates. Nevertheless, the in-utero colonisation hypothesis postulated that the mother's gut microorganisms have been selectively transported to the placenta which colonise the foetus alimentary tract in uterus. In accordance with Perez-Munoz *et al.* (2017), the microbiome of different body site niches in nonpregnant subjects and even found that of these body sites, the oral microbiota was the most akin to the placenta, suggesting a possible correlation of colonisation. Hence, the application of diverse study in the forward application of sequencing data provided a clear understanding and enrichment in the presence of microbiota in the upper genital tract and transportation to the gut microbiota of the foetus.

Gut-microbiota

The gut microbiota has been the system of microorganism in the person's gastrointestinal system that symbiotically aids in supporting energy harvesting, digestion, and immune defense. The human gastrointestinal tract has been referred to harbor complex and dynamic population of microorganisms, the gut microbiota, which exerts a marked influence on the host during homoeostasis and disease prevalence. Considering this existence, multiple factors such as psychological makeup, diet regime, lifestyle, situational and long-term behaviour, and many more contributes to the existence of this gut bacterial existence.

The human GI tract has been representing the largest interface between the host, environmental factors, and antigens in the human body of around 250-400m². In an average lifetime of an individual, around 60 tonnes of food passes through tract where microorganisms focus on maintaining the gut integrity. However, various factors such as genetic background, health status including other determinants, lifestyle habits, emotional pathways, xenobiotics, diet imbalance, and hygiene have been the leading aetiologies for the altered gut-microbiota axis or dysbiosis.

Organism	Habitat	Ecological niche	Genome size (Mb)
<i>Bacteroides thetaiotaomicron</i>	Large Intestine	Saccharolyis	6.3
<i>Bacteroides vulgates</i>	Large intestine	Saccharolyis	5.2
<i>Parabacteroides distasonis</i>	Large intestine	Saccharolyis	4.8
<i>Escherchia coli</i>	Large and small intestine	Fermentation of simple sugars and amino acids	4.5
<i>Roseburia intestinalis</i>	Large intestine	Saccharolyis, butyrate producer	4.2
<i>Eubacterium rectal</i>	Large intestine	Saccharolyis, butyrate producer	3.4
<i>Faccalibacterium prausnitzii</i>	Large intestine	Saccharolyis, butyrate producer	3.1
<i>Bifodabacterium adolescentis</i>	Large intestine	Dietary carbohydrates	2.1
<i>Lactobacillus reuteri</i>	Small intestine	Host specific, fermentation of simple sugars, and 1,2-propandiol	2.0
<i>Methanobrevibacter smitbii</i>	Large intestine	Methanogen	1.9
<i>Helicobacter pylori</i>	Stomach	Utilisation of simple sugars and amino acids	1.67

Table 3: Gut-microbiota based on their size

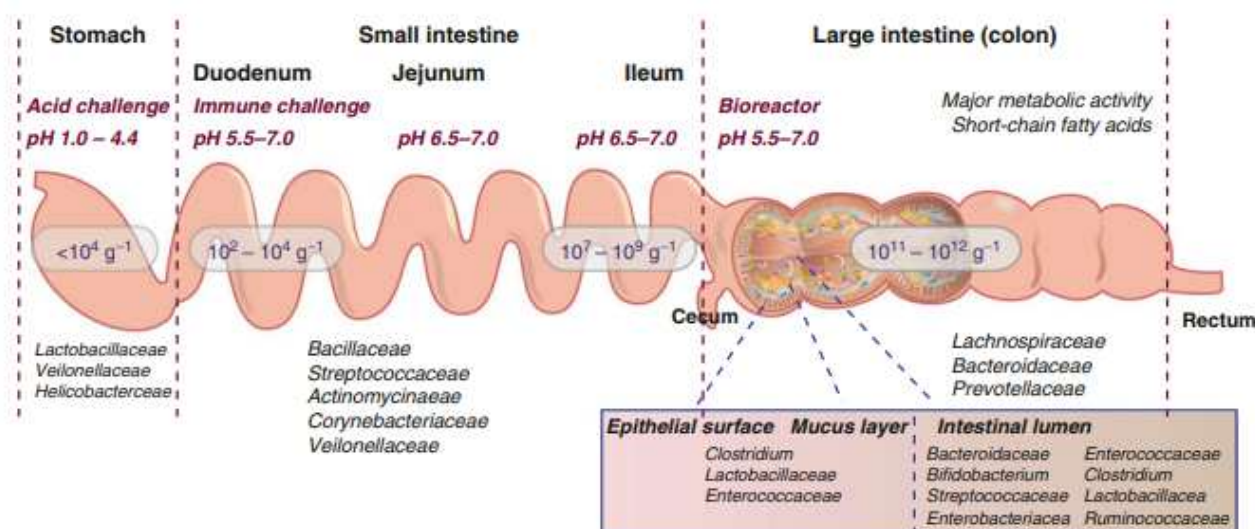


Figure 6: pH of the gut and microbial existence

The above figure justifies the incident that lower the pH higher is the complexity of bacteria and even smaller in the size of bacteria. Furthermore, it could be even evident that the colonisation of intestinal lumen begins at the when the sterile environment of the infant gut has been first colonised by a simple microbial community which develops into a climax community at 2 years of life. Following factors at different life stages impact on the gut microbiota health

- **Foetal stage:** → Maternal microbiota, health status of both parents, lifestyle of mother
- **Birth:** → antibiotic dosage during birth and before, Vaginal Vs C-Section, Term vs Preterm
- **Infancy:** → Geographical location, family environment, breastfeeding vs Formula, Genetics, Complementary food, duration of lactation
- **Adult:** → Diet Lifestyle
- **Elderly** → Diet Lifestyle, Living environment medication

One should note the fact that the first three years of life represents the most critical period for dietary interventions aimed at microbiota modulation to improve child growth and development and positively affect health.

Major Factors	Corresponding Scenario	Bacteria found
Mode of delivery	Vaginal delivery	<ul style="list-style-type: none"> • <i>Lactobacillus</i> and <i>Bifidobacterium</i> spp. • Enterobacteriaceae • <i>Bacteriodes fragilis</i> • 16S rRNA based bacteria
	C-section	<ul style="list-style-type: none"> • Clostridia • Delayed Bacteroidetes colonisation • Skin-associated bacteria <i>Staphylococcus</i> genus and <i>Streptococci</i> genus **The child is only exposed to the diverse of skin and oral bacteria of mother
Antibiotics and preterm birth	Full-term pregnancy	<ul style="list-style-type: none"> • <i>Bifidobacterium</i> sp. • <i>Bacteroides</i> sp.
	Pre-term birth	<ul style="list-style-type: none"> • <i>Enterococcus</i> sp. • <i>Proteobacteria</i> sp
Environment and lifestyle	Uttarakhand, J&K, Sikkim, Himachal Pradesh, Arunachal Pradesh, and other hilly regions of India	<ul style="list-style-type: none"> • <i>Bacillus subtilis</i>, • <i>Bacillus licheniformis</i>, • <i>Actinobacillus hominis</i>, • <i>Streptomyces ruber</i>, • <i>Nocardiosis prasina</i>, • <i>Thermobifida fusca</i>
	West Bengal, Bihar, Jharkhand, Odisha, and UP	<ul style="list-style-type: none"> • <i>Proteobacteria</i> sp. • <i>Acidovorax</i> • <i>Hydrogenophaga</i> • <i>Bosea</i> • <i>Polymorphum</i>
	Southern Indian states including Tamil Nadu, Kerala, Telangana, Andhra Pradesh	<ul style="list-style-type: none"> • <i>Klebsiella pneumoniae</i> • <i>Pseudomonas aeruginosa</i> • <i>Staphylococcus aureus</i> • <i>Coagulase neative staphylococci</i>

Table 4: Major factors affecting the gut microbiota

Discussions

Endometrium microbiota in the modulation of immune tolerance during pregnancy

Endometrial microbiome during embryo implantation

In review of the in-vivo and in-vitro analysis, it has been found that uterine bacteria or endometrial microbiome fragments can influence the endometrial receptivity, inducing an inflammatory response. Indeed, endometrial response during the implantation has been effectively enriching the fertilisation and implantation due to the epithelial receptivity in the uterus. Furthermore, the findings from different research justified that in-utero colonisation have been the major factor for the projection and development of microbiome within the foetus. It has been dependent on the aspects of *Lactobacilli*-dominant flora where the microbes exhibit different regions of the female reproductive system. This hence transforms into the gut of the foetus during implantation or fertilisation.

Role of *Bacteroides Fragilis*

Relevant evidence was found regarding the role of *Bacteroides* (*B.*) *fragilis*. *B. fragilis* has been an important Gram-negative anaerobic bacterium commonly found in the lower gastrointestinal tract; however, as stated previously, it belongs to the endometrial microbiome in non-pregnant women. Thus, the development of *Bacteroides* could be effectively evident in the uterine region and even found in the lower GIT justifying the H1 and hence neglecting the H0.

Role of *Lactobacilli*

Lactobacilli is the major dominative microbe present in the Lower genital tract that aids in the production of lactic acid as major part to maintain the pH and homeostasis of the body. From the previous data, it has been evident that 36.09% of the microbes constitutes of *Lactobacilli* spp. that maintains the inflammatory cytokines and chemokines through toll-like receptor. Hence, with reference from different

articles, it has been found that the *Lactobacilli* is predominately evident in the gut as well as female reproductive system. In nexus with this, the Hypothesis 1 (H1) has been effectively proved and H0 can be neglected.

Conclusion

From different research articles, it could be concluded that endometrial microbiota has been one of the major factors that focuses on the development of gut microbiota in the foetus. However, varied literature even focused on various gaps including the need for DNA sequencing of the uterine region as well as the foetal developing stages in different pregnant women to identify the range of microbes present and pathway for their transformation. Hence, this research scenario justified through literature study that endometrial microbiome has been guiding the development of gut microbiota through the pathways of osmotic transportation increasing the gut microbiota in the intestinal region of foetus.

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