Spring 4-11-2023

Delivery of the Immune System: Understanding the Development of the Human Immune System Based on Birth Mode

Sara Abbas
syabbas001@gmail.com

ARCADIA UNIVERSITY
THE COLLEGE OF GLOBAL STUDIES

Delivery of the Immune System:
Understanding the Development of the Human Immune System Based on Birth Mode

by
Sara Abbas

Arcadia University
INPR 310 - Work in Thought and Action
Dr. Joanna Simos

March 24th, 2023

TABLE OF CONTENTS

KEY WORDS

Vaginal birth, cesarean section, immune system development, neonate, allergies, innate immune system, adaptive immune system, commensal bacteria, cytokine development, regulatory T cells, hypersensitivity, auto-immune disease.

ABSTRACT

The creation of the immune system starts from the womb. The expecting mother plays an immense role outside of the genetic influence in developing a neonate's immune system. The mode of birth has a substantial impact, as the neonate's initial moments can have lasting impacts on the making of a proper immune system. A mother should have the choice in choosing between a vaginal birth or a cesarean section and this paper looks to provide aid in the decision process by contrasting the two modes in terms of immunological impact, especially as the rate of c-sections climb (1,2). The approach of this paper is a collective case study, to understand the similarities and differences between the two modes of birth, vaginal and cesarean section (31). Specifically, it's an instrumental case study as the purpose of the paper is to gain insight overall (32). A combination of primary and secondary research is conducted, using strategies to analyze each source type. Research techniques include an interview, observation, direct interpretation, and categorical aggregation to collect and analyze both qualitative and quantitative data (30,32). The main contrast between the two modes of birth is the creation of the neonate's gut microbiota, as its diversity and amount is directly proportional to the proper development of the neonate's immune system (17). Overall, a c-section can cause the underdevelopment of the immune system increasing risk of short-term and long-term effects on the newborn. Therefore, it is necessary for these consequences to be regarded more highly when choosing a delivery method.

INTRODUCTION

Cesarean section rates have tripled globally since 1990 (1). In fact, c-sections now outnumber vaginal deliveries in parts of Europe, Latin America, and China. Poorer countries have even higher rates of c-sections (1). With c-sections growing so drastically, it is vital to know its impact on the newborn—especially in regards to the development of their immune system.

The immune system is divided into two categories: the innate immune system and the adaptive immune system. These systems have much overlap, to the point of interdependence. The body cannot just rely on one of these branches, both must work in tandem to give the human body its best ability against pathogens. The creation of this system starts from the initiation of human development. The mother plays an essential role in the development of the immune system, separate from genetic influence. The birthing process is complex, as it combines numerous factors to result in a newborn, even down to the method of birth. A newborn's first moments can have long lasting impacts. The birthing process can lead to both short-term and long-term effects on the development of the immune system. This literature review will encompass an understanding of the initial immune capabilities of a newborn and then dive into how the mode of birth can further affect immune system development. Lastly, the discussion section will then include how current literature interprets short-term and long-term changes in regards to differences in the delivery of a newborn.

METHODOLOGY

This project will be approached in a case study manner, for the following two reasons: this project focuses on answering the "how" and "why" of the research topic and because I cannot manipulate the behavior involved in the study directly. (30) A collective case study will be conducted to understand the similarities and differences between the two modes of birth, vaginal and cesarean section (31). This project will use more than one case to understand the involvement of the immune system in each mode of birth, requiring the study to be categorized as collective/multiple-case studies. In Stake's case terminology specifically, this project pursues an instrumental case study as it looks to gain insight to the immune system development of neonates based on their mode of birth. The studies utilized support understanding this concept, in contrast

to simply intrinsic interest. This approach should produce an understanding of the neonatal topic in a robust and reliable manner (30). The structure in reporting the case study will be in a theory building manner (31). Once the topic has been introduced, the initial immune system of the neonate is presented in order to understand the effects of the birthing process, which would be detailed after. The development of the neonate would then be presented for each mode of birth followed by the comparison of each birth mode in the analysis section. The theory would then be built by the conclusion section and the overall impact of the topic would be discussed.

The foundation for this research will include both primary and secondary research. A majority of the project will consist of secondary research, as there is much research existing in the field. The current research has a plethora of knowledge on either side of the spectrum of vaginal or cesarean section but there is not much comparing the two modes of birth directly. The prioritization of utilizing secondary research also comes from the fact that I personally do not have the ability to create a full experiment to compare the modes of birth based on a time and resource strain. Secondary sources will come from online databases provided by the University of Minnesota and using the Google Scholar search engine. Primary data can come from an interview on Dr. Burak Salgin, a Senior Clinical Fellow (ST 4-8 level) in Neonatal Medicine at the Royal London Hospital. This will occur during a shadowing opportunity at the neonatal unit. I hope to gain any information regarding the effects birth mode has on the immune system development of a neonate from Dr. Burak Salgin, especially if he has an opinion on one being better than the other. The timeframe for data collection will be in the month of February, 2023. The month of March will consist of mostly writing out the actual paper.

There will be a mix between the two families of data, quantitative and qualitative, within this research project. The conclusions drawn will mostly be based on quantitative factors, such as the number of children who were found to have allergies. Such a number could be compared between the two birth modes and see if it is an event of causation. It is important to note that qualitative data will also be presented, such as the types of gut microbiomes associated with each birth mode (yes, they allow for different bacterial growth within the neonate!) Using multiple data sources will create data credibility and strategies will be implemented for rigor and

trustworthiness. Three strategies include assessing each source that purposeful sampling has been applied, data collection is systematic, and data analyzation is correct 30).

Some techniques specific to qualitative data include the interview and observations made during that shadowing experience. For an instrumental case study, the techniques of categorical aggregation and direct interpretation will be used (32). With quantitative data, it will utilize direct interpretation more often versus categorical aggregation as the data often is able to speak for itself. Categorical aggregation is done by creating a collection of data based on a specific category to create a meaning within the discussion section (32). This second technique is for both qualitative and quantitative data and can integrate the two. Categorical aggregation will be the central technique of this paper as the purpose to understand the topic in a manner that exposes the relationship between the mode of birth and the neonatal immune system in contrast to exploring the complexity of the issue.

INITIAL IMMUNE STATE OF NEWBORN

The development of the immune system is not a short process (2). At the time of birth, the newborn's first line of defense is fairly developed (2). It is the second and third lines of defense that require further development. The immune system is constantly improving, resulting in an exponential state of growth. Some of the immune development does start in the womb but the mode of birth has the ability to greatly affect the progression of its development (2). It is important to note that a newborn's immediate immunity state is in deficiency as it only has two major maternal factors to help build it: IgG and the amniotic fluid. IgG is the only antibody that can move through the placenta (2). Antibodies are one of the primary connections between the innate and adaptive systems. They are essential to the complement cascade, phagocytic activity, and opsonic activity of the innate system (2). Specificity for both the innate and adaptive must be built and it is done primarily through exposure. This prefaces that the immediate state of the newborn will be severely compromised. To illustrate this, the average C3 level in a newborn is 65%, in comparison to adult levels (2). The low number of antibodies also causes a depletion in phagocytosis and opsonization (2). The clear trend here is that the specificity of the immune system requires further development. In the womb, this is initiated by the amniotic fluid.

The amniotic fluid and vernix caseosa are protective characteristics of pregnancy. But, they differ based on source: amniotic fluid comes from the mother and the vernix caseosa is created by the fetus (3). The amniotic fluid works as a protective, cushioning fluid filled with nutrients for the fetus (3,4). It also contains a plethora of bacterial ribosomal DNA from the mother (5,6). The fetus does ingest some of the amniotic fluid, which in turn can improve the specificity of the immune system (7). The effects of the amniotic fluid are both innate and adaptive, as it can affect pattern recognition receptors from the innate immune system and lymphocytes of the adaptive system (5). Amniotic fluid also creates a pH neutral environment within the newborn's stomach, which allows a suitable environment for commensal bacteria to thrive (8).

Made by the fetal sebaceous glands, the vernix caseosa is a white and creamy biofilm that is formed prior to the third trimester of pregnancy (3). During that last trimester, this lipid-based structure detaches into the amniotic fluid. This is one of the possible ways that the amniotic fluid gains antimicrobial properties (3). Vernix caseosa has numerous benefits to the fetus: from minimization of friction amongst body parts to a direct role in antimicrobial development (4). The vernix caseosa includes a multitude of different proteins that can induce antimicrobial ability, antifungal activity, opsonizing capacity, protease inhibition, and parasite inactivation (3). One of the many peptides found in vernix caseosa is psoriasin, a calcium-binding protein that can cause antimicrobial activity and in turn induce T-lymphocyte and neutrophil migration (3). The principal component of the vernix caseosa is alpha-defensins, a type of innate immunity (3). Paneth cells of the fetus further develop the innate immune system by producing defensins by the 25th week of gestation (9). The vernix caseosa is the main immune defense the newborn has for either mode of birth (10). If anything, the newborn can have more vernix caseosa from a c-section, but typically the practice is to wipe it away soon after the birth (4). So, the effects are quite temporary but necessary.

In regards to immune system development, it is clear that the innate immune system is significantly more developed than the adaptive immune system in a fetus. The innate ability stems from both the creation of barriers and peptides from the vernix caseosa (9). Innate immunity also connects to the adaptive immune system via IgG (2). Amniotic fluid is able to assist the specificity of the innate system. Defensins from the paneth cells and vernix caseosa are examples of the direct

contributions from the fetus to the innate immune system development (9). The limited development of the adaptive immune system results in hampered ability of antigen-presenting cells to activate T cells and a decrease in cytokine amount (2). But overall, the newborn is still considered to be physiologically immunodeficient at the moment of birth.

THE BIRTHING PROCESS & IMMUNE SYSTEM DEVELOPMENT

The main connection between the vaginal birthing process and the immune system development occurs during the movement from the womb to being fully delivered (11). This contact between the mother and the newborn causes maternal vaginal and intestinal flora to be exposed during delivery (12) This bacteria is crucial in creating commensal bacteria within the newborn's intestinal tract. Such bacteria aids the immune system in combating future pathogens and helps the development of the immune system itself. The specific bacteria present in the newborn's initial exposure is linked to many facets of the immune system: from regulatory T cells to cytokines. The microbial environment is then essential to both the innate and adaptive immune system development.

A cesarean section provides a new environment for the newborn. It is performed in conditions when a vaginal birth is not safe for the mother or the baby (13). In the last couple of years, c-sections have increased 10 fold (14). More physicians and patients are starting to elect to have a c-section done, without necessary medical reason (15). A C-section is done by making cuts into the abdomen and uterus, and delivering the baby through the abdominal opening (13). This ushers in a change to the nature of the birthing process: from an originally vaginal environment to an abdominal environment.

A new environment means a difference in what the newborn is exposed to. With a C-section, the newborn is now exposed to GI tract bacteria and the skin flora microbes (16). The C-section also produces a lack of some microbes, as the procedure prompts a sterile environment. Antibiotics cut bacterial exposure, leading to a less diverse gut microbiota (17). This is a clear contrast with vaginal births as the newborn is exposed to vaginal bacteria and the vernix caseosa, so it is nothing near the environment a c-section creates.

In addition to the microbiota environment that the c-section creates, it is also more important to note the "surgical" aspect that the newborn is exposed to. Anesthetics used for a c-section can cross into a placenta and alter the immune system of the newborn (18). The primary effect of this is on the neutrophils, an aspect of the innate immune system that is already suppressed by newborns. However, the effect on the neutrophils is that they are more likely to burst. This can then link the use of lidocaine to hypersensitivity (19). Natural killer T cells, a major facet of adaptive immunity, were found to have lower activity in newborns with c-sections (18,19). The effects of this can also differ based on the type of anesthesia used: lidocaine seems to affect both neutrophils and natural killer T cells, but sevoflurane only seems to affect natural killer T cells (19).

Cesarean section newborns seem to have less cytokines in the long run (20). Cytokines are a type of regulatory molecule that is integral to both the innate and adaptive immune system. One study conducted in 2014 has found decreased production in a wide range of cytokines, including tumor necrosis factor (TNF-α) and IL-1β (20). This is again due to the initial microbial exposure newborns have, *Bacteroides* and *Bifidobacterium spp* are essential to cytokine development (20). A vaginal birth is able to expose the newborn to these microbes, while a c-section newborn is deprived of such contact. Such gram-negative bacteria are needed for the PRR of innate immunity, specifically for the Toll Like Receptors (21). C-section newborns lose some of this innate development (21). Another study from 2018 has found that vaginal delivery newborns have more lipopolysaccharide (LPS) biosynthesis, another detail to the gut microbiota (11). The more diverse representation of LPS allows for higher levels of TNF-α and interleukin 18 (IL-18) (11). In turn, c-sections do not have as much LPS, causing a negative impact on the immune stimulation potential for the newborn (9,11). A depleted amount of cytokines harms the development of the immune system itself as it is connected to cells of both the innate and adaptive immune system.

Regulatory T cells are another part of the adaptive immune system that is affected by the delivery method. These cells are essential in monitoring the immune system, otherwise it can lead to ill events such as hypersensitivity or other forms of auto-immune diseases. Prior to birth, the fetus has a sterile intestinal tract (6, 21). Colonization of the environmental bacteria happens immediately after birth, but the mode of birth can have an effect on what microbes grow.

Regulatory T cells depend on these microbes for development (20). For instance, some of the metabolites created from the commensal bacteria can also influence regulatory T cell activity in the GI tract (22). Changing the type and amount of bacteria has a clear impact on not only the gut based regulatory T cells, but also splenic regulatory T cells (23) . Murine models are able to demonstrate this trend (23) . Cesarean section delivery decreased the amount of splenic regulatory T cells in the mice, as it is linked to the loss of necessary gut bacteria (23). Due to the lack of a microbial environment during a c-section, the effect of regulatory T-cells is suppressed (23). Although a prebiotic diet can improve the activity of these cells, it is not sufficient to fully restore a normal level of regulatory T-cells and their impact (23).

ETHICAL CONSIDERATIONS

The outcomes of this research will impact two major stakeholders. First, expecting mothers will be affected as this research can influence their decision in deciding a mode of birth. It has become more common to have a cesarean section, but is this a trend that mothers-to-be continue to choose? Understanding how the mode of birth may impact the neonate, especially in the sense of immunology, should play a role in the expectant mother's decision. At the very least, this is information that should not be hard for expecting mothers to access.

The second group that will be impacted by this research are health care professionals themselves. The fields of immunology, delivery, and neonatal care can benefit from understanding that the mode of birth can affect the immune system development of a neonate—this can also influence the care that medical professionals give. For example, vaginal seeding is a method to level the playing field between c-sections and vaginal births by exposing the c-section neonates to vaginal bacteria. This is done by swabbing some of the mother's vaginal fluid onto the face of the baby by a medical professional (23). But within the first google search of this topic, one can immediately find an article asking for caution in using such a method. This is because there is an ethical issue in purposefully exposing neonates to bacteria, as it does risk the neonate in receiving an infection.

Thus, it is important to note that the ethics of this paper looks in consideration of its primary stakeholders. Already one of the four pillars of medical ethics, non-maleficence, can be in conflict when presented with the situation of vaginal seeding (34). But all medical practice has the

involvement of risk, from vaginal seeding to the delivery process. Maintaining the medical code of ethics: respect for autonomy, beneficence, non-maleficence, and justice are necessary to stay morally grounded (34). This first ethical consideration is then specific to the second stakeholders, healthcare professionals, who need to maintain this balance of ethics and innovation.

The two stakeholders are involved in an ethical issue together: social implications. Data has been present about the prevalence of racism and sexism in the delivery room (35,36). Sometimes it can come from the patient's surrounding individuals and sometimes it can come from the healthcare professionals as well (36). These ethical issues would then impact the mode of birth as the mother's choice can be affected. This paper is to produce information that can aid the expectant mother's choice but such would most practically be delivered by a healthcare professional. If the healthcare worker is clouded by racism/sexism, such information could be lost and could prevent the mother-to-be from making a well rounded decision. This second ethical concern connects back to Stake's term "issues", isolating the ideals that are wired to political, social, historical, and personal contexts. On the other hand, social implications could also look like disrupting the balance of power between the patient and the provider. The information of this paper could empower the patient into having more control of their care.

A final ethical consideration comes from the study itself. Of course, bias is extremely important to consider during research because it has the ability to distort the findings and understandings that the research presents. Some bias can be unconscious, where individuals do not recognize that they are being biased. Others can be conscious, and such actions can be intentionally very hurtful. To keep away from being ethically inconsiderate, I plan on recognizing the different categories of bias my paper may hold and continue the message of support that the mode of birth is a mother's choice. It is hard to recognize moments of unconscious bias, but there are two notes of possible bias. First, my own identity can provide bias as I am a female and future healthcare professional. This can impact my ability to be critical when presented with either of those contexts. My own identity also contributes to my interest, drive and motivation to complete this paper. My own interests as a future mother and my individual priorities cause me to prefer vaginal birth. I want to focus on the baby's development the best I can and currently I have no health concerns that would need me to do a cesarean section. The original intention of a cesarean section is to be done

when a vaginal birth cannot be done due to delivery complications. Now, the cesarean section has evolved to incorporate the mother's choice—a movement that I fully support. As a researcher then, it is my goal to be as neutral as possible to give my full support to mothers and their choice. In this paper, I provide the developmental perspective of the neonate for both births and give both the positives and negatives of each delivery method. I also add initiatives such as vaginal seeding that gives support to conducting a cesarean section. The end goal of this paper is to provide future mothers with information that can positively impact their delivery choice, thus it is important to recognize the bias that can come from the researcher's personal opinion in order to establish a field of neutrality.

The second category of bias that pertains to this paper is confirmation bias. Earlier in the methodology section, I stated that an interview would be done and I hope to seek information from an expert in the field. I got to shadow at the neonatal unit but I did not get a chance to conduct an interview with Dr. Burak Salgin. But I did get to talk to one of his colleagues who is a Nurse Practitioner. The NP was not aware of the immunology specifics, but did note how cesarean section neonates often would go through more respiratory distress than vaginal birth. This respiratory distress is linked with the mechanics of the mode of birth. While the neonate is in the womb, it is important to recognize they are surrounded by fluid for protection and sometimes that fluid can go into their lungs. During the process of the vaginal birth, this lung fluid would be literally pressed out during delivery from the pressure of the vaginal canal. Cesarean section neonates would not go through such a process, risking some of that fluid to remain in the lung and cause an infection...impacting the proper development of the immune system. This observation made during shadowing is supported by current literature and shows a totally different lens on how the mode of delivery can impact the neonate and their immune system (37). The presence of confirmation bias occurs here because going into the conversation, I was looking for information specific to the cellular side of impact and not the mechanical aspect at all! Until talking to my professor Dr. Joanna Simos, I would have left such information out because it was inconsistent with my interview goals. I am glad that I was able to revisit this observation and see that it was still quite impactful to this project.

The presence of ethics is ever present in the growing medical field, especially when covering new boundaries. Ethical issues also arise in the social sense as this study can provide power to the individuals linked to the delivery room causing potential positive or negative change. Exploring possible biases are essential for having a well rounded paper as some degree of bias can always be present. The overall outcome of this research is to aid the risk assessment of both mothers to be and healthcare professionals in deciding care of the neonate during the birthing process; ethical issues are important to consider in order to achieve such an outcome.

DISCUSSION

Some research has shown that the differences in gut bacteria are temporary, suggesting no long-term impacts. A study done in 2017 found that any differences between c-section newborns and vaginal delivery newborns would no longer be substantial by 8 or 24 weeks of age (24). Yet, another study found the mode of delivery can affect the gut microbiota in children even at 7 years of age (16)! These c-section children were also noted to have slower development of gut bacteria (16). This trend of contradiction is unusually common in much of this subject field. A variety of linkages are discussed back and forth. These include the connection between delivery of birth and likelihood of diabetes to the effectiveness of prebiotics to reverse the effects of c-sections.

One article discusses an increased risk of diabetes with a cesarean section while another shows that the difference in birth delivery has no impact on developing Type 1 diabetes (23,26). The same notion is apparent in the countermeasures for c-sections: prebiotics and vaginal seeding (23). A few articles will support the countermeasures while others are still unsure of its actual benefits (23). Both attempt to restore the normal gut bacteria for the newborn: prebiotics stimulate the growth of microbes in the gut while vaginal seeding places some of the vaginal flora the newborn would have been originally in contact with (23, 27). There are so many factors of the birthing process and not one contributes solely to the immune system development. In current research, not all of these factors are accounted for. From anesthesia to evaluating breast milk as another countermeasure, these details may not be apparent in all research and can lead to contrasting conclusions. For example, Neu discusses this effect by presenting two similar studies with contradictory results caused by the influences of breast milk versus formula (6).

It is better to recognize the actual impacts of the gut bacteria than to compare its conditions based on the mode of birth. The magnitude of impact that the mode of birth places on gut bacteria is substantial: it allows points of irregularities within the immune system. Improper development of the immune system can lead to both long-term and short-impacts. In the long run, it is possible the irregularities within the immune system can lead to hypersensitivity cases, such as allergies (14,16). The short-term aspect is that a baby's immune system that is already underdeveloped, is now even worse off. This situation further hinders the immune system and can be crucial to the survival of the child.

Studies have shown a link between Sudden Infant Death Syndrome (SIDs) and hypersensitivity of the immune system (28). Several studies have noted increased activity of the innate immune system in cases of SIDs, relating it to the anaphylaxis event (29). But again, contradictions appear in deciding the sequence of SIDs (28,29).

All of these examples of contradiction, point to the need for additional research. New research must be conducted in a method that can isolate variables, allowing it to be comparable and combined in effort. There are various factors of the development of the immune system that can affect a conclusion and are not necessarily specific to the birthing process. This is how the comparison and discussion between literature can become skewed. The overwhelming evidence does show that the delivery method does have an effect, but more research is needed in order to evaluate and compare the effects found. A majority of the research done for newborn immunity development regards short-term effects. For this topic, an emphasis on long-term research is needed to better understand the total effect of the birthing process. This can in turn potentially support treatment of autoimmune conditions: from hypersensitivities to Type 1 diabetes (14, 16, 23, 24). Overall, the underdevelopment of the immune system caused by c-section can lead to probable short-term and long-term effects on the newborn. Therefore, it is necessary for these consequences to be regarded more highly when choosing a delivery method.

CONCLUSION

Initially, the newborn has very limited immune capabilities in regards to both innate and adaptive systems. The little ability the newborn has is primarily innate, through creating the

vernix caseosa. Maternal factors that kickstart some development come from IgG and the amniotic fluid. But overall, the newborn is physiologically immunodeficient at birth.

The major difference between the mode of birth is the type of microbes the newborn becomes exposed to: a vaginal birth causes exposure of vaginal and intestinal flora while a c-section leads to contact with skin flora and the GI tract bacterium. The gut bacteria of the newborn is essential for further development of its immune system. The environmental differences, including antibiotics and anesthetics used during the procedure, can lead to changes of the newborn's gut bacteria and immune cells.

Cytokines, toll-like receptors, and regulatory T cells are a few of the major components of the immune system that get altered due to the delivery method and affect the gut bacteria of the newborn. These components of the immune system are crucial in regulation as they prevent hypersensitivities and other ill outcomes. Cesarean section effects on the newborn's immune system development can be short-term and long-term: increasing the possibility of SIDs, allergies, and autoimmune diseases.

With c-sections having more harms than benefits to the newborn's immune system development, it offers caution in the world's increased rate of c-sections. However, there are plenty of cases where c-sections must be used. In those cases, countermeasures should be applied. These include non-pasteurized breast milk, prebiotics, and vaginal seeding in efforts for the newborn regain its gut microbiota (23,30). Breast milk that is unpasteurized or comes directly from the maternal source can often contain bacteria that aids in regaining the newborn's gut microbes (30). This is also a reason why some articles may clash in determining if the difference in gut microbiota is truly substantial: because the research did not account for major confounding factors such as breastfeeding (6). This trend is true for many different topics within this field, from establishing the difference over time for gut bacteria to the possibility of a delivery method linking it to type 1 diabetes. The immune system is not built on one single foundation but many facets. This is why this topic can provide difficulty in gaining a clear picture on the relationship between delivery mode and immune system development. But, it appears that a majority of the research points to the existence of a difference between vaginal births and c-sections in regards to gut bacteria. This

paper shows how the mode of birth, vaginal birth or cesarean section, clearly impacts the deliverance of the neonate's immune system.

BIBLIOGRAPHY

1. Doucleff, M. (2018, August 12). Rate Of C-Sections Is Rising At An “Alarming” Rate, Report Says. Retrieved April 29, 2022, from https://www.npr.org/sections/goatsandsoda/2018/10/12/656198429/rate-of-c-sections-is-rising-at-an-alarming-rate
2. Lumen Candela. (n.d.). Development of the Immune System | Boundless Anatomy and Physiology. Retrieved April 29, 2022, from https://courses.lumenlearning.com/boundless-ap/chapter/development-of-the-immune-system/
3. Yoshio, H., Tollin, M., Gudmundsson, G. *et al.* Antimicrobial Polypeptides of Human Vernix Caseosa and Amniotic Fluid: Implications for Newborn Innate Defense. *Pediatr Res* 53, 211–216 (2003). https://doi.org/10.1203/01.PDR.0000047471.47777.B0
4. Singh, G., & Archana, G. (2008). Unraveling the mystery of vernix caseosa. *Indian journal of dermatology*, *53*(2), 54–60. https://doi.org/10.4103/0019-5154.41645
5. Neu, J., & Rushing, J. (2011). Cesarean Versus Vaginal Delivery: Long-term Infant Outcomes and the Hygiene Hypothesis. *Clinics in Perinatology*, *38*(2), 321–331. https://doi.org/10.1016/j.clp.2011.03.008
6. Neu, J. Developmental aspects of maternal-fetal, and infant gut microbiota and implications for long-term health. *matern health, neonatol and perinatol* 1, 6 (2015). https://doi.org/10.1186/s40748-015-0007-4
7. Roy M. Pitkin, W. Ann Reynolds, Fetal ingestion and metabolism of amniotic fluid protein, American Journal of Obstetrics and Gynecology, Volume 123, Issue 4, 1975, Pages 356-363, ISSN 0002-9378, https://doi.org/10.1016/S0002-9378(16)33436-6.
8. Avery GB, Randolph JG, Weaver T. Gastric acidity in the first day of life. Pediatrics 1966;37:1005–7.
9. Zasloff, M. Vernix, the Newborn, and Innate Defense: Commentary on the article by Yoshio *et al.* on page 211. *Pediatr Res* 53, 203–204 (2003). https://doi.org/10.1203/01.PDR.0000047470.04132.87
10. Nishijima, K., Yoneda, M., Hirai, T., Takakuwa, K., & Enomoto, T. (2019). Biology of the vernix caseosa: A review. *Journal of Obstetrics and Gynaecology Research*, *45*(11), 2145-2149. https://obgyn.onlinelibrary.wiley.com/doi/pdf/10.1111/jog.14103?casa_token=ONq972m0MyYAAAAA:kbrr5JkuuztvIL5ryiFUtPLpcHe5wry_MezQbowYP2Zhnp7diibPoLcIxAw_zRK939zVXZX93NhGw8He
11. Wampach, L., Heintz-Buschart, A., Fritz, J.V. *et al.* Birth mode is associated with earliest strain-conferred gut microbiome functions and immunostimulatory potential. *Nat Commun* 9, 5091 (2018). https://doi.org/10.1038/s41467-018-07631-x

12. Zachariassen, L. F., Krych, L., Rasmussen, S. H., Nielsen, D. S., Kot, W., Holm, T. L., ... & Hansen, C. H. F. (2019). Cesarean section induces microbiota-regulated immune disturbances in C57BL/6 mice. *The Journal of Immunology*, *202*(1), 142-150.
13. *C-section - Mayo Clinic*. (2020, June 20). Www.mayoclinic.org. https://www.mayoclinic.org/tests-procedures/c-section/about/pac-20393655#:~:text=Cesarean%20delivery%20(C%2Dsection)
14. Huurre, A., Kalliomäki, M., Rautava, S., Rinne, M., Salminen, S., & Isolauri, E. (2007). Mode of Delivery – Effects on Gut Microbiota and Humoral Immunity. *Neonatology*, *93*(4), 236–240. https://doi.org/10.1159/000111102
15. Deutchman, M., Connor, P., Gobbo, R., & FitzSimmons, R. (1995). Outcomes of cesarean sections performed by family physicians and the training they received: a 15-year retrospective study. *The Journal of the American Board of Family Practice*, *8*(2), 81-90.
16. Salminen, S. (2004). Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut*, *53*(9), 1388–1389. https://doi.org/10.1136/gut.2004.041640
17. Tormo-Badia, N., Håkansson, Å., Vasudevan, K., Molin, G., Ahrné, S., & Cilio, C. M. (2022). Antibiotic Treatment of Pregnant Non-Obese Diabetic Mice Leads to Altered Gut Microbiota and Intestinal Immunological Changes in the Offspring. *Scandinavian Journal of Immunology*, *80*(4), 250–260. https://doi.org/10.1111/sji.12205
18. Cho, C. E., & Norman, M. (2013). Cesarean section and development of the immune system in the offspring. *American Journal of Obstetrics and Gynecology*, *208*(4), 249–254. https://doi.org/10.1016/j.ajog.2012.08.009
19. Gasparoni, A., Ciardelli, L., De Amici, D., Castellazzi, A. M., Autelli, M., Bottino, R., Polito, E., Bartoli, A., Rondini, G., & Chirico, G. (2002). Effect of general and epidural anaesthesia on thyroid hormones and immunity in neonates. *Paediatric Anaesthesia*, *12*(1), 59–64. https://doi.org/10.1046/j.1460-9592.2002.00752.x
20. Hansen, C. H. F., Andersen, L. S. F., Krych, Ł., Metzdorff, S. B., Hasselby, J. P., Skov, S., Nielsen, D. S., Buschard, K., Hansen, L. H., & Hansen, A. K. (2014). Mode of Delivery Shapes Gut Colonization Pattern and Modulates Regulatory Immunity in Mice. *The Journal of Immunology*, *193*(3), 1213–1222. https://doi.org/10.4049/jimmunol.1400085
21. Lotz, M., GütleD., Walther, S., MénardS., Bogdan, C., & Hornef, M. W. (2006). Postnatal acquisition of endotoxin tolerance in intestinal epithelial cells. *Journal of Experimental Medicine*, *203*(4), 973–984. https://doi.org/10.1084/jem.20050625

22. Hill, C. J., Lynch, D. B., Murphy, K., Ulaszewska, M., Jeffery, I. B., O'Shea, C. A., Watkins, C., Dempsey, E., Mattivi, F., Tuohy, K., Ross, R. P., Ryan, C. A., O' Toole, P. W., & Stanton, C. (2017). Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome*, *5*(1), 4. https://doi.org/10.1186/s40168-016-0213-y
23. Zachariassen, L. F., Krych, L., Rasmussen, S. H., Nielsen, D. S., Kot, W., Holm, T. L., Hansen, A. K., & Hansen, C. H. F. (2018). Cesarean Section Induces Microbiota-Regulated Immune Disturbances in C57BL/6 Mice. *The Journal of Immunology*, ji1800666. https://doi.org/10.4049/jimmunol.1800666
24. Butler, É. M., Chiavaroli, V., Derraik, J. G. B., Grigg, C. P., Wilson, B. C., Walker, N., O'Sullivan, J. M., & Cutfield, W. S. (2020). Maternal bacteria to correct abnormal gut microbiota in babies born by C-section. *Medicine*, *99*(30), e21315. https://doi.org/10.1097/md.0000000000021315
25. Salminen, S. (2004). Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut*, *53*(9), 1388–1389. https://doi.org/10.1136/gut.2004.041640
26. Tormo-Badia, N., Håkansson, Å., Vasudevan, K., Molin, G., Ahrné, S., & Cilio, C. M. (2014). Antibiotic Treatment of Pregnant Non-Obese Diabetic Mice Leads to Altered Gut Microbiota and Intestinal Immunological Changes in the Offspring. *Scandinavian Journal of Immunology*, *80*(4), 250–260. https://doi.org/10.1111/sji.12205
27. Vandenplas, Y., De Greef, E., & Veereman, G. (2014). Prebiotics in infant formula. *Gut microbes*, *5*(6), 681–687. https://doi.org/10.4161/19490976.2014.972237
28. Kevin D. Forsyth, Immune and inflammatory responses in sudden infant death syndrome, *FEMS Immunology & Medical Microbiology*, Volume 25, Issue 1-2, August 1999, Pages 79–83, https://doi.org/10.1111/j.1574-695X.1999.tb01329.x
29. Opdal SH. Cytokines, Infection, and Immunity. In: Duncan JR, Byard RW, editors. SIDS Sudden Infant and Early Childhood Death: The Past, the Present and the Future. Adelaide (AU): University of Adelaide Press; 2018 May. Chapter 30. Available from: https://www.ncbi.nlm.nih.gov/books/NBK513388/
30. Mohammadkhah, A. I., Simpson, E. B., Patterson, S. G., & Ferguson, J. F. (2018). Development of the Gut Microbiome in Children, and Lifetime Implications for Obesity and Cardiometabolic Disease. *Children (Basel, Switzerland)*, *5*(12), 160. https://doi.org/10.3390/children5120160
31. Baxter, P., & Jack S. (2008). Qualitative Case Study Methodology: Study Design and Implementation for Novice Researchers. The Qualitative Report, 13(4), 544-559.
32. Yin, R. K. (2003). Case study research: Design and methods (3rd ed.). Thousand Oaks, CA: Sage.
33. Stake, R. E. (1995). The art of case study research. Thousand Oaks, CA: Sage.

34. Careers, B. (2022, August 12). Ethical Guidance for Doctors. BMJ Careers. https://www.bmj.com/careers/article/ethical-guidance-for-doctors#:~:text=The%204%20Pillars%20of%20Medical,or%20during%20your%20medical%20education.
35. Alhusen JL, Bower KM, Epstein E, Sharps P. Racial Discrimination and Adverse Birth Outcomes: An Integrative Review. J Midwifery Womens Health. 2016 Nov;61(6):707-720. doi: 10.1111/jmwh.12490. Epub 2016 Oct 13. PMID: 27737504; PMCID: PMC5206968.
36. Vedam, S., Stoll, K., Taiwo, T.K. et al. The Giving Voice to Mothers study: inequity and mistreatment during pregnancy and childbirth in the United States. Reprod Health 16, 77 (2019). https://doi.org/10.1186/s12978-019-0729-2
37. Ramachandrappa A, Jain L. Elective cesarean section: its impact on neonatal respiratory outcome. Clin Perinatol. 2008 Jun;35(2):373-93, vii. doi: 10.1016/j.clp.2008.03.006. PMID: 18456075; PMCID: PMC2453515.