Vaginal Seeding in Cesarean Born Infants: An Integrative Review

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Introduction

Thirty-two percent of all births in the United States were via cesarean according to data from 2023 (Fast Stats Births Method of Delivery 2023). This percentage has grown significantly since the 1990s, as medical advancements make outcomes more favorable for both moms and babies who undergo the invasive procedure. However, as the rate of cesareans increases, medical professionals have begun to question the long-term effects that this type of birth has on babies' overall wellbeing. According to the American Academy of Pediatrics, babies born by cesarean have higher rates of asthma, systemic connective tissue disorders, juvenile arthritis, inflammatory bowel disease, immune deficiencies, and leukemia. (Sevelsted et al., 2015). Until recently, the question of why that is had remained unanswered.

One study has shown that a potential problem with cesarean deliveries is that babies born this way do not come into contact with the mother's natural vaginal bacteria, which is responsible for kick starting the baby's own healthy microbiome. In a peer reviewed article published by the United States Department of Health and Human Services, a link was drawn between maternal and infant microbiomes.

> Regardless of intrauterine exposure, massive bacterial colonization of the newborn occurs at birth upon exposure to vaginal, fecal, and skin microbiota. During vaginal delivery, facultative anaerobic species such as Escherichia coli, Staphylococcus, and Streptococcus colonize the infant gut and produce anaerobic environs in the first few days of life that allow strict anaerobes like Bacteroides and Bifidobacterium spp. to thrive (Mueller et al., 2015).

The bacteria that make up our natural microbiome largely outnumber even our own cells and play a large hand in our overall wellbeing as adults. Impairing the founding of this microbiome puts babies at risk not only immediately postpartum, but past their childhood and into adulthood, as

evidenced by the American Association of Pediatrics (Sevelsted et al., 2015). While birth by cesarean may be a necessary and lifesaving intervention, these long-term complications has led to the question, "how can we mitigate the risk for babies long term health following cesarean birth?" One proposal that seeks to answer this question is that of vaginal seeding. Vaginal seeding is a procedure in which a piece of gauze is placed in the mother's vagina before a planned cesarean section, and then once the baby is born that gauze is removed and swabbed over the baby's face, mouth, eyes, and body, transferring natural bacteria from mother to baby and simulating the environment produced by a vaginal birth (*Vaginal Seeding*, 2024). There are pros and cons for this practice, and we will explore the literature on both sides of this proposal in the paper to follow.

Background

The notion of vaginal seeding is a relatively new one in the world of obstetric medicine. The practice was first explored in a 2016 pilot study by Dominguez-Bello et al titled "Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer". This study observed babies born via cesarean in their first month of life following a vaginal seeding procedure. While their study was unconclusive for the long-term effects of this procedure, they were able to determine that "by exposing the infant to the maternal vaginal microbiota, the bacterial communities of newborns delivered by cesarean can be partially restored to resemble that of vaginally delivered babies" (Dominguez-Bello et al., 2016). However, their study was with a small cohort of mothers and babies over a very short period of time, and the procedure is not without its risks. At the time of writing this paper, the children who were subjected to vaginal seeding in the very first exploratory pilot study are only 8 years old, so we have no idea what kind of effects, either positive or negative, of this procedure extend into adulthood. The American College of Gynecology does not recommend vaginal seeding due to a lack of research on long term effects. They issued the following statement in 2022.

The American College of Obstetricians and Gynecologists does not recommend or

> encourage vaginal seeding outside of the context of an institutional review board-approved research protocol, and it is recommended that vaginal seeding otherwise not be performed until adequate data regarding the safety and benefit of the process become available. (*Vaginal seeding* 2022)

Purpose of the study

This particular study was conducted alongside my mentor, Dr Rebecca Bagley, CNM, is an integrative review of the literature both in support of and against the practice of vaginal seeding. Our goal was to intensively review the existing studies that have been done, from the original pilot study in 2016 to the most current research, and to provide a comprehensive exploration of the risks and benefits of this procedure. Our approach is intended to condense the facts and data from a multitude of sources and create a document that can be used to look at both sides of the issue.

Research question(s) or hypotheses

There are several questions guiding our research. I used the PICO format to write our guiding questions. The first and foremost question we hope to answer is "In infants born via cesarean section, is vaginal seeding a safe and effective way to establish a microbiome similar to that of vaginally delivered babies?" Another question we aim to tackle is "In infants born via cesarean section, does vaginal seeding reduce the incidence of long term autoimmune and metabolic complications?" These questions will help us to focus our research and hopefully come to a more concrete conclusion at the end of our process.

Methodology

For this particular project we conducted an integrative review. We used the East Carolina University library's online database, ProQuest, as well as PubMed, to gather our literature. I developed a list of key search terms, and applied those terms to our selected databases. Key search terms included vaginal seeding, cesarean and newborn microbiome. These keywords lead to 168 scholarly articles to

be reviewed between the three databases. After going through the inclusion and exclusion process, 8 articles passed and were included in the analysis. I then sorted, organized, and reviewed all final sources in their entirety. This process took up the majority of my time working on the project, as there was a substantial amount of research to get through. I then extracted the relevant data and wrote up a thematic analysis of the findings to summarize and condense the existing literature's findings.

Findings

After searching using our list of key words, 110 relevant articles were found on PubMed, and fifty--eight on ProQuest. Of these one hundred sixty-eight total articles, eight key research studies were identified that would be the most useful to our research. Five of these were randomized controlled trials, one was an observational longitudinal study, one retrospective study and one was a systematic review. There was a mix of conclusions, with some studies showing little evidence of benefit, and others showing that babies who underwent vaginal seeding more closely resembled vaginally delivered babies in skin and fecal bacterial content. There remains a need for further research later on in life, as all of the studies I read only followed up for the first two years of life at maximum.

The first relevant study found during the search of the literature was the pilot study that began the larger-scale debate on the potential benefits of vaginal seeding. This randomized controlled trial consisted of 171 pregnant women who agreed to be a part of the study. Out of the 171, 124 were excluded due to testing positive for infectious pathogens, being administered antibiotics, delivering preterm, experiencing intrapartum fever, or from the cesarean group if they experienced spontaneous rupture of membranes, or emergency cesarean delivery. Infants were excluded who had congenital abnormalities at birth, or APGAR scores less than 7 at 5 minutes. After exclusions were made, 12 infants received the vaginal seeding procedure, 13 received a placebo, and 22 were born vaginally and used as a control group. There was no observed difference in the microbial composition of infant stool samples between the vaginally seeded and placebo groups at one month (primary outcome, $p = 0.90$)

or three months of age (p = 0.18) (Wilson et al., 2021). Although this study consisted of a relatively short period of time, it did not show significant differences between the vaginally seeded and placebo group.

In contrast to the previous results, a double-blind randomized placebo controlled trial conducted in Virginia in 2023 by Mueller et al did find measurable differences between control and vaginally seeded groups. This study was the first double blind placebo study to be done on the topic of vaginal seeding, and it offers some compelling results. Twenty pairs of mothers and infants were randomized to either receive vaginal seeding or the placebo procedure using sterile water on gauze. 10 infants received the vaginal seeding and 10 received the placebo. Samples of both groups were then taken at two to three days of life, and again at 30 days of life. Their results were as follows.

> Vaginal seeding significantly increased bacterial load in the skin (forearm; average, 32 copies/μL versus 14 copies/μL, in seeded and control babies, respectively; P = 0.033) but not in the transitional stool (P = 0.21) or day 30 stool (P = 0.32). Compared with the control, vaginal seeding caused a significant reduction in alpha diversity (Shannon index) in the skin at day 1and transitional stool of the neonates (Fig. 2C) (P = 0.047 for skin; P = 0.02 for stool), an effect that persisted in the infant stool microbiota by day 30 (Fig. 2C) (P = 0.01). (Mueller et al., 2023)

The results of this study provide some insight into the possible effectiveness of the vaginal seeding procedure in terms of infant microbiome, but lack long term follow up on the infant's wellbeing beyond the first days of life. This lead me to seek out longer term studies that focus on infant health beyond the newborn stage.

A similar study was performed in Greece in 2023 by Anthuoulaki et al. The goal of this study, like the one published by Mueller et al, was to compare infant microbiomes from different delivery methods, vaginally, via cesarean alone, and cesarean with vaginal seeding. Infants were split into three groups. Group

A included 110 neonates born by cesarean who underwent vaginal seeding. Group B included 85 neonates born by cesarean who did not undergo vaginal seeding. Group C included 95 neonates born by vaginal delivery. Mothers in vaginal seeding group A had a sterile gauze inserted in the vagina 20 minutes before their cesarean delivery, and the gauze applied to the neonate's face, mouth and skin. After 5 days neonatal feces were tested for microbial composition, and the groups were compared to each other. Their results were as follows.

> Vaginal seeding in neonates born with cesarean resulted in gut microbiome which was similar to the gut microbiome of neonates born by vaginal delivery (including lactobacillus species and Bacteroides). On the contrary, gut microbiome of neonates born by cesarean without vaginal seeding was "limited". In terms of nasal microbes, neonates delivered vaginally have statistically significant more often E. Coli (P=0,037) & Bifidobacterium (P=0,015) than that deliver with cesarean. (Anthuoulaki et al, 2023)

The results of this study further supported the results of the previous study conducted by Mueller et al, that vaginally seeded infants more closely resembled vaginally delivered infants than those who did not receive the procedure. However, the same drawback applies to this study with the lack of follow-up. Samples from these groups were only taken at 5 days of life, and no other time. At this point it is still unknown if these positive outcomes would continue later on in childhood, and if the bacterial profile of vaginally seeded infants would result in improvements of overall health.

One of the most detailed studies was performed in 2021 by Song et. al, across multiple countries. During this study, 177

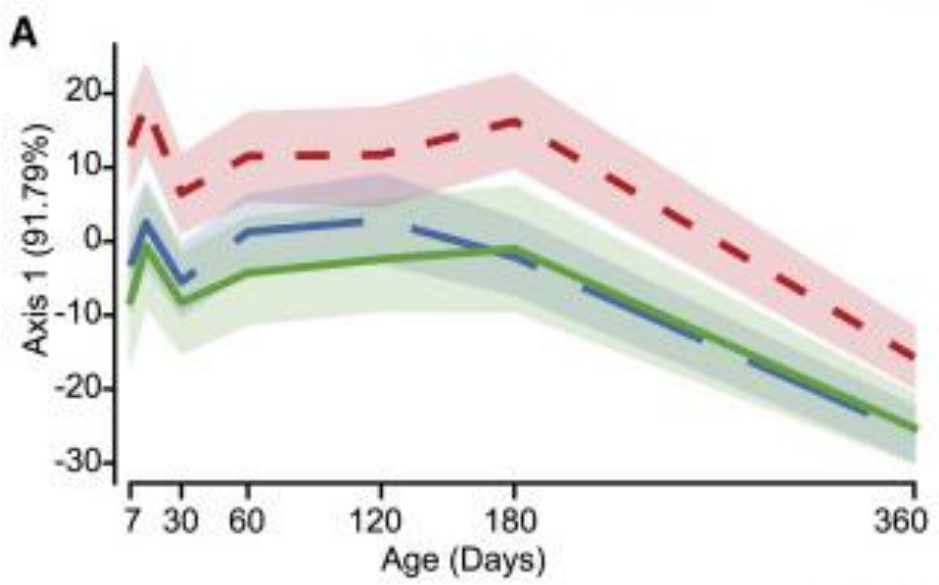

Figure 1: Skin microbiota development during the first year of life (Song et all 2021)

infants born in the United States, Chile, Bolivia, and Spain were observed. After inclusion criteria and screening, 79 infants were included who were delivered via cesarean. Thirty of those 79 underwent the vaginal seeding procedure, and 98 were delivered vaginally. Stool, mouth, and skin swabs were taken of all three groups over their first year of life, and it was found that the vaginally seeded group had a developmental trajectory that more closely resembled that of vaginally born infants, particularly in fecal samples. Their results were as follows,

> A stepwise redundancy analysis based on the first three principal components of compositional tensor factorization ordination—confirmed that birth mode significantly contributed to differences in microbial community structures in the gut and on skin but not in the mouth, with effect sizes of 0.17 (R^2) in fecal samples and 0.09 in skin samples. (Song et al., 2021)

A line graph was provided to visualize the bacterial data collected. (See above). The red line represents the density of bacteria in infants born via ceserean who did not undergo the vaginal seeding procedure. The blue line represents vaginally delivered infants. The green line represents the vaginally seeded infants. As you can see, from seven to 360 days of life, the vaginally seeded group and vaginally delivered group are much closer than the vaginally seeded group and the control cesarean birth group. This trajectory supports the theory that vaginal seeding results in an infant microbiome that closely resembles that of a vaginally delivered infant.

Another study conducted at Liuyang Maternal and Child Health Care Hospital in Hunan, China by Liu et al (2022), offered an opposing conclusion. The study included 120 singleton, term women who delivered via cesarean. Of these 120, 60 infants received the vaginal seeding procedure, and 60 received standard, routine care. The focus of the research was on obesity and allergies developed in the first two years of life. This study's longer term follow-up allowed me to gain insight to the effects of vaginal seeding on childhood development. Body mass index scores as well as body mass index z-scores were observed at 6, 12, 18, and 24 months of age. The other primary outcome was of allergy risk scores (scored from 0-6) at 18 and 20 months of age for 20

common childhood allergens. Their results were as follows.

> At 6 months: the mean standard deviation body mass index was 17.5 (1.4) kg/m^2 and 17.8 (1.8) kg/m^2 in the vaginal seeding and control groups, respectively (mean difference, −0.31 kg/m^2 [95% confidence interval, −0.91 to 0.28]; *P*=.30). The body mass index z-score was 0.2 (1.0) and 0.4 (1.1), respectively (mean difference, −0.20 [95% confidence interval, −0.58 to 0.18]; *P*=.31). The median total allergy risk score was 1.5 (interquartile range, 0.0–4.0) in the vaginal seeding group and 2.0 (interquartile range, 1.0–3.0) in the control group (median difference, 0.00 [95% confidence interval, −1.00 to 1.00]; *P*=.48). For infants from the vaginal seeding group, the relative abundance of genera *Lactobacillus* and *Bacteroides* in the gut microbiota was slightly yet non significantly elevated at birth and 6 months, and the risk of overweight/obesity was lower at 6 months (0/57 vs 6/59; relative risk, 0.03 [95% confidence interval, 0.00–0.57]; *P*=.03) though not at subsequent time points. Other secondary outcomes did not differ between groups. (Liu et al., 2022)

These findings suggested that vaginal seeding did not have significant impact on body mass index or allergy risk, at least in the first two years of life. However, further follow-ups are needed on larger groups over longer periods.

Another study published in Cell Host and Microbe by Zhou et al (2023), explored the neurological effects of vaginal seeding. While most studies examine metabolic and immune responses to infant microbiota, this study focuses on improvements in neurological function, which is a perspective I have not seen in any other literature. A triple blind randomized controlled trial was performed, in which 68 pregnant women participated. Two gauzes were prepared for each woman, one with vaginal fluid and the other with sterile saline. Half of the cesarean group was swabbed with the gauze impregnated with vaginal fluid, and the other half with the sterile saline gauze. The group swabbed with sterile saline gauze was used as a negative control to compare to the vaginally seeded infants. Thirty-three pregnant women who experienced a vaginal delivery also

participated, to see how similar their microbiomes were to the vaginally seeded group. Fecal samples were collected from the infants and studied at 3, 7, 30, and 42 days after birth to determine bacterial content. The results of those fecal samples were as follows:

> A higher proportion of the mature microbiota (cluster 1) was observed in the vaginal microbial transfer group compared with the control group ($p = 0.044$, Figure 3D). Moreover, we found that the vaginal delivery group had a more mature microbiota than the cesarean birth group, corresponding to previous reports that cesarean birth could stunt gut microbiota maturation. We then wondered whether the gut microbiota in the vaginal microbial transfer group was more similar to that in the vaginal delivery group than the Con group was (Zhou et. al., 2023).

Groups of infants were compared on days three, seven, 30, and 42, and on all of these days the vaginally seeded group more closely resembled the vaginally delivered group than the control cesarean group. This similarity supports the hypothesis that vaginal seeding can perhaps partially recreate the microbiome which naturally occurs in vaginally delivered infants (Zhou et. al., 2023).

The connection between gut microbiome and neurological development is a concept that is also still being explored. Zhou et al, (2023) examined the gut's impact on the brain with the use of vaginal seeding and found certain metabolic pathways to be closely related to proper neurologic function. The connection between these metabolic pathways is a hot topic of research in the field of neurological medicine. A study by Zhou et al (2023) explored the neonatal gut's impact on the brain, and the outcome of vaginal seeding. They found that vaginal seeding has the potential to improve neurological function in infants. The establishment of a microbiome similar to that of a vaginally delivered infant can regulate essential metabolic pathways formed in the gut, which have been shown to be correlated with improvement of neurological function. One key metabolite examined in the study is phenylglycine, which plays a role in diseases where N-methyl-d-aspartate

receptor activity is reduced. The most notable of these diseases, is Alzheimer's. The exact means by which these neural receptor pathways are affected by gut metabolites is still unknown, however, the fact that vaginal seeding contributes to healthy gut development suggests that perhaps neurological outcomes much later in life could be improved by the procedure. (Zhou et. al., 2023)

It will be exciting to see in coming years if the impact of infant gut can be correlated to neurological development. Further studies in this realm are certainly needed, and the results of this study, while optimistic, do not confirm whether or not vaginal seeding can have a positive impact on infant health.

The final piece of literature examined is a systematic review conducted by Inchingolo in January of this year (2024). This review analyzed the results of 12 randomized controlled trials, with a common theme of the effects of birth mode on the neonatal microbiome. This made it possible to effectively review a large amount of data so that observations of trends could be made. What was found varied greatly from study to study. While the general consensus is that birth mode plays a large role in microbiome development, the best and safest interventions are still a topic of debate. The results of the review remained largely inconclusive. While some studies did not find significant changes in the microbiome of vaginally seeded infants, others did. None of the studies examined in the review were able to say definitively whether or not the intervention was effective. Additionally, concerns about risk for infection was discussed. There is a potential for adverse effects if an infant is exposed to harmful pathogens contained in the matern~~ner~~al vaginal fluid, such as Group B strep, and sexually transmitted infections. In the conclusion of the review, alternative strategies for supporting neonatal microbiome development, for example, administering probiotics and delaying administration of routine prophylactic antibiotics, may offer safer options (Inchingolo, 2024). This systematic review also facilitated exploration into alternatives to vaginal seeding that have been proposed, such as encouraging breastfeeding and administering probiotic and symbiotic supplements.

Discussion

Upon reviewing the literature, it appears the effectiveness of vaginal seeding is still undetermined.

While four~~4~~ studies showed a measurable difference in microbiome as a result of vaginal seeding, only ~~1~~one showed the potential for any difference in long term outcome. There has also yet to be a large enough study to determine in absolutes the safety and effectiveness of the procedure. Many medical professionals have expressed concern for possible disease transmission through the procedure, but it should be noted that in reviewing the literature adverse outcomes as a result of vaginal seeding were not observed, due to the extensive maternal testing that occurred before birth.

Since the first pilot studies on vaginal seeding are only eight years old, even the oldest children who have undergone the vaginal seeding procedure have yet to enter adolescence. Follow up on children who participated in these studies as they enter adulthood could be extremely beneficial in determining if there are any lifelong effects worth investing further research studies into. This follow up could result in significant changes in the standard of care provided to infants born via cesarean if positive impact was found.

Closure

Cesarean birth may be a lifesaving intervention for both mothers and babies around the world. As the procedure becomes more and more commonplace in modern medicine, we are discovering that the long-term complications that result from cesarean birth are more extensive than previously estimated. As we learn more about our body's natural microbiome, and how it affects our everyday health and functioning, it is becoming evident that intervention is necessary. Arroya et al (2018) found that 195 children die from asthma each year. The prevalence of food allergies in children under 18 years of age is 7.6%, and 42.3% of those children have experienced a severe reaction (Allergy statistics in the United States, 2024). Though the incidence of autoimmune disorders in the United States is not widely known, according to John Hopkins Department of Pathology, it is estimated that all autoimmune diseases combined affect about three percent of the United States population**,** which amounts to approximately ten million people" (Prevalence of autoimmune diseases 2024). Since connections have been drawn between rates of developing these disorders and the health of the human microbiome during infancy, it leads us to wonder how an intervention such as vaginal seeding might affect the rate

of children developing disorders such as asthma, allergies, and autoimmune conditions. Health begins with birth, and as research continues to surface, we come closer to bettering the health of humanity as a whole.

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