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Microbiome and Fetus: A Relationship for Life

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Abstract: The impact of the microbiome has become relevant to the world of pre- and perinatal psychology and medicine. The microbiome is defined as the microbes which inhabit our body. Many scientists consider the microbiome to be a "super organ," which plays a much greater than expected role in our health. The startling new realization over the last decade or so is that the acquisition of microbiome begins *in utero*. Since the 1900's, it has been presumed that the fetus is sterile and the first acquisition of microbes occurs as the infant is delivered. Over the last decade, research has provided strong evidence that the fetus is not sterile in the womb, and bacteria are "transmitted" from mother to fetus primarily through the placenta. This paradigm shift—that the womb is not sterile—opens a new frontier in exploring our complex relationship with microbes. Given the research suggesting significant effects of microbiome on infants, children, and adults, what might we find is the effect of those microbes on the developing human fetus? This review article looks at some of the areas of study in this vast topic and proposes additional questions for further research.

Keywords: microbiome, fetal microbiome, dysbiosis, symbiosis, maternal transmission, fetal immunity

The microbiome has finally come of age. The newest revelations show that the microbes we carry in our gut affect our behavior and well-being in unexpected ways. The gut microbiome has been associated with psychiatric, gastrointestinal (GI), autoimmune, allergy, and cardiovascular disorders, to name a few. The research on the microbiome grows exponentially in multiple arenas—medicine, neuroscience, and psychiatry, as well as microbiology, neuroimmunology, and genetics. The microbiome will prove to be a vast frontier of new understandings about our health, behavior, and development. Recently, the microbiome has been proven to be an important component of the prenatal environment, and therefore should be of interest to those in the field of pre- and perinatal psychology. The documentary film, *Microbirth*, has popularized the importance of infant's early microbiome, and starts with childbirth. The premise of the

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movie is that the fetus grows in a sterile environment and receives its first "inoculum" of bacteria as it traverses the birth canal, or skin in Caesarean section delivery. The main point of the movie is that the microbes from the vagina should be the first source of microbes for the baby, and that aberrations in gut microbial development occur following Caesarean section births. The producers suggest that indeed the high prevalence of Caesarean deliveries may be the specific cause of dysbiosis, and the disorders in our society which are related to it. The majority of the data on which the conclusions are based come from the work of Dr. Dominguez-Bello and colleagues (2010) who appear frequently in the documentary. It would not be my intention to argue against vaginal delivery, however, I suspect acquisition of the microbes is not one of the major reasons to support it. Considering the covering of the newborn with vernix caseosa, a lipid-and-protein-rich insulating and antimicrobial layer designed to protect the infant against microbial invasion, I would guess that microbe acquisition is not primary in nature's intent for the delivery process. Most importantly in this paper, we will reconsider the fundamental notion that the "womb is sterile." The evidence available suggests the opposite, that the womb has a plethora of important beneficial bacteria actually transmitted to fetus by mother for very important developmental reasons.

Re-examining our relationship with bacteria and consideration of the fetus and microbiome relationship will be the main topic of this paper. There has been an explosion of research in this area in the last ten years and many of the papers cited here will be from 2011 through 2015. The full depth of the vast research in this area, in multiple subspecialties, is beyond the scope of this paper. But I am hoping to introduce this fascinating and important "paradigm shift" of the non-sterile womb, and to suggest how we may further investigate the microbiome in fetal development.

The Sterile Womb Hypothesis

The sterile womb hypothesis originated in the 1900s with a French pediatrician named Henri Tissier. It has been stated that he "declared" or "asserted" that the fetus grew in a sterile environment until it traversed the birth canal. His ability to know the fetus was sterile was limited. Bacteria were discovered with a single lens microscope by Leeuwenhoek in 1674 but the germ theory of disease was not formulated until the 1870's. In the early 1900's Paul Ehrlich made the connection between bacteria, disease, and the immune system defense. Ehrlich also began one of the first searches for targeted antimicrobial therapy against syphilis. Targeted antimicrobial therapy, against specific organisms, is of course

prevalent today, as is hand washing and "sterile procedures" in hospitals. Though, in the 1900's, Dr. Eli Metchnikoff noted the longevity and health of peasants who ate yogurt frequently, and thus made a connection between bacteria and health, most of our modern conception of bacteria relates to disease.

In the fields of obstetrics and perinatology, bacteria and inflammation have been aligned with adverse conditions such as premature delivery, pre-eclampsia, and maternal/fetal infection.

Our general perception of bacteria is that they are "bad" for us. We have spent an inordinate amount of time and resources trying to be clean. Recently, we are using cleaning agents, antibacterial soaps, and other products which may expose us to adverse toxins. We deprive our children of outdoor play and loathe the "dirty hand" to such a degree that some worry this lack of proper exposure to antigens has created the vast number of allergies we see in children today.

But what if bacteria were found in non-inflammatory conditions and seemed to be good for us? This is the current basis for the paradigm shift regarding our microbiome; that some bacteria are not only good for us, but necessary for us. Furthermore, that they originate in the fetus in the womb.

In experiments as early as 1978, bacteria were injected into the mother's bloodstream and subsequently found in the fetus and fetal tissues prior to birth (Coid, Sandison, Slavin, & Altman, 1978). In fact, some bacteria seemed to preferentially infect the placenta/fetus over other maternal organs. Many scientists began to question the sterile womb hypothesis (Wasseenaar & Panigrahi, 2014). Important public research comes from studies of the placenta by the NIH and, specifically, the work of Dr. Aagaard (2014; Ma, et al., 2014). Dr. Aagaard cultured the placenta using sterile techniques and found a host of colonizing microbes. To clarify, the difference between "colonizing" and "infecting" microbes is the inflammatory reaction that they create: infecting microbes are the cause of inflammation and disease states; colonizing microbes seem to live in cooperation with the host cells in a non-inflamed environment. The colonizing placental microbes Dr. Aagaard found actually benefited the host. They were predominantly microbes that were involved with metabolism (digestion) of substances, and often produced useful proteins and vitamins as the by-products. In other words, these colonizing bacteria were helpful and beneficial to the host organism. These beneficial bacteria are called "symbiotic" bacteria, and having such bacteria as the predominant types in one's microbiome is called symbiosis.

The "sterile womb hypothesis" continues to be challenged as more researchers have studied the placenta (Stout et al., 2013; Guttmacker,

Maddox, & Spong, 2014)**,** the surrounding fetal tissues of the chorion and amnion, the cord blood (Jimenez et al., 2005), and ultimately the fetal gut itself as evidenced by fetal meconium.

Sophisticated measurement techniques have found that, when the meconium is tested within two hours of birth, before feeding, and before an effect could be obtained by delivery method, the newborn already has a substantial colonization of microbes in the gut (Jimenez et al., 2008). Joseph Neu (Mshvildadze et al., 2010) cultured the microbes in the meconium and found a wide population of microbes in the meconium of both full-term and preterm infants regardless of delivery method. Quite extensive categorization of the fetal microbiome at various gestational ages shows that it continues to advance during fetal maturation (Qin et al., 2010). Surgical studies of the fetal intestine immediately following delivery, not only demonstrated microbial populations in the gut, but provided the additional evidence that there was already some of the adult-pattern localization of gut microbes even as they are acquired *in utero* (Romano-Keeler et al., 2014).

As genetic methods of testing become more sophisticated, a vast and diverse population of microbes in fetal tissues prior to delivery has been revealed. The contribution of this symbiotic population of microbes is yet to be fully understood, but it is clear microbes play an important role in immune system development while preparing the fetus for independent life after birth. An extensive review article on the state of the current knowledge is available in an article by Romano-Keeler and Weitkamp (2015).

The accumulating data clearly stood in the face of the "sterile womb hypothesis" (Collins, 2014; Kaiser, 2014; Grens, 2014). The newness and contrary nature of the current findings against the old sterile womb dogma has meant some have remained skeptical. Even in articles written in the last several years, authors may state in the introduction that the fetus is sterile in the womb as a foundational belief, but do not cite supporting evidence. Interestingly, perhaps no part of the body is truly sterile; not urine, not the brain, not anything. There is evidence of microbes or their antigens everywhere. However, the exploration of the character, source, purpose, and full consequence of the microbiome is just beginning, and includes the important question of "What is the inter-relationship of the microbiome and the fetus in life?

The field of pre- and perinatal psychology knows that the fetus is "conscious" and, rather than being protected, is actually exposed to and reacts to the emotional, experiential, and chemical environment of the womb. As we will discover, the maternal microbiome should also be considered an aspect of this environment to which the fetus is exposed.

Characterizing the Microbiome

The microbiome is considered by some to be a "super organ." The vast majority of microbes live in the gut and number approximately 100 trillion. One of the complexities of microbiome analysis is the vast number of microbes involved. In the body, the numbers of bacteria are ten times greater than the number of tissue cells. The bacteria are very small compared to tissue cells, so, although they vastly outnumber our cells, bacteria *only* contribute two to five pounds of our body weight. However, the bacterial DNA in our body is 30 times greater than our cellular DNA (about 25,000 genes to 750,000 genes). So these cells outnumber our tissue cells in both number and genetic "power."

Despite general similarities in phyla and species of bacteria among humans, each individual person has a unique microbial "signature" with different proportions of microbes. Bacteria are classified according to differences in structure, function, and capability into phyla, class, order, family, genus, species, and strain. There are between 23 to 59 phyla, depending on classification, but each phyla contains *many* subclassifications. Only a few phyla are represented in the human microbiome, but there are about 500 to 1200 different species represented. For example, in the human intestine, most symbiotic bacteria are from the Bacteriodetes and Firmicutes phyla, but between 400 and 1000 different species may be represented. Additionally, there are many strains within each species; so, it is the strain which provides the more unique identification of a bacteria. When studies are done to show direct transference of microbes from mother to fetus, the identical strain of the bacteria is what will prove that it is the same bacteria. Jimenez et al. (2008) used genetically labelled gut bacteria to show that there was direct transference of a specific Enterococcus from mother, who ingested it orally, to fetal meconium. These are difficult experiments to accomplish and few studies have gone to this level of specificity. This complexity of bacterial nomenclature makes interpreting comparisons of microbial content in the literature difficult; it is not always clear exactly which bacteria are being identified.

To make matters even more complicated, there are several accepted methods for characterizing members of microbial populations. The methods used currently employ genetic techniques to identify bacteria by segments of their genomes, rather than culture techniques. The gold standard is the Sanger method with amplification of the V4 region of the 16s rRNA subunit; this has been the most complete and least inaccurate technique. It is important to realize that, not only do researchers use

variations of this method, all these methods contain inherent measurement errors due to the extraction, amplification, and other procedures which are necessary components of the whole process.

A new technology, called Metagenomic (or "shotgun") sequencing, is possibly more consistent, complete, and accurate in its findings; it is able to assay a wide variety of the microbial genes present in a microbiome without introducing error from amplification or extraction, as in the Sanger method. It also gives a profile of function of the microbes, because of the genes identified. However, there are still other potential inaccuracies of characterization of the microbes in a sample because of variations of the depth to which a sample is analyzed. Metagenomic sequencing is also very expensive, but its use seems to be increasing. In time, an optimum method will hopefully be promoted and become more universally available, affordable, and standardized, so that more consistent data will emerge across research platforms.

The Fetal Source of Microbes: Maternal Transmission

Once the fetus was identified as containing microbes, the search for the maternal source of that transmission was underway. As to the question of a vaginal or skin source of the fetal microbiome, Dong et al. (2015) investigated the microbial contents of initial meconium and placenta in the delivery room in two infants, one delivered by Caesarean and one vaginally. He found that both infants had similar bacterial communities, and that they were both most similar to what was found in their placentas rather than the vagina or skin.

Micrococcineae was most abundant in the meconium and also prominent in the placentas; the vaginas were dominated by *Lactobacillus*. Also, both infants had more diversity of gut bacteria than was present in the vagina. Therefore, he concluded the microbes originated from another source and the placenta seemed to be a primary source.

Ongoing research has continued to support the normal placenta and fetal tissues as having a microbiome prior to delivery. Due to the anatomy of the placenta, and the proximity and intermingling of fetal and maternal blood, it is apparent that bacteria in the bloodstream could easily reach the placenta and thereby the fetus. Where did the placental microbes originate from?

The NIH Human Microbiome project (HMP) has begun to characterize the microbes of our bodies, including those living in our nasal passages, oral cavities, skin, GI tract, and urogenital tract. Dr. K. Aagaard, the 2007 recipient of a NIH Director's New Innovator Award, and associate professor of obstetrics and gynecology at Baylor and Texas Children's

Hospital, found that, when compared to microbes elsewhere in mother's body, the placental microbes most closely resembled the microbes in mother's mouth and, in fact, not the skin or the vaginal microbes as had been postulated by others (Aagaard, 2014)

Ongoing research has continued to support the normal placenta and fetal tissues as having a microbiome prior to delivery and the microbes as originating from mother's enteral tract (mouth and gut). Jimenez and colleagues (2008) performed a study in mice using specific genetically labelled microbes. They obtained an Enterococcus strain from the pregnant mouse intestine, genetically labelled the Enterococcus so that it would be easily tracked, and orally fed it again to the pregnant mouse. After delivery, the researchers were able to retrieve the same genetically labelled Enterococcus from the meconium of the newborn mouse offspring, even following Caesarean delivery. There was no contamination in the newborn mice of control animals who had not been fed the bacteria. This study concluded that there was transference of microbes from the mother to the baby prior to delivery, and the mouth was suspected to be the source.

Due to the anatomy of the placenta, and its close inter-relationship with fetal tissues, it is apparent that bacteria in the bloodstream could easily reach the placenta and thereby the fetus. But how do bacteria enter the bloodstream from the mouth and gut? Aside from the breeches on integrity of the oral mucosa due to poor dental hygiene, is there an inherent mechanism of transport of bacteria and/or bacterial antigens to the fetus via the bloodstream? Both the oral and gut source theories propose that dendritic cells (in addition to goblet and M cells) are the initiators of transport of bacteria (and/or bacterial antigens) to the womb. These dendritic cells are very prevalent in the mouth and intestine and appear to be specifically designed to usher the microbes in mouth and gut through the surface mucosa and directly into the lympathic system where they are transported by bloodstream to the placenta (Fardini, Chung, Dumm, Joshi, & Han, 2010; Rescigno, Rotta, Valzasina, & Ricciardi-Castagnoli, 2001). To further support that somehow this transmission of bacteria is intentional, it has been noted that immune cells are more likely to harbor bacteria during pregnancy than at other times of life (Funkhouser & Bordenstein, 2013; Perez et al., 2007). Mother is believed to be providing her baby *in utero* with the microbial basis to live independently in the environment which mother currently finds herself in.

Are the dendritic cells selective about which microbes that they acquire and transport, or are the microbes picked up randomly? The answer to this important question is not yet known; however, the placenta has been found to have an abundance of beneficial bacteria which supply

vital nutrients and vitamins for the growing fetus. The beneficial bacteria seem to have an important purpose that supports fetal development and therefore, their occurrence is not likely accidental (Guttmacker, Maddox, & Spong, 2014; Ma et al., 2014; Stout et al., 2013).

Maternal microbial transmission to the offspring has been found to be a universal phenomenon in the animal kingdom and recently is thought to play a critical role in evolutionary development (Funkhouser & Bordenstein, 2013). It appears that all animals studied—mammals, amphibians, fish, sponges, birds, insects, and even some invertebrates in the hydrothermal springs deep in the ocean floor—have a mechanism for bacterial transmission from mother to progeny. There are a variety of methods by which this occurs, but it is recognized that in each case, most of the bacteria are beneficial to the offspring and often perform some vital task for the newborn.

Why would it be advantageous for mothers to transmit bacteria to her baby in the womb?

The Purpose of Symbiotic Microbiome

The microbiome has been likened to a "super organ" of the body, its own functions integrated with needs of the host. The microbiome is believed to have complex communication capabilities with the host and with members of its own group. In many ways the communication found among bacteria is similar to neural connections in the brain; specifically, ion channels open and close to send "signals" to other bacteria. In some organized bacterial colonies, or biofilms, ion channels open and close in coherent patterns. In the example of tartar, which is a bacterial biofilm in the mouth, the pattern of opening and closing ion channels allows all the bacteria in the colony—rather than just those at the periphery—access to the nutritional source, glutamate, in the mouth. This communication helps to support the existence of the entire group.

We may need to regard bacteria in a much more complex way than we have in the past. These microscopic creatures are actually fully integrated into our physical being, and may play a role in our physical and emotional lives beyond anything imagined. While no one would claim to have all knowledge about our inter-relationship with bacteria, persistent inquiry has led to some remarkable new understandings.

I will digress briefly to introduce the concepts of mutualism commensalism (symbiosis), and parasitism. In mutualism, two organisms live together in ways the benefit both organisms. In commensalism, two organisms live together and one benefits, but the other is neither harmed nor benefited. Both are considered symbiotic relationships. In parasitism,

two organisms live together, but one gets all the benefits at the expense of the other. Many examples of these can be found in nature. For example, the parasitic wasp lays eggs inside the caterpillar. The caterpillar eats excessively and behaves differently to protect itself so that the eggs may hatch and the wasp larvae eat and mature within it. At some point the caterpillar is overwhelmed by the numbers of larvae and dies. At that point the caterpillar is fully consumed by the wasp larvae, who then fly off. These wasp larvae are considered parasitic because they have used the caterpillar for their own purpose of survival, to the detriment of the caterpillar.

An interesting example of mutualism is in the relationship between mitochondria and our tissue cells. Mitochondria are the energy producing organelles in each of our cells. Mitochondria are dependent for their existence on the home provided by the cell and, likewise, the cells depend on the mitochondria for the energy they use to perform their functions. This relationship began millennia ago in a process called endosymbiosis. Mitochondria actually originated as bacteria (*Rickettsia*) which invaded cells early in evolutionary history. Rather than kill the cell, or be killed by it, the bacteria and cell co-adapted to a mutually beneficial relationship. Endosymbiosis is considered a key step in evolution. Without the co-adaptation of cell and mitochondria, complex living organisms may never have had the necessary energy resources to evolve.

Mutualism and commensalism are common themes with beneficial microbes. The host (the gut, for example) provides protection and sustenance (food) for the bacteria, while the bacteria may have a variety of functions. In humans, bacteria are known to be beneficial in multiple ways. These have been discussed in various publications, including Perlmutter's (2015) book *Brain Maker,* which discusses brain health and the adult microbiome. The following key functions have been attributed to symbiotic (beneficial) bacteria in the human intestine:

1. Bacteria metabolize otherwise indigestible foods consumed by the host.

   What is particularly interesting is the example of the oligosaccharides contained in breast milk. For years these seemed to be a useless by product of breast milk production, until it was discovered that the oligosaccharides are a unique food source for beneficial bacteria which live in the infant intestine. The presence of oligosaccharides promotes a healthy microbiome.

In the Hadze hunter-gatherer societies, the people eat a plant diet composed of complex and dense fibrous structures which is only possible to digest because of a unique bacteria living in their intestinal microbiome.

2. Bacteria produce important vitamins and proteins for the host.

   Vitamins K, B12, and a number of other B vitamins, are good examples of metabolites from intestinal microbes which are necessary for humans.

3. Bacteria maintain the integrity of the intestinal mucosal surface.

   Beneficial gut microbes maintain the closure of the tight junctions between mucosal cells of the intestinal wall. When these junctions are not tight, leaky gut occurs, allowing pathogens and proteins to penetrate into the host bloodstream. This is presumed to be a mechanism by which a high number of food allergies occur.

4. Bacteria protect against other pathogens.

   Some bacteria produce toxins which directly kill pathogens. Other bacteria stimulate an immune reaction from the host to defeat pathogenic bacteria. In both cases, beneficial bacteria are an important line of defense against pathogens which might infect host tissues.

5. Bacteria maintain the immune balance of the intestine.

   Beneficial bacteria are instrumental in training and regulating the host immune system. Bacteria both activate and mitigate immune reactions to potential pathogens.

6. Bacteria influence a wide variety of genes in the host.

   Beneficial bacteria directly influence the genetic code on/off switches which regulate production of proteins.

7. Bacteria produce proteins which function as neurotransmitters.

   Beneficial bacteria actually produce more neurotransmitters than the brain itself. These neurotransmitters operate locally in the gut to control motility and secretion of hormones, but also they seem to have an effect on brain function through the gut-brain axis, mediated by the vagal nerve. Psychiatric disorders have been associated with abnormalities in the gut microbiome.

Evidence of all of these bacterial functions begins in fetal life, suggesting bacteria play a critical role in the health and viability of the fetus, as well as in the adult. Bacteria are more complex than previously realized, and they may have a larger impact on our health and behavior than we were previously aware. With respect to fetal development, symbiotic bacteria play a critical role in development of fetal immune

function, regulation of the fetal inflammatory state, and even fetal brain development.

Development of Fetal Immunity

The main function of the immune system is to protect the body by generating an inflammatory response to a pathogen. An inflammatory response is vital to the body, as is a military to a nation. The fetus also is able to mount an inflammatory response *in utero*: what we would refer to as a systemic inflammatory response in postnatal life would be called a fetal inflammatory response in the fetus. The development of immune function starts at about the seventh week of gestation; it is functional, but not fully formed, at the time of birth. The fetal immune system has attack cells and cytokines with which to kill invasive bacteria, but still relies on mother's immunoglobulins until later in infancy when the baby produces more of its own. To function properly, the immune cells must recognize self from other, and they typically attack other but not the self. When and how does the immune system learn this distinction?

Compelling evidence suggests that this process begins *in utero*, and the creation of a functional immune system, which distinguishes self from other, is a critical part of fetus' preparation for life separate from mother (Romano-Keeler & Weitkamp, 2015). The baby needs an immune system to wage an attack on potential pathogens. As the baby is birthed, it will begin to encounter a myriad of microbes, even as it passes through the birth canal. Without some protection and preparation *in utero*, it could be fatal for the baby. The baby needs an immune system to wage an attack on potential pathogens

The other important feature of immune competence is knowing what NOT to attack. This also must be taught. The baby's body must be able to recognize beneficial bacteria and support them as the healthy symbiotic microbiome. The mother not only passes on the beneficial bacteria to the fetus, but somehow also her immune knowledge so that the fetus develops an innate capability to recognize the beneficial bacteria and not attack them. Appropriate stimulation of the fetal immune system *in utero* allows the baby to develop a tolerogenic (appropriately tolerant) immune environment so that the infant will not react to everything with an immune-inflammatory response. This phenomenon in the gut is called oral tolerance whereby some substances are tolerated and not attacked.

In this sense, there are both activating and modulating components in our normal immune function. Manifesting an appropriate immune reaction is part of immune system regulation. Bacterial imprinting is the term given to the process of teaching the fetal immune system about the

beneficial bacteria. The mechanism by which this occurs is not fully understood, but the overwhelming conclusion of scientific research is that beneficial bacteria help tutor the immune system with fetal Treg immune cells as part of this process. This modulating effect of beneficial bacteria may have many lasting benefits to the fetus and infant.

Fetal Inflammatory Response (FIR) and Prematurity

The fetal inflammatory response is an immune response and has as its main feature production and release of cytokines and other inflammatory markers, such as prostaglandins. Cytokines important to the fetus include IL-6, TNF, and IL-B1. These are primarily pro-inflammatory cytokines, although anti-inflammatory cytokines exist as well. Both human fetal membranes and the fetal intestine are active participants in this fetal inflammatory response, but some suspect the fetal intestine as the primary site of origin for the fetal inflammatory response, given its active role immune development during gestation (Ardissone et al., 2014).

The pro-inflammatory cytokines associated with FIR in the fetal tissues contribute to pregnancy complications of abortion, stillbirth, and prematurity. In the last decade, intrauterine inflammation has been identified as a leading cause of preterm delivery. The degree of prematurity often correlated experimentally with increased microbes as well as an abundance of cytokines and inflammatory elements in meconium and amniotic fluid (Ardissone et al., 2014; Flores-Herrera et al., 2012; Raj, Bonney, & Phillippe, 2014; Uchide, Ohyama, Bessho, Takeichi, & Toyoda, 2012; Uchide, Ohyama, Bessho, & Toyoda, 2005; Uchide, Suzuki, Ohyama, Bessho, & Toyoda, 2006). Since the amniotic fluid is swallowed continually by the fetus into the gut, this similarity is not unexpected.

Causes of increased inflammation in the fetal meconium or amniotic fluid need to be explored in depth. Local infection such as chorioamnionitis certainly would lead to inflammation and is associated with prematurity. But what about remote effects of inflammation that mother carries? We know that maternal periodontal disease has been associated with an immune inflammatory response in the feto-placental unit (Madianos, Bobetsis, & Offenbacher, 2013) and is also a risk factor for prematurity. We know that maternal obesity, a highly inflammatory state, is also associated with prematurity, among other complications of pregnancy. Inflammatory bowel disease is another systemic disorder in mothers prone to having premature babies. How do these conditions impact the fetus? Is it through the fetal microbiome and generation of FIR, or direct

impact of the maternal inflammatory markers themselves on the fetus? These questions deserve further study.

Fetal Inflammatory Response and Brain Development: Autism and Cerebral Palsy

Even in the absence of prematurity, the presence of fetal cytokines impacts organ development, including brain development, and may lead to a number of neurodevelopmental disabilities (Elovitz et al., 2011). Maternal chorioamnionitis is correlated with cerebral palsy and autism, as well as a higher risk of preterm delivery. An excessive immune inflammatory response, from either mother or fetus, may be enough to cause injury to the developing fetal brain. Even in the absence of prematurity, the presence of fetal cytokines impacts organ development, including brain development, and may lead to a number of neurodevelopmental disabilities (Elovitz et al., 2011). What is clear in the literature is that an excessive immune inflammatory response, from either mother or fetus, may be enough to cause injury to the developing fetal brain. Could inflammation in the fetus be a cause of many unexplained developmental issues in our children today?

Direct infection of the fetus causing inflammation and abnormal development of the fetal brain has been recognized for decades. Cytomegalovirus, toxoplasmosis, rubella, herpes simplex, syphilis, and HIV (TORCH) have been known to cause identifiable brain disease in the fetus. Microcephaly, intracerebral calcifications, hydrocephalous, and gyral malformations are among the structural abnormalities which lead to life-long disabilities (Adams Waldorf & McAdams, 2013). These brain abnormalities due to direct infection are macroscopic and can be seen on imaging such as head ultrasound and brain scans. But other brain abnormalities are on a "microscopic" level and not visible with current imaging techniques. These invisible abnormalities may have important neuro-behavioral consequences resulting in behaviors such as hyperactivity, attention deficits, learning disabilities, and autism, all of which are typically associated with normal imaging studies. These are areas where fetal inflammation may be having its greatest impact.

In general, there is a strong correlation of early inflammation with a number of prevalent health disorders in our society today, including cerebral palsy, autism, ADHD, autoimmune disorders, and inflammatory bowel disorders. Autism is a particular concern, as its prevalence continues to increase from one in 150 in the year 2000 (CDC data) to one in 68 children in 2014 (CDC data) to a suggested one in 45 children in a 2015 National Health Interview Survey. Large case studies have shown

autism has a high association with maternal inflammation and infection (Abdallah et al., 2012; Goines et al., 2011). Chorioamnionitis has been suggested as an independent risk factor for autism. These studies and others show that in the presence of inflammation in the fetal womb, a higher than expected number of children will have autism as a diagnosis (Atladóttir et al., 2010; Pinelli & Zwaigenbaum, 2008).

Another major concern is cerebral palsy, a significant motor disability which affects about three in 1,000 children. In the past, its occurrence was frequently blamed on obstetricians, assuming that the process of delivery was faulty and led to unnecessary perinatal injury to the fetal brain. This has perpetuated the use of fetal heart monitoring, supine positioning of mothers, and use of Caesarean deliveries in efforts to improve fetal outcomes. But, despite all these changes in obstetric practice, the risk of cerebral palsy has not lessened. Many children with cerebral palsy have had inflammation as the underlying condition which may have led to increase in cell injury and cell death (Rees, Harding, & Walker, 2011; Svigos, 2001; Yoon et al., 1997). Chorioamnionitis has been shown to be an independent risk factor for brain injury causing cerebral palsy (McAdams & Juul, 2012; Wu & Colford, 2000). Also, the presence of inflammatory cytokines worsens the brain outcomes of infants exposed to hypoxic (low oxygen delivery) - ischemic (low blood flow) injury during delivery (Eklind, Mallard, Arvidsson, & Hagberg, 2005; Eklind et al., 2001; Larouche et al., 2005; Wang et al., 2007). Fetal inflammation may be the background condition for the adverse effects on the fetal brain.

What is the experimental evidence to support the correlation between autism, cerebral palsy, and fetal inflammation? The role of cytokines in central nervous system development of the fetus is increasingly appreciated. One of the most significant findings is that cytokines, such as IL-6, TNF-a, and IL-1B, have a disruptive effect on neurogenesis, stem cell maturation and synaptic development in the brain (Burd et al., 2010), and are associated with development of phenotypes in the mouse of the psychiatric disorders, schizophrenic and autism (Hsiao & Patterson 2011; Patterson, 2009). Research conducted with pregnant mice has provided experimental evidence that antigens (lipopolysaccharides) from bacteria are able to trigger immune responses in the fetus. The affected baby mice demonstrated a behavioral phenotype which is characterized by hyperactivity, spatial and memory difficulties. When these babies' brains were autopsied, activated immune cells were found infiltrating the brain, and hippocampal neurons were decreased in quantity. Hippocampal neurons are known to be important in memory and learning, and their diminished numbers correlated with the behavioral phenotype (Burd, Balakrishnan, & Kannan, 2012).

Other experimental research has similarly found that when exposed to intrauterine conditions of inflammation activated by a viral mimetic, neurons have fewer dendritic processes (decreased synaptic connectivity), decreased cortical neurons and poorly organized cortical layers (Atladóttir et al., 2010; Pinelli & Zwaigenbaum, 2008). In a study by Soumiya, Fukumitsu, and Furukawa (2011), it was demonstrated that this deleterious effect on cortical progenitor cells was mediated by an effect on gene expression (Pax6) which plays a role in neuronal maturation and development. Hsiao et al. (2013) injected pregnant mice with polyinosinic:polycytidylic acid (poly I:C), which mimics a viral infection. The offspring of those mice exhibited autism-like behaviors and neuropathology on brain autopsy. In the same model, the maternal immune activation was shown to produce altered intestinal microbial population and a leaky gut in the offspring. Remarkably, treatment of the babies with *Bacteroides fragilis*, an important component of a healthy gut microbiome, corrected the gut permeability (no longer leaky), microbial population in the gut, and the autistic-like behaviors!

There is a large body of data to support the relationship of fetal inflammation and brain injury associated with cerebral palsy. White matter damage to the fetal brain (periventricular leukomalacia), leading to cerebral palsy, occurred after inoculation with bacteria in the presence of bacteria antigens, or in the presence of pro-inflammatory cytokines in amniotic fluid even without bacterial presence (Bashiri, Burstein, & Mazor, 2006; Patrick et al., 2004; Yoon, Park, & Chaiworapongsa, 2003). If we could reduce inflammation that fetuses are exposed to, we may be able to reduce cerebral palsy incidence as well.

What Can We Do?

What we are beginning to recognize is that one of the most critical periods of brain development is during gestation. The capacity of the gut microbiome to influence health and disease, even *in utero*, is believed to be vast and is just beginning to be fully explored (Collado, Cernada, Baüerl, Vento, & Pérez-Martínez, 2012; Shreiner, Kao, & Young, 2015). What we are discovering is that when the fetal immune response *in utero* is activated, there may be a profound effect on the developing fetal brain. The microbiome in the fetus may have a critical role in that immune activation.

Contemporary mothers may not have predominantly healthy microbes. Mothers will transmit to offspring microbes that they have, healthy or not. From the body's point of view, those are the microbes that are deemed necessary for survival in the environment mother lives in. If

mother is living with a pro-inflammatory microbiome, that is what she will transmit. Mother herself may be suffering with an overly active inflammatory state. The fetus may be affected then in two ways; direct exposure to the inflammatory elements of mother, and transmission of the maternal gut microbiome which may perpetuate inflammation. If we are unable to control inflammation in mother and adjust her microbiome to a less inflammatory one, the fetus is at risk for brain developmental abnormalities which result from inflammation.

Obesity, hypertension, diabetes, and stress are all too common among women today, and that includes woman of childbearing age. Obesity is one of the hallmarks of an inflammatory state. Obesity is associated with a physiologic state of chronic, low-grade inflammation with elevated levels of circulating inflammatory markers such as C-reactive protein (CRP), interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)-a. Obesity in pregnancy is also associated with inflammatory cytokines (Madan et al., 2009). Not surprisingly, obese pregnant women have higher rates of birth complications including prematurity, low Apgars, placental insufficiency, neonatal mortality, seizures, and cerebral palsy (Schmatz, Madan, Marino, & Davis, 2010). An experimental study in macaque monkeys showed that a high fat maternal diet alters the microbiome of the offspring adversely (Ma et al., 2014). Maternal nutritional habits during pregnancy and the use of antibiotics appear to influence the type of microbes present in the meconium at birth. Infants whose mothers consumed an organic or biodynamic diet seemed to have improved microbiome compared to mothers eating regular foods. We must recognize that the abnormal microbiome that accompanies poor eating habits and obesity in particular, may be affecting the inflammatory state of mothers and babies.

Ways to reduce inflammation and improve the microbiome in the adult include reduction of stress and improvement in diet. An anti-inflammatory diet is generally one with whole grains containing complex carbohydrates which have low glycemic index; fats predominantly from olives, avocados, seeds, and nuts; vegetables and fruits which are full of fiber and colorful; fermented foods (such as yogurt, sauerkraut, Kefir, kimchi); and limited meat/fish/dairy protein. The term “whole food” can be applied and also alludes to the process-free aspect of foods in this diet. An anti-inflammatory diet includes probiotics, for beneficial bacteria supplementation, and pre-biotics which are the fibers that the healthy bacteria thrive on. Probiotics generally contain limited species of microbes and have not been shown to benefit newborn infants, although they seem beneficial for adults and can reduce risk for preterm delivery in pregnant women (Myhre et al., 2011). Prebiotics are contained in fibrous plants/nuts and seeds. Prebiotics are already suggested for pregnant

woman to help control gastrointestinal symptoms such as bloating, constipation and cramping.

Normal weight, healthy diet, exercise, and stress reduction are important targets for any remedial program to improve one's microbiome, but other therapies are also being considered. Studies are underway to investigate the benefits of fecal transplant in a variety of disorders, including autism, but a fecal transplant program has not been recommended for pregnant women at this time. However, as research expands our understanding, new therapies are likely to emerge for treatments of maternal microbiome. However, the first simple dietary steps can be undertaken individually with clients in your practices to improve their own eating practices.

If we increase awareness of the effects of maternal microbiome on inflammation and the well-being of the fetus, we may gain attention and interest of young women we have otherwise been unable to reach. Movement toward healthy eating and healthy lifestyles for young women of childbearing age would undoubtedly go a long way towards increasing the general health of our children and lowering the incidence of some of the rampant societal disorders affecting the brain, such as autism and cerebral palsy (Marques, Bjørke-Monsen, Teixeira, & Silverman, 2015; Melillo, 2012).

Conclusion

Health is considered to be related to the degree of inflammation in our bodies. Whatever we can do to reduce inflammation, particularly in pregnancy, will help both women and babies (Englund-Ögge et al., 2014; Trosvik, Stenseth, & Rudi, 2010). The microbiome—as existent in the mother and transmitted to the fetus—is considered to be the intermediary by which much excessive inflammation is occurring.

Given the important findings of microbial populations in the placenta and fetus, the discovery of purpose for these microbes in developing immune function, and the commonality of maternal microbial transmission among almost all known maternal-offspring dyads, it is hard to imagine that nature intended the infant's first microbial exposure to be the birth canal as stated by some authors. Although there may be a transient difference in microbes related to delivery mode (not all researchers have not confirmed this; Yoon, Park, & Chaiworapongsa, 2003), it is critical that the microbial transmission *in utero* be recognized as existent and significant in fetal development.

Recent scientific evidence argues that the fetal womb is not sterile. In fact, there is important evolutionary benefit in fetal microbial exposure.

The establishment of a healthy microbiome and development of fetal immunity is vital to the well-being of the child, both in intrauterine and extrauterine life. This new paradigm means we leave behind the century old dogma of a sterile womb, and begin to fully investigate the impact that our endogenous microbes play in life in the womb.

The new paradigm must recognize that a healthy microbiome is critical for our own health and for the health of our babies. The inflammation associated with dysbiosis is well recognized in adult diseases, and is increasingly realized as a source of neuro-developmental disabilities for children such as autism and cerebral palsy. The new paradigm needs to include the possibility that the microbiome of the mother will have perhaps a lifelong effect on the brain development and general health of her baby.

Our intimate and interdependent relationship with microbes is an example of co-evolution and co-development of species that cannot be ignored. The research in this area is vastly and rapidly expanding, and the studies and findings presented in this article represent the tip of the iceberg. We stand at the threshold of deeper understanding of ourselves and our relationship to microbes. There may be a significant opportunity for us to improve our own health, and to improve the lifetime health and development of our babies, by the attention we give to establishing a healthy microbiome.

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