



# The matter of the reproductive microbiome

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## Abstract

The preconceptional presence of microbiota in the female and male reproductive organs suggests that fertilization is taking place in a nonsterile environment and contributes to reproductive success. The concept of embryonic development in a sterile uterus has also been challenged with recent reports of the existence of a microbiome of the placenta, amniotic fluid and the fetal gut in normal, uncomplicated pregnancies. The maternal origins of the microbiota colonising the fetus and its surroundings are unknown as are the mechanisms of maternal-to-fetal transfer. In this review, we aim to highlight the preconception male and female microbiome, the maternal vaginal and gut microbiome during pregnancy and the fetal microbiome, including their possible roles in reproduction, and maternal and neonatal pregnancy outcome.

## Keywords

Microbiome, maternal–fetal medicine, embryo, placenta, pregnancy, periconception

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## Introduction

The presence of microbes during pregnancy has for decades been associated with inflammation, disease and adverse pregnancy outcomes, such as preterm birth.<sup>1</sup> However, this was under the long prevailing assumption that the human body, including the uterus and placenta were sterile sites. Recent evidence, however, demonstrates a unique set of microbiota in the vagina,<sup>2</sup> uterine cavity<sup>3</sup> and placenta<sup>4</sup> in healthy pregnant women, which challenges this old adage that the embryo and fetus develop in a sterile environment.

The microbiota of the human body include bacteria, viruses, fungi, yeast and archaea. The term microbiome refers to the total genomic content of these microorganisms, but has nowadays become interchangeable with the term microbiota. Most of the microbiome research has focussed on the human gastro-intestinal tract hosting most of the microbiota across the body sites.<sup>5</sup> It is known that the local gut microbiome is involved in the digestion of foods, synthesis of vitamins, host metabolism, defense against pathogens, and immune responses, such as suppressing inflammatory responses and promoting immunological tolerance via the innate immune system.<sup>6,7</sup> The host-microbiome relationship can be beneficial, symbiotic and well-balanced, whereas a disruption in the local balance or composition of the microbiome can result in an unhealthy state of dysbiosis, which is among others associated with inflammation and disease (reviewed in Knight et al.<sup>8</sup>).

The Human Microbiome Project<sup>2,9</sup> revealed the presence of a microbiome in different human anatomical niches, such as gut, oral cavity, skin and vagina. Besides a diversity in the residing commensal microbes (alpha-diversity) at each site within each individual, there is also a difference between individuals in diversity in the microbial composition at each site (beta-diversity).<sup>2</sup> These differences are influenced by diet (Table 1), host genetics, body mass, early microbial exposure and antibiotics (reviewed in Turnbaugh et al.<sup>8</sup>). For a few

years, it has been known that also the endometrium,<sup>3</sup> placenta,<sup>4</sup> amniotic fluid,<sup>10</sup> seminal fluid<sup>11</sup> and fetal gut<sup>12</sup> have their own diverse and unique set of microbiota.

The amount of biomass (the number of microbiota of a tissue or a sample) is a continuous matter of controversy and debate. It is almost impossible to prevent contamination of a sample with DNA present in the air<sup>13</sup> or laboratory reagents.<sup>14,15</sup> Because of the high number of microbiota of various microbiome niches, the impact of contamination will be limited. However, in samples with a low biomass, such as seminal fluid,<sup>11</sup> placenta,<sup>4</sup> amniotic fluid<sup>10,16</sup> and meconium,<sup>17</sup> the possibility exists that the contaminating DNA will dominate the result of the microbiome analysis.<sup>15</sup> The challenge in interpreting microbiome research data is to approach contradicting results with this in mind.

In the present review, we aim to give an overview of the literature about the preconceptional and pregnancy microbiome at different sites and its impact on reproduction, fetal development and pregnancy outcome, with a focus on bacteria.

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## Preconception

The preconception period is important for pregnancy outcome, since preconception conditions (Table 1), such as diet and lifestyles have significant impact on reproductive success (reviewed in Steegers-Theunissen et al.<sup>18</sup>) and influence the composition of the gut microbiome during pregnancy.<sup>19</sup> Also, at the core of successful conception and subsequent pregnancy lies the preconception maturation of female and male gametes. Therefore, it could be assumed that the microbiome during the preconception period contributes to pregnancy outcome. Herewith in line are the recent reports showing that the microbiome of the follicular fluid<sup>20,21</sup> and endometrium are associated with implantation success.<sup>22</sup> In the next paragraphs, we describe the preconceptional microbiome of follicular fluid, semen, uterus and vagina.

### Microbiome of follicular fluid

Oocyte maturation occurs within the environment of the follicular fluid of the developing follicle. Pelzer et al.<sup>20,21</sup> showed that the human follicular fluid in women undergoing IVF cycles is not sterile, but colonized with microbiota. To discriminate between procedure-related contamination and in vivo colonisation, the follicular fluids were classified as either contaminated when the detected microbiota in follicular fluid were also detectable in the vagina during transvaginal oocyte retrieval and colonized when the microbiota were only present within the follicular fluid. The most prevalent microbiota detected in colonized follicular fluids were *Lactobacillus* spp. (*L. crispatus*, *L. gasseri*, *Actinomyces* spp. and *Propionibacterium* spp.). It has been suggested that the follicular fluid microbiome influences the outcome of IVF treatment, since the presence of *Lactobacillus* spp. is associated with better embryo quality leading to significantly higher rates of embryo transfer and pregnancy.<sup>21</sup> The antimicrobial properties of lactic acid, the major acid metabolite produced by *Lactobacillus* spp., can protect against adverse microbiota during oocyte maturation.<sup>23</sup> Interestingly, the follicular fluid of the left compared to the right ovary contains significantly more microbiota, perhaps due to the asymmetrical vascularisation of the gonads,<sup>21</sup> pointing towards distribution of microbiota via the blood stream.

### Microbiome of semen

Up until now, the microbiome of the interior milieu during spermatogenesis, such as that of the testis or epididymis, have not been reported. However, the microbiome of the ejaculate has been analysed and most likely represents the presence of microbiota from all contributing sites of the male reproductive tract, including

epididymis, seminal vesicles, prostate, bulbourethral glands and urethra. It revealed that semen contains the strictly anaerobic *Prevotella* and high proportions of facultative anaerobic bacteria of which the most abundant bacteria are *Lactobacillus*, *Pseudomonas*, *Gardnerella*, *Finegoldia*, *Corynebacterium* and *Staphylococcus*.<sup>11,24</sup> Based on the microbiome composition of the semen samples analysed so far, different seminal types can be identified, dominated by either *Lactobacillus*, *Pseudomonas* or *Prevotella*.<sup>11,24</sup> However, it is still unknown whether the microbiome of semen is associated with male fertility or semen quality, although Weng et al.<sup>24</sup> found a positive association of a *Lactobacillus* dominated microbiome with semen quality. The latter could be explained by the high levels of lactic acid synthesized by *Lactobacillus*, which could protect against the negative influence of Gram-negative bacteria during spermatogenesis.<sup>23,24</sup>

The seminal microbiome has a lower biomass, but a higher diversity of the microbiome compared to the vaginal microbiome (see below) and induces a significant change in the vaginal microbiome after intercourse.<sup>25</sup> As the seminal fluid and its microbiome get into contact with the endometrial fluid, a similar change in the endometrial microbiome could also occur. Hypothetically, an endometrial microbiome change and mixing of the endometrial and seminal fluid can influence the maternal-embryonic interaction during implantation and placentation. Supportive of this hypothesis, are experiments in rodent models that show an essential role for seminal fluid in enabling successful embryo implantation and optimal placental development (reviewed in Robertson and Sharkey<sup>26</sup>).

### The uterine/endometrial microbiome

Verstraelen et al.<sup>3</sup> showed in a group of women ( $n = 19$ ) with a variety of reproductive conditions by endometrial biopsies that the uterine microbiome mainly seems to consist of three bacterial phyla, in particular *Proteobacteria*, *Firmicutes* and *Bacteroidetes*, with *Bacteroidetes* being the most dominant phylum in the majority of the women included.<sup>3</sup> Importantly, species diversity and richness of the endometrium were significantly higher than that in the adjacent vaginal compartment (see below). The endometrium is suggested to be colonized with both vaginal and gut microbiota, since microbiota associated with the gastrointestinal tract, such as *Bacteroidetes* (*Bacteroidetes xylanisolvens*, *Bacteroidetes thetaiotaomicron*, *Bacteroidetes fragilis*) and *Proteobacteria*, as well as microbiota associated with the vaginal microbiome, such as *Lactobacillus iners*, *L. crispatus* and *Prevotella amnii*, were all identified in the endometrial samples.<sup>3</sup>

A different microbiome was found in endometrial fluid aspirates, with the most abundant genera being *Lactobacillus*, *Gardnerella*, *Bifidobacterium*, *Streptococcus* and *Prevotella*.<sup>22</sup> Importantly, a

**Table 1.** Exposures or conditions that affect the human maternal microbiome during the preconception period and pregnancy.

Exposure/condition	Timing	Affected microbiome
Maternal diet <sup>19,94</sup>	Preconception	Gut
Antibiotics <sup>95</sup>	Preconception	Gut
BMI <sup>42,86,96,97</sup>	Preconception	Gut, meconium (vaginal delivery)
Overweight / obesity <sup>96,98,99</sup>	Preconception	Gut
Diabetes mellitus <sup>100</sup>	Preconception	Meconium
Pregnancy <sup>34,35,37,40,41</sup>	Pregnancy	Gut, vagina
Maternal diet <sup>19,101</sup>	Pregnancy	Gut, placenta, meconium
Probiotics <sup>101,102</sup>	Pregnancy	Placenta, meconium, Vagina
Overweight/obesity <sup>42,45,46</sup>	Pregnancy	Gut
Gestational diabetes <sup>100,103</sup>		Gut, placenta, meconium
Gestational weight gain <sup>19,42,45,96</sup>	Pregnancy	Gut, placenta
Chorioamnionitis <sup>4,66,104</sup>	Pregnancy	Placenta, amniotic fluid

*Lactobacillus*-dominated microbiome of the aspirates was associated with higher chance of live birth, whereas the presence of other bacteria, especially for *Gardnerella* or *Streptococcus* genera, seemed to be correlated with implantation failure or early pregnancy loss.<sup>22</sup>

## Microbiome of vagina

The non-gravid vaginal microbiome is complex and in most women dominated by four *Lactobacillus* spp., i.e. *L. crispatus*, *L. iners*, *L. jensenii* or *L. gasseri*.<sup>27–29</sup> Some women show a vaginal microbiome less dominated by *Lactobacillus* spp. and have more strict anaerobes.<sup>27–29</sup> Ethnicity is of influence on the vaginal microbiome, since several studies showed that healthy, non-pregnant women from European descent have a *Lactobacillus*-dominated vaginal microbiome, whereas healthy non-pregnant African-American and Hispanic women have a non-*Lactobacillus*-dominated microbiome.<sup>28–30</sup> In addition, the vaginal microbiome is dynamic and seems to be affected by the menstrual cycle, the dominant microbiota and sexual activity.<sup>27</sup>

The diversity of the non-pregnant vaginal microflora may be important for pregnancy outcome, since at the time of embryo transfer, the vaginal microflora seems to be an important factor in the success of the IVF-embryo transfer procedure: A vaginal microbiome composed solely of *Lactobacillus* (*L. crispatus*, *L. iners*, *L. jensenii*, *L. gasseri* or other *Lactobacillus* species) at the moment of menses before embryo transfer is associated with a successful outcome of the IVF-ET procedure.<sup>31</sup>

In summary, recent research has repeatedly demonstrated the existence of a microbiome in the male and female reproductive organs and changed the perception of sterile fertilization. The increasing evidence that the presence of microbiota in the female reproductive tract plays a role during the process of implantation, indicates that residing microbiota of the male and female reproductive tract around the conception could influence, both beneficially or detrimentally, local immune responses and direct the process of

embryo implantation and subsequent placental development and function.

## Pregnancy

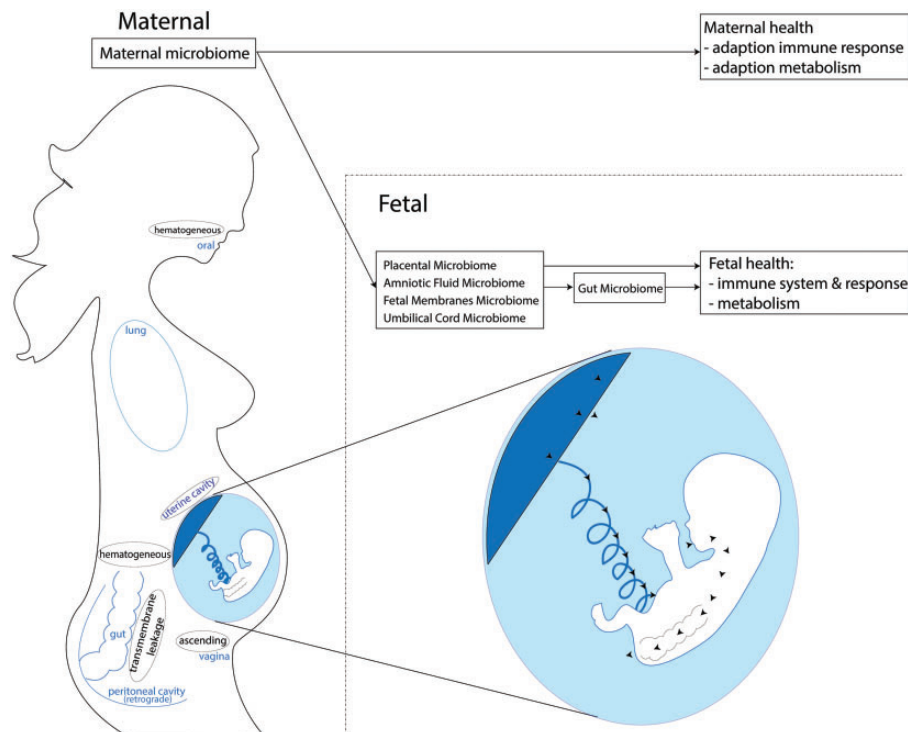
Around fertilization and during pregnancy, the maternal body undergoes a series of physiological adaptations to prepare for support, protect and nurture the embryo and fetus.<sup>32,33</sup> Besides anatomical, biochemical and immunological adaptations, the residing microbiome in the maternal body undergoes changes in parallel (Table 1). We are only beginning to understand which roles the maternal and paternal microbiota are playing during pregnancy. In line with the current knowledge about the interactive roles of the gut microbiome, not only dysbiosis in the gut, but also dysbiosis in the endometrium, vagina and placenta during pregnancy, could potentially lead to inflammation or disease. These changes may increase risk of adverse pregnancy outcomes, such as miscarriage and preterm birth. In the next paragraphs, we describe the pregnancy microbiome at different locations (Figure 1).

## Maternal microbiome

### Microbiome of the vagina

After implantation, the dynamics and stability of the vaginal microbial community change during the course of pregnancy (Tables 1 and 2). During the first trimester, a dominance of genus *Lactobacillus* in the vaginal microbiome of Caucasian women is observed.<sup>34–37</sup>

As pregnancy progresses beyond the first trimester, lesser diversity and richness combined with an increased stability are seen.<sup>34–39</sup> Also, various species of *Lactobacillus*, such as *L. iners*, *L. crispatus*, *L. jensenii* and *L. johnsonii*, are enriched in pregnancy.<sup>34</sup>



**Figure 1.** Overview of the maternal and fetal microbiome during pregnancy, health effects and maternal–fetal microbiome axes.

**Table 2.** Dynamics of local microbiomes preconceptionally and during pregnancy.

Location	Timing	Dynamics
<b>Maternal-preconception</b>		
Ovary <sup>20,21</sup>	Preconception	Unknown
Uterine/endometrium <sup>22</sup>	Preconception	Stable
Vagina <sup>27,34,40</sup>	Preconception	Highly dynamic
Gut <sup>2,41,42</sup>	Preconception	Stable – diet dependent
<b>Maternal-pregnancy</b>		
Vagina <sup>34,39,40</sup>	Pregnancy	Increased stability with each trimester
Gut <sup>41,42</sup>	Pregnancy	Dynamic – trimester dependent, increase microbial load
Oral <sup>105</sup>	Pregnancy	Dynamic – trimester dependent, increase microbial load
<b>Fetal</b>		
Placenta <sup>4</sup>	Pregnancy	Unknown
Amniotic fluid <sup>10</sup>	Pregnancy	Unknown
Fetal membrane <sup>10</sup>	Pregnancy	Unknown
Umbilical cord <sup>64</sup>	Pregnancy	Unknown

With continuation of pregnancy, in some women, a shift from one *Lactobacillus*-dominated to another *Lactobacillus*-dominated composition occurs,<sup>40</sup> whereas in other women, a specific increase in *Lactobacillus* is accompanied by a decrease in anaerobic microbiota.<sup>37</sup> While the vaginal microbiome varies during the course of pregnancy, around term, the vaginal microbiome seems to return to a non-pregnant composition.<sup>34</sup>

Women with a relatively stable vaginal microbiome throughout pregnancy, in terms of richness and diversity, seem to deliver more often at term, whereas women with a decrease of richness and diversity of the vaginal microbiome early in pregnancy seem to be at risk of preterm birth.<sup>35,36,39</sup> So far there are no indications that the composition of the vaginal microbiome before pregnancy predicts the dynamics during pregnancy and pregnancy outcome.<sup>39</sup>

### Microbiome of the gastrointestinal tract

During the first trimester of pregnancy, the maternal gut microbiome is comparable to the microbiome in the preconceptional period.<sup>41</sup> However, with progression of the pregnancy, the total number of microbiota increases (Table 1), paralleled by an overall increase in *Proteobacteria* and *Actinobacteria*.<sup>41,42</sup> It has also been shown that levels of *Faecalibacterium* are decreased during the third trimester of pregnancy.<sup>41</sup> Whereas within the gut of individual women the general diversity and richness decrease (alpha diversity), between women the diversity increases (beta diversity).<sup>41</sup> Another study, however, showed no changes in the gut microbiota during pregnancy or upon delivery,<sup>35</sup> indicating that more research is needed into the maternal microbiome during pregnancy.

Animal research showed that germ-free mice transplanted with third trimester stool microbiota of pregnant women as compared to germ-free animals treated with first trimester stool microbiota of pregnant women had reduced glucose tolerance and more weight gain.<sup>41</sup> These effects suggest that the maternal gut microbiome has an active role in the metabolic changes observed during pregnancy. In view of the effect of the gut microbiota on immune responses,<sup>6,7</sup> changes in the maternal gut microbiota during pregnancy could also have an impact on the maternal immune system. Adaptations of the maternal immune response during pregnancy are necessary to accept and prevent rejection of the semi-allogenic fetal tissues, while simultaneously maintaining its defence mechanism against microbes to ensure maternal and fetal survival during the same period (reviewed in Mor et al.<sup>43</sup>).

Also during pregnancy,<sup>44</sup> the maternal gut microbiome is strongly influenced by dietary patterns.<sup>19</sup> A recent study showed that the maternal diet in mice during the preconception period and during

pregnancy led to significant changes in the gut microbiome and metabolic pathways throughout pregnancy. In addition, obesity during pregnancy is associated with a different maternal gut microbiome composition (Table 1),<sup>42,45,46</sup> characterized by increased *Bacteroides* and *Staphylococcus*.<sup>42</sup>

Changes in the gut microbial composition between the first and third trimester are suggested to be involved in supporting the changing metabolic and immunological needs of the maternal body. However, there are indications that microbiota are also important for fetal growth and development, since as described below, transfer of microbiota from the mother to the fetus takes place.

### Embryonic and fetal microbiome

After reaching the uterine cavity, the blastocyst hatches so that the trophoblast, the outer extra-embryonic layer, is able to invade the maternal endometrium and reach the local microbiome. The three embryonic cell lines, the epiblast, trophoblast and hypoblast, will give rise to the different embryonic and extra-embryonic tissues. Epiblast and trophoblast chorion most likely contribute to the chorion,<sup>47</sup> whereas the epiblast will give rise to the embryo proper and the amnion.<sup>47</sup> Around 17–20 weeks of gestation, the inner amniotic membrane will fuse with the outer chorion to form the double-layered chorioamniotic membrane.<sup>48</sup> In order to form the placenta, cells of the trophoblast will invade the maternal spiral arteries to remodel them. Initially, these cells accumulate in the lumen to form trophoblast plugs<sup>49</sup> blocking maternal blood flow to the placenta until the end of the first trimester, when they disintegrate.<sup>50</sup>

Many pregnancy complications, such as preeclampsia, fetal growth restriction and abruption placentae, but also preterm birth, premature rupture of membranes and late spontaneous abortion are associated with disorders of deep placentation.<sup>51</sup> Suboptimal invasion of the endometrium and aberrant spiral artery remodelling will result in placental insufficiency,<sup>52,53</sup> which will often lead to (iatrogenic) preterm birth because of fetal distress caused by either inadequate placental attachment and uteroplacental ischemia or premature placental detachment. As such, these pregnancy complications often find their origin during embryonic implantation.<sup>52</sup>

Implantation is accompanied by an interactive immunological dialogue between local cytokines, chemokines, the maternal immune system and the developing embryo. More recently, Mor et al.<sup>43</sup> have hypothesized a role for the local microbiome in this interactive immunological dialogue. Although unknown so far, it is feasible that also the seminal and endometrial microbiome may play a role in the process of implantation and placentation.



In the following paragraph, we will give a summary of the microbiome of the fetus and its environment (Figure 1).

### Microbiome of the placenta

Stout et al.<sup>54</sup> used high magnification imaging in combination with the use of histologic tissue staining and observed intracellular microorganisms in the basal plates of 27% of both preterm and term placentas. More importantly, the apparent evidence of the presence of microbiota was found without any histological or pathological signs of infection. Parnell et al.<sup>55</sup> found significant differences between the microbiome of the basal plate and the placental villi. *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, and *Spirochaetes* were detected in the basal plate, whereas the placental villi harboured *Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Spirochaetes*. This may be related to different susceptibility of the different spatial compartments of the placenta to infection and specific pathogens.<sup>56–59</sup>

The combination of 16S rDNA and whole-genome shotgun metagenomic techniques showed that the placenta exhibits its own unique microbiome, with a low abundance but a metabolically rich microbiome. The placental microbiome largely consists of nonpathogenic commensal microbiota from the phyla of *Firmicutes*, *Tenericutes*, *Proteobacteria*, *Bacteroidetes*, and *Fusobacteria*<sup>4</sup> and mostly resembles that of the oral cavity<sup>4</sup> and the deep endometrium of non-pregnant women<sup>3</sup> rather than that of the adjacent vaginal microbiome.<sup>2</sup> This is in contrast to another study which failed to identify a distinct placental microbiome and ascribed the detected bacteria of the placenta samples to bacterial contamination of the environment or the reagents used.<sup>60</sup>

Although, the presence of commensal, symbiotic placental microbiota seems increasingly valid, the presence of pathological microbes or a potential local dysbiosis can lead to adverse pregnancy outcomes. A different microbiome, as compared with normal pregnancy outcome, has been detected in placentas of pregnancies complicated by preterm birth.<sup>61</sup> The microbiota found in the preterm placenta were similar to those commonly residing in the vagina (*Prevotella bivia*, *Lactobacillus* spp., *Peptostreptococcus magnus*, or *Gardnerella vaginalis*). In placentas of other pregnancy complications such as preeclampsia or fetal growth restriction (FGR), the microbiota *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella*, *Escherichia*, *Klebsiella pneumoniae*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* ssp., *Porphyromonas gingivalis* and *Prevotella intermedia* have been cultured or sequenced.<sup>61–63</sup>

Since fetal viability, growth and development are completely dependent on optimal placental function, the recent finding of a placental microbiome in healthy pregnancy may implicate a role for the bacteria in normal fetal growth and development.

### Microbiome of fetal membranes, umbilical cord and amniotic fluid

Already in 2005, the presence of several different microbial DNA was reported in the amniotic membranes of term healthy pregnancies of women in or outside labour.<sup>63</sup> More recently, the composition of the microbiota in the fetal membranes was shown to consist of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Spirochaetes*. Although, most of these microbiota were also detectable in the basal plate and placental villi of the placenta attached, the ratio of the different microbiota and total composition of the microbiome was significantly different between basal plate and placental villi.<sup>55</sup>

In 2005, Jimenez et al.<sup>64</sup> reported the identification of bacteria in umbilical cord blood from term neonates born via elective caesarean section. The microbiota identified were *Enterococcus faecium*,

*Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Streptococcus sanguinis*, which are regarded as commensals in healthy neonates.<sup>64</sup>

In healthy pregnant women, bacteria were found not only in the placenta and umbilical cord blood, but also in the amniotic fluid. A recent, elegant study comparing the microbiome of different maternal and fetal samples collected during elective term caesarean section showed that the microbiota in amniotic fluid were similar to the placental microbiota.<sup>10</sup> The most prevalent phylum, *Proteobacteria*, showed low abundance, low richness and low diversity, but with high consistency across different individuals. Other phyla detected were *Enterobacter*, and *Escherichia/Shigella*, *Propionibacterium*, *Lactobacillus*, *Streptococcus* and *Staphylococcus*. Importantly, besides microbial DNA, also viable microbiota were detected.<sup>10</sup> In addition, in amniotic fluid aspirated from healthy women between a gestational age of 16 until 20 weeks and who delivered at term, *Ureaplasma* spp. was detected.<sup>65</sup>

Although the reported bacterial findings seem not to be related to adverse pregnancy outcome, different compositions of the microbiome of the amniotic fluid are associated with chorioamnionitis (Table 1) and could even be used for prediction of preterm birth.<sup>66</sup>

The developing fetus is not only immersed in amniotic fluid, but the swallowing and exchange of amniotic fluid are indispensable for proper development of the fetal lung and gastrointestinal tract.<sup>16,67</sup> Around term, the fetus will swallow around 1000 ml amniotic fluid per day including the microbiota.<sup>68</sup> The presence of amniotic bacteria may thus aid in bacterial colonisation of the fetal gut. Indeed, continuous fetal amniotic fluid ingestion could then lead to colonization of the fetal gut<sup>69</sup> (see also below).

### Microbiome of the fetal gut

In line with the observations that the amniotic fluid contains bacteria, also the meconium as product of the fetal gut is not sterile.<sup>10,12,17</sup> The presence of *Streptococcus*, *Enterobacteriaceae*, such as *Enterobacter* and *Escherichia*, *Propionibacterium*, *Lactobacillus*, *Bacillales* has been reported; however, the dominant microbiota in meconium samples seems to differ between studies.<sup>10,17</sup> Although the meconium microbiome has a unique microbial composition, it shows significant resemblance with both the microbiota of the amniotic fluid<sup>10,17</sup> and the placenta.<sup>10</sup> These data suggest, in contrast to the general belief, that colonization of the gut already occurs during pregnancy.

It thus seems increasingly evident that the fetal compartment, including the placenta and membranes, are not sterile, but colonised during pregnancy. The fetal gut may be colonised via the placenta and amniotic fluid, since the meconium microbiome resembles the microbiome of the placenta and amniotic fluid. The function of the fetal microbiome is unknown; however, considering the effect of the adult microbiome on immune responses and metabolism, it may be speculated that the fetal microbiome is important for maturation of the fetal immune response as well as for optimal setting of the fetal metabolism (Figure 1).

### The maternal–fetal microbiota axes

The mechanisms by which microbiota transfer from the mother to the fetus are still unclear. However, it is not unthinkable that the routes for seeding of maternal microbiota to the fetal environment are the same as the suggested potential routes of intrauterine infection (Figure 1).<sup>70</sup> Although they could potentially contribute, iatrogenic routes such as chorionic villus sampling, amniocentesis or blunt external trauma, are not plausible as standard.

### The systemic blood circulation

The most obvious and effective route would be spreading via the systemic blood circulation. During pregnancy, the cardiac output directed toward the uterine arteries increases from 3.5% at the beginning of pregnancy to 12% around term.<sup>71</sup> The combined effect of all maternal pregnancy adaptations leads to an increased uteroplacental blood flow of 20–50 ml/min to 450–800 ml/min at term in singleton pregnancies,<sup>72</sup> which may contribute to delivering diverse microorganisms to the fetal–maternal interface.

Direct evidence for a haematogenous maternal gut–fetal route comes from animal studies showing that oral intake of genetically labeled *Enterococcus Fecium* by pregnant mice could be traced back in the amniotic fluid<sup>64</sup> and meconium,<sup>73</sup> whereas it could not be detected in non-inoculated pregnant mice.<sup>64,73</sup> Maternal dendritic cells and leukocytes are suggested as trafficking cells for bacterial uptake by placenta.<sup>74</sup> Dendritic cells can cross the paracellular space of the intestinal epithelium to directly ingest bacteria from the intestinal lumen. However, dendritic cells are relatively ineffective at killing internalized organisms, and bacteria can by way of dendritic cells hematogeneously spread to other locations, such as the placenta<sup>75</sup> and be transferred to the fetus via the paracellular pathway of the placental barrier<sup>76</sup> into the amniotic fluid or the fetal blood circulation via the umbilical cord.

The similarity of composition of the placental microbiome to the microbiome of oral cavity<sup>4</sup> and the longstanding association between periodontal disease and adverse pregnancy outcome supports the hematogenous shedding of periodontitis-associated bacteria (reviewed in Cobb et al.<sup>77</sup>).

### The peritoneal cavity

While all abdominal organs are enclosed in the subperitoneal cavity by the peritoneum, the fallopian fimbriated ends are structures that open into the peritoneal cavity.<sup>78</sup> This makes retrograde spreading of microbiota present in the peritoneal cavity fluid to the fetal compartments another possible route of transfer of bacteria from the mother to the fetus.

Increased intestinal permeability due to a change in microbiome or inflammation<sup>41</sup> could result in gut bacterial leakage into the peritoneal cavity.<sup>79</sup> Inflammatory cells are known to spread across the one cell-layer peritoneum into the peritoneal cavity.<sup>78</sup> However, whether the inflammatory state and shift in gut microbiome during pregnancy leads to increased gut permeability and transfer of the gut microbiome into the peritoneal cavity is unknown; however, the ability of dendritic cells to cross the intestinal lumen<sup>74</sup> suggests it is possible.

### The lower reproductive tract

Ascending maternal vaginal microbiota could also be a potential source of microbiome of the fetal compartment,<sup>80</sup> since DNA from vaginal microbes has been found in amniotic fluid.<sup>81</sup> The fetal membranes are thought to form a barrier to ascending microorganisms, which is supported by the fact that after prolonged (premature) rupture of the membranes, intrauterine infection and preterm labour often occur.<sup>82</sup> Since the fusion of the fetal membranes to form a barrier does not occur until 17–20 weeks of gestation,<sup>48</sup> the uterine cavity could still be accessible for ascending vaginal microbiota, hereby potentially contributing to the early placental and fetal microbiome.

### Uterine cavity

The presence of a local residing uterine and endometrial microbiota<sup>3,22,83</sup> indicates that during pregnancy, a local microbiome may also be present. Since with implantation the fetal and maternal compartment come into close contact, it is feasible that the uterine and endometrial microbiome, and perhaps even the seminal microbiome, could contribute to the initial placental microbiome.

The main source of the microbiome of the fetal compartment during pregnancy has to be maternal, however, despite different theoretical routes of spreading, it is unknown which, what and how the maternal microbiome contributes to the microbiome of the fetal compartments.

### Birth

Despite microbial colonization of the fetal compartments, including the fetal gut, the main maternal source of the postnatal microbiome of the newborn immediately after birth is probably provided during birth. Depending on the birth mode, vaginal delivery versus caesarean section, the neonate has to respectively pass through the vagina or the abdominal skin. The mechanistic process of being born exposes the newborn to commensal bacteria colonizing the vagina, perineum and skin, subsequently resulting in neonatal gut and skin colonization.

Vaginal delivery leads to colonization of neonatal gut with microbes resembling the maternal vaginal microbiota, such as *Lactobacillus*, *Prevotella* and *Bifidobacterium*.<sup>84,85</sup> On the other hand, neonates delivered by caesarean section showed an intestinal absence of vaginal microbes, e.g. *Bifidobacterium*,<sup>84</sup> but the presence of microbes resembling the microbial communities of the maternal skin, such as *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp.<sup>85</sup>

### Conclusion

The physiological state of pregnancy demands a significant adaptation of the maternal body, including changes in the residing microbial compositions and establishing a placental and fetal microbiome. In addition, during the preconception period, the composition of the male and female microbiome may also interfere with reproductive success. However, it is still difficult to determine which microbial composition is detrimental and optimal for the host or what the exact function of the microbiome at each location is.

The changes in the maternal microbiome seem to support maternal health, since it affects maternal metabolism,<sup>41</sup> while also influencing maternal immune responses. On the other hand, changes in the maternal microbiome could also be important for colonisation of the fetus, most likely necessary for the development of fetal immune responses and for inducing the metabolic settings (Figure 1).<sup>86,87</sup> It is therefore essential to understand the routes of microbial seeding and how the fetal microbiome is established.

The potentially important role of the maternal and fetal microbiome in maternal, fetal and neonatal health prompts the question of antibiotic use during pregnancy. Antibiotic treatment is associated with disruption of the microbiome<sup>88,89</sup> and with a large number of health problems, such as metabolic and immunological diseases.<sup>88</sup> Recent studies have shown that prenatal antibiotic use is associated with an increased risk of childhood asthma<sup>90</sup> and childhood obesity.<sup>91</sup> There are speculations that this is due to the effect of prenatal antibiotics on the maternal and fetal microbiome, resulting in dysbiosis, with detrimental effects on health. In line with the DOHaD theory,<sup>92</sup> cautious use of antibiotics should therefore be indicated, since the effect of in utero exposure on the establishment of the fetal microbiome and postnatal development and health in later life

is unknown, while it is known that the desired effect of preventing adverse pregnancy outcome is minimal.<sup>70</sup>

In order to put the roles of the microbiome in maternal and fetal physiology and pathology in perspective, the next steps in microbiome research should also include the interactive effects of other microorganisms present, such as bacteria, viruses, fungi, yeast and archaea. Racicot et al.<sup>93</sup> showed that the cervix of pregnant mice is more susceptible to viral infection as compared to the cervix of non-pregnant mice. More importantly, a viral infection of the cervix during pregnancy reduces the protection against ascending vaginal bacteria. Besides, expanding the range of microbiota, combining microbiome research with whole genome sequencing, metagenomics and metabolomics are essential to interpret the potential effects of the microbiome on maternal metabolism and epigenetics.

For the microbiome research focussing on the period of pregnancy, we would like to propose to include the periconception period. Not only do the results of the endometrial<sup>22</sup> and vaginal microbiome<sup>39</sup> suggest an interactive role around and during pregnancy, effective lifestyle interventions during the preconception period increase not only pregnancy chances, but also health in maternal postpartum and neonatal life.<sup>18</sup>

This knowledge could lead to the development of pregnancy or periconceptional novel dietary interventions, such as treatment with pre- or probiotics, to prevent or correct certain dysbiotic states. Preconceptional or early pregnancy microbiome data could be future biomarkers to determine obstetrical and fetal health. The ultimate goal would be, if necessary, to postpone a pregnancy to correct a suboptimal and dysbiotic maternal (and paternal) microbiome composition to optimally prepare a couple for a future healthy pregnancy and child.

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