Research Article / *Araştırma Makalesi*

Ege Journal of Medicine / *Ege Tıp Dergisi* 2021; 60 (1): 83-89

Amniotic fluid microbiome in asymptomatic pregnants at second trimester

İkinci trimesterde asemptomatik gebeliklerde amniyotik sıvı mikrobiyomu

Özge Kömürcü Karuserci[1] Yasemin Zer[2] Seyhun Sucu[1] Elif İşbilen[3]
Hanifi Bademkıran[1] Hilmi Taşdemir[1]

[1] Department of Gynecology and Obstetrics, Gaziantep University, Gaziantep, Turkey
[2] Department of Microbiology, Gaziantep University, Gaziantep, Turkey
[3] Department of Biochemistry, Gaziantep University, Gaziantep, Turkey

ABSTRACT

Aim: The purpose of this prospective cohort study is to evaluate the possible microbiome of the amniotic cavity in the second trimester in asymptomatic pregnant women by the culture-based technique.

Materials and Methods: This prospective cohort study was conducted in Gaziantep University Gynecology and Obstetrics Clinic between October 2017 and November 2019. 100 pregnant women who had amniocentesis for genetic screening in the fetus, and who had no complaints or clinical symptoms of vaginal or chorioamnionitis infection, were included in the study. While culture tests were performed by using the amniotic fluids of these pregnant women, glucose and leukocyte levels of their amniotic fluids were also measured. At the same time, culture and gram staining analyses were performed by collecting vaginal swab specimens from the patients.

Results: 12 patients (12%, 95% confidence interval, 0 to 41%) had positive amniotic fluid culture results. The most observed bacteria were E. coli (5%). No findings of clinical infection were observed in the patients with positive amniotic fluid culture results. These patients delivered healthy babies with no complications.

Conclusion: The amniotic fluid has its own microbiome, and the vaginal flora plays a role in the formation of this microbiome. This is a preliminary study; therefore, larger studies and targeted broad range molecular methods are needed to find the variety of the possible flora of the amniotic fluid.

Keywords: Microbiome, amniotic fluid, pregnancy, culture, vaginal fluid.

ÖZ

Amaç: *Bu prospektif kohort çalışmasının amacı, ikinci trimesterde asemptomatik gebelerde amniyotik kavitenin olası mikrobiyomunu kültür temelli teknikle değerlendirmektir.*

Gereç ve Yöntem: *Bu prospektif kohort çalışma, Gaziantep Üniversitesi Kadın Hastalıkları ve Doğum Kliniği'nde Ekim 2017- Kasım 2019 tarihleri arasında gerçekleştirildi. Fetüste genetik tarama için amniyosentez yapılan, vajinal enfeksiyon veya koryoamniyonite dair klinik semptomu olmayan 100 gebe kadın çalışmaya dahil edildi. Bu gebelerin amniyotik sıvıları kullanılarak kültür testleri yapılırken, amniyotik sıvılarının glikoz ve lökosit düzeyleri de ölçüldü. Aynı zamanda hastalardan vajinal sürüntü örnekleri alınarak kültür ve gram boyama analizleri yapıldı.*

Bulgular: *12 hastada (%12, %95 güven aralığı, %0 ile %41) amniyotik sıvı kültürü pozitif çıktı. En sık görülen bakteri E. coli idi (%5). Amniyotik sıvı kültürü sonucu pozitif olan hastalarda klinik enfeksiyon bulgusu izlenmedi. Bu hastalar hiçbir komplikasyon olmaksızın sağlıklı bebekler doğurdu.*

Corresponding author: Özge Kömürcü Karuserci
Department of Gynecology and Obstetrics, Gaziantep University, Gaziantep, Turkey
E-mail: *ozgekomurcu@hotmail.com*
Application date: 24.11.2020 Accepted: 04.02.2021

Sonuç: *Amniyotik sıvının kendi mikrobiyomu vardır ve vajinal flora bu mikrobiyomun oluşumunda rol oynar. Bu bir ön çalışmadır; bu nedenle, amniyotik sıvının olası florasının çeşitliliğini bulmak için daha büyük çalışmalara ve geniş kapsamlı moleküler yöntemlere ihtiyaç vardır.*

Anahtar Sözcükler: *Mikrobiyom, amniyotik sıvı, gebelik, kültür, vajinal sıvı.*

INTRODUCTION

Microbiome plays an important role in the early period of the life of the baby by affecting immune functions (1). The effect of microbiome on metabolism and immune system has become evident in the recent years. There is an increasing curiosity in understanding whether microbial relationships begin in the fetal environment, and if these relationships start in the intrauterine period, what is the source of the microorganisms?

Most of the studies conducted starting from the early twentieth century until today have demonstrated that amniotic fluid is sterile during a normal pregnancy (2). Therefore, any bacterial isolation from the amniotic fluid has been considered to be a pathological finding so far.

In fact, the amniotic fluid has been studied during labor, cesarean delivery or preterm labor in most of the studies conducted so far (3-6). But what about the condition of the amniotic fluids of healthy pregnant women who have no complaints and pathological findings or risk of preterm labor in the second trimester? What if there is a microbiome in the intra-amniotic space? It is now known that the placenta has its own endogenous microbial flora (7, 8). If the placenta has its own endogenous flora, why not the amniotic space, too? These questions have attracted many scientists' curiosity for at least a century, and no clear answer has been found so far.

The purpose of this prospective cohort study is to evaluate the possible microbiome of the amniotic cavity in the second trimester in asymptomatic pregnant women by the culture-based technique.

MATERIALS AND METHODS

This prospective cohort study was conducted in Department of Obstetrics, Gaziantep University, Turkey Clinic between October 2017 and November 2019 with the ethics committee approval number 2017/226. Informed consent was obtained from the patients before they were included in the study.

100 pregnant women aged between 18 and 40, who had singleton pregnancies on weeks 15.0-20, who had amniocentesis for genetic screening in the fetus, and who had no complaints or clinical symptoms of vaginal or chorioamnionitis infection, were included in the study. While culture tests were performed by using the amniotic fluids of these pregnant women, glucose and leukocyte levels of their amniotic fluids were measured at the same time. At the same time, culture and gram staining analyses were performed by collecting vaginal swab specimens from the patients. The data obtained were evaluated together with the pregnancy results, maternal clinical and demographic attributes.

The exclusion criteria were chronic diseases such as membrane rupture, cervical effacement or dilation, major fetal abnormality, medical indication for miscarriage, active viral or bacterial infection, hypertension, diabetes, and connective tissue diseases.

Transabdominal amniocentesis was performed for all patients under the same conditions and by the same team using a full antiseptic skin preparation with 25 G disposable needle (Hanaco Medical Co., Ltd., Saitama, Japan). 10 cc excess fluid was collected from the amniotic fluid collected for genetic examination. 5 cc of this fluid was put in an aerobic blood culture bottle (Bactec 9240 BD, USA). The remaining 5 cc fluid was divided into 2 separate tubes and sent to the biochemistry and microbiology laboratories for biochemical evaluation and microscopic examination (cell count and gram staining). Vaginal swab specimens were simultaneously collected from the patients and Thayer-Martin agar (Oxoid, UK), chocolate agar (BD, USA) and blood agar (BD, USA) culture tests were performed.

Amniotic fluid specimens collected into blood culture bottles were kept in the system for an incubation period of 5 days. 5% sheep blood agar (BD, USA) and eosin methylene blue agar (BD, USA) culture analyses were performed for the reproduced bacteria and they were incubated under aerobic conditions and in 10% CO2 incubator at 37°C for 48-72 hours. Vitek2 (Biomerieux, France) identification system was used to identify the detected bacteria.

WBC (aCell) and glucose (aGlucose) levels in the amniotic fluid specimens were measured in the

biochemistry laboratory. aCell was determined using a vertical microscope (ECLIPSE Ci-L; Nikon, Tokyo, Japan) and expressed as the number of cells per cubic millimeter. aGlucose was analyzed automatically using a LABOSPECT 008 (Hitachi High-Technologies Corporation, Tokyo, Japan).

All patients included in the study were routinely followed up until the end of pregnancy. Chorioamnionitis, premature rupture of the membrane, preterm birth, presence of genital infections; and additionally, birth week, weight, Apgar score and any signs of clinical infection in the newborn were recorded. Treatment was not administered to any patient with no suspected clinical findings during pregnancy. Patients whose vaginal culture tests were positive were treated through the vaginal route with appropriate antibiotic therapy for 7 days.

Although this was a prospective study, it was not a comparative study. Therefore, we present only the descriptive statistical analysis of the culture results.

Statistical analysis

Continuous variables were summarized as median and range, categorical variables as numbers and percentages. Odds ratios (OR) and 95% confidence intervals (CI) were estimated by using logistic regression models. Chi-square test was used in the analysis of the data presented as N (%) and Kruskal-Walla ANOVA [sic: Kruskal-Wallis ANOVA] was used in the analysis of the data presented as median (interquartile interval). All statistical analyses were performed by using the JMP software version 9 (SAS Institute, Car, NC, USA) and SPSS version 16.0J for Windows Base System SC (SPSS Japan, Tokyo, Japan). $p<0.05$ value was considered statistically significant.

RESULTS

Table-1 shows the maternal characteristics, demographic and clinical data of 100 patients who constituted the study population.

12 patients (12%, 95% confidence interval, 0 to 41%) had positive amniotic fluid culture results. The most observed bacteria were E. coli (5%) in the amniotic fluid. Table-2 shows the results of patients who had reproduction in their amniotic fluids compared to the vaginal culture results.

Four patients continued smoking during pregnancy; however, no reproduction was observed in the specimens collected from the amniotic fluid or vagina in these patients.

No findings of clinical infection were observed in the patients with positive amniotic fluid culture results. These patients delivered healthy babies with no complications.

Preterm labor was observed in eleven patients (<37 weeks), but none of them had reproduction in their amniotic culture. Reproduction occurred in two of the patients who had preterm labor (Candida spp., S. agalactiae). The mean gestational age of preterm deliveries was 34 weeks.

Table-1. Maternal characteristics, demographic data and clinical data of patients.

	Median (IQR) or % (n/N)
Maternal age (years)	25 (19.2 - 32.3)
Body mass index (kg/m^2)	25 (24 - 31)
AF glucose (mg/dL)	32 (24 - 35)
AF white blood cell count (cells/mm^3)	4 (0 - 8)
GA at amniocentesis (weeks)	18 (15 - 20)
GA at delivery (weeks)	37 (31 - 40)
Birth weight (grams)	2950 (2450 - 3900)
AF culture positive	12 (12/100)
AF gram stain positive	25 (3/12)
Vaginal culture positive	15 (15/100)
Vaginal gram stain positive	60 (9/15)

AF: amniotic fluid; GA: gestational age; IQR: interquartile range

Table-2. Results of patients who had reproduction in their amniotic fluids compared to the vaginal culture results.

Sample number	Amnion culture	Vaginal culture	Gram stain
1	P. spp.	P. spp	Gram positive cocci
2	P. spp	C. spp.	Gram positive cocci
3	P. spp	None	None
4	E. coli	E. coli	Gram positive cocci
5	E. coli	E. coli	Gram positive cocci
6	E. coli	S. agalactiae	None
7	E. coli	C. spp,	Gram positive cocci
8	E. coli	None	None
9	S. agalactiae	S. agalactiae	Gram positive cocci
10	S. agalactiae	E. coli	Gram positive cocci
11	S. epidermidis	S. agalactiae	Gram positive cocci
12	S. epidermidis	None	None

Abbreviations: S. agalactiae: Streptococcus agalactiae; E. coli: Escherichia coli; S. epidermidis; Staphylococcus epidermidis; P. spp: Peptostreptococcus spp; C. spp: Candida spp

Table-3. Maternal clinical information and pregnancy outcome of culture-positive and culture-negative groups.

	Amnion Culture positive (n=12)	Amnion Culture negative (n=88)	p value
Maternal age (years)*	30 (21-36)	32 (28-35)	0.27
Nulliparity[+]	3 (25)	21 (24)	0.38
GA at amniocentesis (week)*	18 (17-19)	17 (16-18)	0.64
Ga at delivery (week)*	38 (38-40)	37 (37-40)	0.72
Birthweight (grams)*	2950 (2600-3400)	3100 (2780-3800)	0.86
1 minute Apgar<7[+]	2 (17)	11 (12.5)	0.71
5 minute Apgar<7 [+]	1 (8)	5 (6)	0.61
Body Mass index (kg/m^2)	26 (24-29)	27 (24-31)	0.66
AF glucose (mg/dL)	33 (25 - 34)	29 (27-35)	0.13
AF white blood cell count (cells/mm^3)	3 (1 - 8)	5 (0-6)	0.23

[+]Data presented as n (%) and analyzed by Chi square

* Data presented as median (interquartile range) and analyzed by Kruskal-Walla

None of the babies delivered by the pregnant women included in this study had no complications directly related to the infection. None of them had neonatal death and severe long-term neonatal morbidity, tocolysis, tocolysis resistant preterm delivery, fetal distress and placental abruption.

No statistical difference was observed in the demographic characteristics of the patients with and without bacterial growth in the amnion culture (p> 0.05). Table-3 shows the pregnancy outcomes of the groups with positive and negative culture test results with maternal clinical and demographic data.

DISCUSSION

Although there are some studies asserting the idea that the amniotic fluid is sterile (9-11), according to the results of our study, reproduction was observed in the culture tests of the mid-trimester amniotic fluids of the healthy pregnant women with a rate of 12% and this result was partially consistent with the rare culture-based studies conducted on this subject (12, 13). However, molecular-based studies investigating the amniotic fluid of the pregnant women with complicated conditions such as preterm labor, preeclampsia, infants with low birth weight, premature rupture of the membranes have also observed bacteria in the amniotic fluid, but most of the studies conducted on this subject were

conducted with pregnant women with complications (14-19). Recently, bacterial sequences have been detected in the amniotic fluids collected from 15 healthy term pregnancies using new generation sequencing; this suggests that human amniotic fluid has a microbial community and that microbial effects on the infant health and development begin before birth (20-21). Similarly, there are different studies confirming the presence of bacterial populations in other components of the utero environment including placenta, cord blood, and meconium (22-24).

Microorganisms can reach the amniotic cavity iatrogenically during interventional procedures such as migration from the genital system, hematogenous spread through the placenta or amniocentesis (25, 26). According to the results of previous culture studies, the most common way of bacterial invasion of the amniotic cavity is through the ascending vaginal infections (27). The mechanism causing bacterial invasion and subsequent preterm labor starts with the colonization of microorganisms, and progresses with inflammation of the amniotic membranes and pro-inflammatory cytokine activation, prostaglandin production, premature contractions, and preterm delivery, respectively (28). Microorganisms can also induce preterm labor directly by producing some enzymes or by causing prostaglandin synthesis (29). In our study, both the clinical and laboratory findings of the patients and the white blood cell and glucose values obtained as a result of the biochemical examination of the amniotic fluid were not in favor of infection in none of the patients who had reproduction in their culture and no preterm labor was observed in none of these patients.

In this case, if microorganisms in the amniotic fluid do not cause any infections, can these microorganisms be considered members of the microbiome? Or can a new terminology such as colonization or “transient amnionemia” be used? It seems very exciting to answer this question.

In our study, Peptostreptococcus spp reproduced in the amniotic fluids of 3 patients, E. coli in 5 patients, Strep.agalactiae in 2 patients, and S. epidermitis in 2 patients. All bacteria other than S. epidermitis among the bacteria that reproduced were members of the normal vaginal flora. The different aspects of this study compared to the studies conducted until now are as follows: blood culture method, which is a reproduction method, was used when evaluating the amniotic fluid, vaginal culture tests were also simultaneously performed and evaluated, and the study was conducted in healthy pregnant women who had no clinical problems or signs of preterm labor in the early pregnancy week. There are studies with different outcomes in the literature as well. In a previous culture-based study evaluating the amniotic fluids of 166 preterm pregnant women, 2 Peptostreptococcus spp. and 1 Streptococcus agalactiae reproduced (17); in another study with 62 patients, Ureoplasma urealiticum reproduced only in 1 patient (18), and in another study in which the specimens collected from 52 patients with intrauterine growth restriction, no reproduction occurred in the amniotic fluid (19). The study which was most similar to our results was the study of the pregnant women diagnosed with preterm labor (17), and the pregnant women in our own patient group did not have any preterm labor diagnosis or suspicion. In a study of 50 women who were terminated before the 20th week of gestation, four Mycoplasma hominis and two Staphylococcus epidermidis were isolated from the amniotic fluid. In this study, the authors assumed that M. hominis was transmitted through the vagina and that S.epidermitis was associated with skin contamination (1). In another study, 21-26% bacteria were isolated from the placenta of the pregnant women, who delivered healthy term, by culture method and viable bacteria (Enterococcus, streptococcus, staphylococcus or Propionibacterium) were isolated from the cord blood of the healthy newborn babies (10). In our study, a bacterial growth with a rate of 12% was observed. S. epidermidis was isolated in two pregnant women and although it was collected under aseptic conditions, we think that there might be skin contamination based on the reproduction of the bacteria even in small amounts in the blood culture bottles we used for bacterial isolation. Other detected factors are largely similar to the results of the vaginal culture tests and it was observed that they formed a profile similar to the vaginal flora spectrum. The possibility of this transition due to changes in permeability without the micropore or defect occurring in the amniotic membrane are subjects awaiting discussion and proof. We think that the fact that bacteria such as Chlamydia trachomatis (12), Mycoplasma, Ureoplasma (28), which have been found in the culture-based studies so far, are bacteria with

vaginal and cervical colonization supports this hypothesis. Therefore, if there is a microbiome in the amnion, its source may most likely be the vaginal flora. However, this hypothesis must be proven at the DNA level by molecular tests.

In this study, we aimed to investigate the presence of bacterial population and/or microbiome in the amniotic fluid of pregnant women in the early period by using culture-based methods. Because we thought that the results we obtained would have a major impact on our understanding of how the baby intestine or placenta was full of microbes initially. Although dethroned by the modern molecular techniques, culture-based bacterial isolation is still the first choice as a low-cost and easy method. Although culture methods also provide a much stronger microbial analysis than other methods morphologically and biochemically (30), more than 99% of the complex microbial groups observed microscopically cannot be reproduced in the culture analyses. Because pure culture methods are only suitable for the growth of a limited microbial population with no numerical dominance or clinical significance. In this case, in addition to culture-based methods, molecular methods such as polymerase chain reaction (PCR) can provide more detailed and clear results (31, 32).

CONCLUSION

We can conclude that the amniotic fluid can have its own microbiome, and the vaginal flora plays a role in the formation of this microbiome. This is a preliminary study; therefore, larger studies and targeted broad range molecular methods are needed to find the variety of the possible flora of the amniotic fluid.

Conflict of interest

There is no conflict of interest.

Acknowledgements

Thanks to Tanyeli Güneyligil Kazaz for statistical contrubutions.

References

1. Romero R, Espinoza J, Chaiworapongsa T, Kalache K. Infection and prematurity and the role of preventive strategies. Semin Neonatol 2002; 7 (4): 259-74.
2. Harris JW, Brown JH. The bacterial content of the uterus at cesarean section. AJOG 1927; 13 (2): 133-43.
3. DiGiulio DB. Diversity of microbes in amniotic fluid. Semin Fetal Neonat M 2012;17 (1): 2-11.
4. Zhou X, Brotman RM, Gajer P, et al. Recent advances in understanding the microbiology of the female reproductive tract and the causes of premature birth. Infect Dis Obstet Gynecol. 2010.
5. Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med 2014; 6 (237): 237ra65-237ra65.
6. Antony KM, Ma J, Mitchell KB, Racusin DA, Versalovic J, Aagaard K. The preterm placental microbiome varies in association with excess maternal gestational weight gain. AJOG 2015; 212 (5): 653. e1-653. e16.
7. Chu D, Stewart C, Seferovic M, et al. 26: Profiling of microbiota in second trimester amniotic fluid reveals a distinctive community present in the mid trimester and predictive of the placental microbiome at parturition. AJOG 2017; 216 (1): S18-S19.
8. Gisslen T, Harris RA, Sweeney EL, et al. The placental microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis.2016.
9. Pelzer E, Gomez-Arango LF, Barrett HL, Nitert MD. Maternal health and the placental microbiome. Placenta 2017; 54: 30-7.
10. Lim ES, Rodriguez C, Holtz LR. Amniotic fluid from healthy term pregnancies does not harbor a detectable microbial community. Microbiome 2018; 6 (1): 1-8.
11. Montuclard B, Guibert M, Ville Y, Frydman R, Fernandez H. Does asymptomatic amniotic infection in the second trimester really exist? J Gynecol Obstet Biol Reprod 1996; 25 (2): 186-91.
12. Mändar R, Loivukene K, Ehrenberg A, et al. Amniotic fluid microflora in asymptomatic women at mid-gestation. Scand J Infect Dis 2001; 33 (1): 60-2.
13. DiGiulio DB, Romero R, Amogan HP, et al. Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PloS one 2008; 3 (8): e3056.
14. Gervasi MT, Romero R, Bracalente G, et al. Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and

associations with spontaneous early (< 32 weeks) and late (> 32 weeks) preterm delivery. J Perinat Med 2012; 40 (4): 329-43.
15. Gerber S, Vial Y, Hohfeld P, Witkin SS. Detection of Ureaplasma urealyticum in second-trimester amniotic fluid by polymerase chain reaction correlates with subsequent preterm labor and delivery. J Infect Dis 2003; 187 (3): 518-21.
16. Nguyen DP, Gerber S, Hohlfeld P, Sandrine G, Witkin SS. Mycoplasma hominis in mid-trimester amniotic fluid: relation to pregnancy outcome. J Perinat Med 2004; 32 (4): 323-6.
17. Rowlands S, Danielewski JA, Tabrizi SN, Walker SP, Garland SM. Microbial invasion of the amniotic cavity in midtrimester pregnancies using molecular microbiology. AJOG 2017; 217 (1): 71. e1-71. e5.
18. DiGiulio DB, Gervasi M, Romero R, et al. Microbial invasion of the amniotic cavity in preeclampsia as assessed by cultivation and sequence-based methods. J Perinat Med 2010; 38 (5): 503-13.
19. DiGiulio DB, Gervasi MT, Romero R, et al. Microbial invasion of the amniotic cavity in pregnancies with small-for-gestational-age fetuses. J Perinat Med 2010; 38 (5): 495-502.
20. DiGiulio DB, Romero R, Kusanovic JP, et al. Prevalence and diversity of microbes in the amniotic fluid, the fetal inflammatory response, and pregnancy outcome in women with preterm pre-labor rupture of membranes. Am J Reprod Immunol 2010; 64 (1):38-57.
21. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep 2016;6:23129.
22. Willyard C. Could baby's first bacteria take root before birth? Nature 2018;553:7688.
23. Perez-Muñoz ME, Arrieta MC, Ramer-Tait AE, Walter J. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: implications for research on the pioneer infant microbiome. Microbiome 2017; 5 (1): 48.
24. Stout MJ, Conlon B, Landeau M, et al. Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations. AJOG 2013; 208 (3): 226. e1-226. e7.
25. Jiménez E, Fernandez L, Marin ML, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. Curr Microbiol 2005; 51 (4): 270-4.
26. Lauder AP, Roche AM, Sherrill-Mix S, et al. Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. Microbiome 2016; 4 (1): 29.
27. Combs CA, Gravett M, Garite TJ, et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. AJOG 2014; 210 (2): 125. e1-125. e15.
28. Mendz GL, Kaakoush NO, Quinlivan JA. Bacterial aetiological agents of intra-amniotic infections and preterm birth in pregnant women. Front Cell Infect Microbiol 2013;3:58.
29. Stinson LF, Payne MS. Infection-mediated preterm birth: Bacterial origins and avenues for intervention.Aust N Z J Obstet Gynaecol 2019; 59 (6): 781-90.
30. Suff N, Karda R, Diaz JA, et al. Ascending vaginal infection using bioluminescent bacteria evokes intrauterine inflammation, preterm birth, and neonatal brain injury in pregnant mice. Am J Pathol 2018; 188 (10): 2164-76.
31. Rehbinder EM, Carlsen KCL, Staff AC, et al. Is amniotic fluid of women with uncomplicated term pregnancies free of bacteria? AJOG 2018; 219 (3): 289. e1-289. e12.
32. Kuperman AA, Zimmerman A, Hamadia S, et al. Deep microbial analysis of multiple placentas shows no evidence for a placental microbiome. BJOG 2020; 127 (2): 159-69.